MECHANISMS OF FATIGUE DURING PROLONGED EXERCISE IN THE HEAT

By

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ABSTRACT

Increase in body temperature is a major factor limiting endurance performance in the heat and it is shown in this thesis that the effects of raised body temperature on performance, perception and neuroendocrine response to exercise are mediated by an interaction of body temperatures. Prolactin has been used as an indicator of hypothalamic activity and the pathways regulating its release have been investigated using pindolol as a 5-HT_{1A} antagonist. The prolactin response to a buspirone challenge has been shown to be approximately 50% serotonergic and 50% dopaminergic, but with a wide inter-subject variation. Passive heating is a potent stimulus for prolactin release and it was shown that 5-HT_{1A} stimulation plays virtually no part in this process, raising the possibility that prolactin release during hyperthermic exercise may also be largely due to withdrawal of dopamine inhibition. A comparison of exercise tolerance in the heat and the sensitivity of central serotonergic and dopaminergic pathways further indicates the importance of dopamine in central fatigue. The action of caffeine in enhancing endurance performance has been shown not to involve the hypothalamus and this draws attention to other pathways that may be involved in central fatigue including the basal ganglia and limbic system.

It doesn't matter what temperature the room is; it's always room temperature.

Steven Wright - US comic

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The action of caffeine and perception of exertion during prolonged exercise.
M.W. Bridge, J. Broom, G. Besford, T. Allen, A. Sharma, D.A. Jones
The Journal of Physiology (2000) 523P, pp.224P

Effect of ambient temperature on exercise-induced prolactinaemia.
M.W. Bridge, T. Allen, A. Sharma and D.A. Jones
The Journal of Physiology (1999) 521P; pp 100P

Chapter 1

Fatigue, Thermoregulation and the Brain

1.1 Fatigue during exercise

Fatigue is defined in the Oxford English dictionary as; ***a)*** Lassitude or weariness resulting from either bodily or mental exertion; ***b)*** a condition of muscles, organs, or cells characterized by a temporary reduction in power or sensitivity following a period of prolonged activity or stimulation. These two definitions correspond quite well with what, in the physiological world, have come to be known respectively as, central and peripheral fatigue. Another term sometimes used is 'objective fatigue' (Layzer, 1998) which is seen as a deterioration of performance, either during a specific type of competition or as a task is repeated from one day to another.

Edwards (Edwards, 1983) described the major possible causes of fatigue in relation to the chain of command for voluntary muscle activations (Figure 1.1). The separation of these possible causes into peripheral and central effects occurs at the level of the motoneurone. A failure of transmission along the peripheral nerve, at the neuromuscular junction or within the muscle itself is therefore considered peripheral fatigue and is the main problem during short duration high intensity exercise. Many studies have addressed the biochemical mechanisms of peripheral fatigue and while the details have yet to be elucidated, there is broad agreement that the loss of force and power is due to decreased calcium release and slowing of cross bridge cycling consequent upon metabolic changes within the muscle fibre (Allen *et al.*, 1995; Jones, 1999).

Chain of command   **Possible mechanisms of fatigue**

Impaired:

'Psyche'/brain

↓   ←————————   Motivation
                i.e. recruitment of motor units

Spinal cord

↓   ←————————   Reflex drive

Peripheral nerve

↓   ←————————   Neuromuscular transmission

Muscle sarcolemma

↓   ←————————   Muscle action potential

Transverse
tubular system

↓   ←————————   Excitation

Calcium release

↓   ←————————   Activation

↓   ←————————   Energy supply

Actin-myosin interaction/
cross-bridge formation

↓

Muscle contraction

↓

Force/power output

Figure 1.1 The chain of command for voluntary contractions and the major causes for fatigue. From (Edwards, 1983).

Endurance (i.e. the time to volitional fatigue) during exercise is negatively related to the exercise intensity during both dynamic and static contractions (Sahlin, 1992). The relationships are not linear but have a strong curvilinear appearance (Figure 1.2).
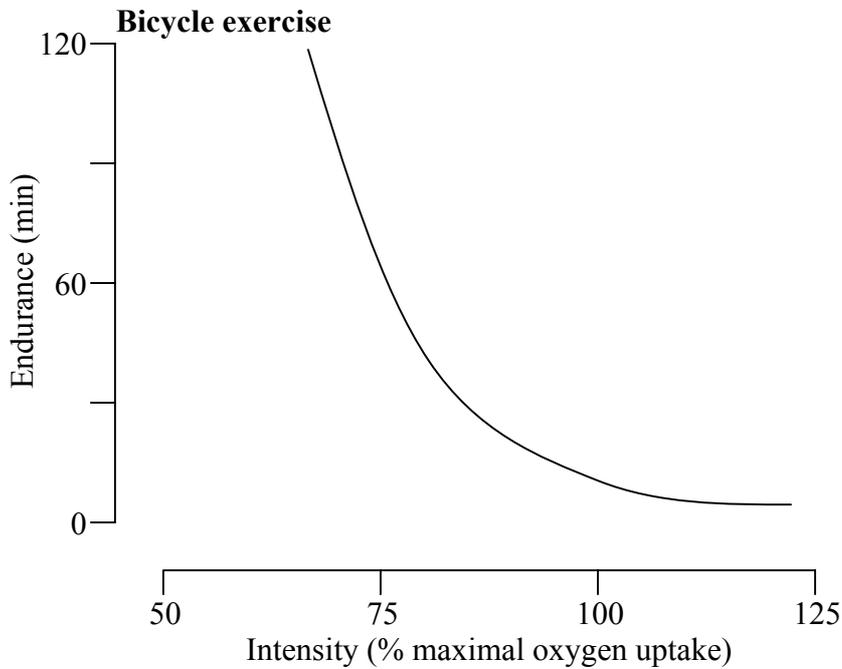
Figure 1.2 Estimated endurance time during cycle ergometry in relation to work intensity, from (Sahlin, 1992).

1.1.1 Prolonged exercise

The very short durations of exercise at intensities greater than 100% maximal oxygen uptake, are clearly limited by the inability of the muscle to supply energy at a sufficient rate to meet the demands and will lead to peripheral failure. However, for exercise below 100% maximal oxygen uptake, the mechanism of fatigue is not so clear. The energy demands are within the aerobic capacity of the body and, in theory, exercise should continue until the fuel supply is exhausted and, at low intensity, where energy is supplied mainly by fatty acid oxidation, this could continue for days. At higher workloads in well-fed subjects, carbohydrate stores could support exercise for several hours. When the stores of oxidative fuels are depleted, anaerobic sources will be called upon and metabolic failure will occur within minutes. However these upper limits of endurance are achieved only by some individuals and in some circumstances, notably by experienced and trained subjects exercising at moderate intensities in cool conditions. It appears, therefore, that there may be at least two mechanisms limiting submaximal exercise. One is a peripheral mechanism related to the exhaustion of energy reserves, while the other is of less obvious origin.

1.1.1.1   Peripheral Fatigue

At exercise intensities between 60 and 90% of maximal oxygen uptake, fatigue has been associated with depleted stores of muscle glycogen (Saltin & Karlsson, 1972). Depletion of muscle glycogen poses a metabolic problem for the muscle but it also puts a strain on the liver and, consequently, on blood glucose levels and hypoglycaemia also has a detrimental effect on exercise. Many studies have manipulated muscle glycogen content through diet. Elevation of pre-exercise muscle glycogen has generally been found to be of benefit only in events lasting longer than 60-90 minutes where delays in fatigue of ~20% have been reported (Hawley *et al.*, 1997).

Feeding carbohydrate during exercise is one way of counteracting the reduction in muscle and liver glycogen during exercise and maintaining blood glucose. This has been demonstrated to improve exercise performance and time to fatigue in a large number of studies, to the extent that the relationship between improved performance and carbohydrate feeding can be considered true (Jeukendrup & Jentjens, 2000). However even with carbohydrate feedings perceived exertion still increases during exercise, and fatigue cannot be delayed indefinitely.

The hints and suggestions of central fatigue mechanisms during prolonged exercise under thermo- neutral conditions are much more evident when exercise is carried out in hot and/or humid conditions. Perceived exertion is higher and fatigue occurs before carbohydrate levels become limiting (Galloway & Maughan, 1997, 2000) during exercise in the heat. It is likely, therefore that fatigue during exercise in the heat is largely due to central factors whereas in cooler environments peripheral mechanisms of fatigue are more important (see section 1.3).

1.1.1.2   Perceived exertion and central fatigue

Whether we perceive exercise as easy or hard depends on many factors, only some of which are related to the physical or mental demands, while others depend on our interest and motivation. The perceived difficulty of a task is continuously monitored and if this increases there is a natural tendency to reduce the demands by slowing

down or stopping. The perception of muscular effort is not an absolute physical reality but something that is modulated by a multitude of internal and external factors. Internal factors may be the sensitivity with which afferent information is perceived and processed; external factors might include distractions, rewards or threats. An example of heightened perception of exercise is the disorder of chronic fatigue syndrome whose suffers, in the extreme case, find even the mildest activity demanding and tiring and yet have no impairment of muscle function (Lloyd *et al.*, 1991; Gibson *et al.*, 1993). At the other extreme, highly motivated athletes competing for the highest prizes will endure conditions that most would find intolerable.

These internal and external considerations seem to set the overall gain or sensitivity of the processes linking perception and motivation. But, no matter what the absolute level of perception, all subjects report an increase in the perception of exertion as exercise continues even when the exercise is constant and apparently non-fatiguing, as judged by the signs that normally signify peripheral fatigue (e.g. Robertson, 1982; Borg *et al.*, 1987; Robertson *et al.*, 1990; Maw *et al.*, 1993; Glass *et al.*, 1994; Travlos & Marisi, 1996; Marvin *et al.*, 1997; Garcin *et al.*, 1998; Szmedra & Bacharach, 1998; Held & Marti, 1999; Utter *et al.*, 1999; Nielsen *et al.*, 2001; Nybo & Nielsen, 2001c).

It would appear, therefore, that there is some factor that accumulates during exercise and increases the perception of exertion. This factor, or factors, could be the accumulation of some metabolite or product of activity such as heat, it may be the summation of some constant signal, such as stretch of tendon organs, or an increasing signal from inflamed or damaged receptors in muscles, tendons or joints. There is one further possibility, and that is the summation of feed-forward efferent drive from the motor cortex or from the cardiovascular and respiratory centres.

Electrical stimulation was first used by Merton (1954) to determine whether it is possible to fully activate skeletal muscles by a voluntary effort and the technique of twitch superimposition or " twitch occlusion" was further developed for experimental and clinical applications by a number of groups (Bigland-Ritchie *et al.*, 1978; Rutherford *et al.*, 1986 and see Gandevia, 2001 for review). Most of these studies have been concerned with high force isometric contractions, primarily because it is

technically difficult to stimulate and record from a muscle that is moving although James et al (1995) and Beelen et al (1995) managed to use the technique during isokinetic knee extension and cycling, respectively. In these cases electrical stimulation was applied to the peripheral nerve or branches within the muscle which means that it is not possible to distinguish failure at the motoneurone from failure at high centres.

The introduction of transcranial magnetic stimulation (TMS) has allowed the techniques to be safely extended to the motor cortex to assess muscle activation (Hollge *et al.*, 1997). Liepert et al (1996) compared TMS and peripheral electrical stimulations after fatiguing finger abduction exercise in controls and patients suffering from central nervous system (CNS) lesions and subjective fatigue. The authors concluded that the fatigue resulting from the exercise could not be attributed to either intramuscular processes or to a reduced spinal excitability, but that it probably reflected a supraspinal, probably cortical, phenomenon. Gandevia et al (1996) stimulated different levels of the motor pathway during prolonged fatiguing contractions. They found no limitation to maximum voluntary contraction (MVC) occurred in the motor cortex, α–motoneurones or within the muscle fibres. The one area that may be involved in the genesis of central fatigue during repetitive exercise is an inadequate neural drive 'upstream' of the motor cortex (Gandevia *et al.*, 1996; Gandevia, 1999) although the cause of this inadequate drive is unknown (Taylor *et al.*, 2000). So far, studies using TMS, as with electrical stimulation, have concentrated on single muscle groups of the upper body primarily looking at high force contractions and have not been applied to prolonged exercise.

Nybo & Nielsen (2001a) assessed the ability of subjects to maintain activation of voluntary isometric contractions following cycling exercise in thermoneutral and hyperthermic conditions and showed that hyperthermic subjects had difficulty fully activating their muscles. Interestingly this difficulty applied both to the quadriceps, the major muscle group used in the exercise, but also to handgrip strength, which presumably was not heavily used. This implies a global reduction in motor activation rather than specific inhibition of the working muscles.

A recent study looked at the effects of an ultramarathon (65 km) on neuromuscular function (Millet *et al.*, 2002). The authors found that both MVC and maximal voluntary activation decreased significantly after the ultramarathon and suggested that these changes were mostly due to central fatigue, possibly resulting from changes in intracortical excitability or some effect of afferent feedback from the muscle.

Another technique that has been used to assess changes in the motor output of the brain with exercise is the measurement of brain EEG. Several studies have looked at changes in activity during prolonged sub-maximal exercise (Kubitz & Mott, 1996; Nielsen *et al.*, 2001; Nybo & Nielsen, 2001c) and found increases towards the end of exercise suggesting that a greater central drive was required to maintain the same motor output.

Whilst it still remains difficult to obtain direct evidence of central fatigue during prolonged dynamic exercise, the evidence strongly suggests that, as exercise continues, a greater central effort is required to generate the same output from the motor cortex. This increased drive to the motor cortex may well impinge on consciousness, increasing the sense of effort through a feed-forward mechanism and eventually resulting in fatigue.

1.1.1.3 Summary

The literature suggests that fatigue during prolonged endurance exercise has a significant central component as is apparent during constant load exercise when perceived exertion increases steadily long before muscle glycogen or other causes of peripheral fatigue become limiting. Central fatigue is unlikely to be the only cause of loss of performance in every situation since the relative importance of central and peripheral factors will vary with the type and intensity of exercise and the physical and psychological characteristics of the subjects. It is evident, however, that central fatigue can be an important factor limiting performance and, moreover, the extent of the problem and the underlying mechanisms are poorly understood in comparison to peripheral muscle fatigue that has been the subject of considerable research over the last 50 years.

1.2 The neuroendocrine system and fatigue

The inability to continue exercising in some circumstances may be due to a loss of central drive or motivation and changes in brain motor activity, as discussed above. The role of changes in central neurotransmission in fatigue and the loss of motivation during prolonged sub maximal exercise has recently become the subject of a great deal of interest. It was suggested by Newsholme and colleagues (1987) that changes in plasma amino acid concentrations could play a role in central fatigue by altering the synthesis, concentration and release of neurotransmitters and, in particular, 5-Hydroxytryptamine (serotonin, 5-HT) in the brain. Although Newsholme's ideas about the role of fatty acids and branch chain amino acids regulating tryptophan entry into the brain have been largely discounted as a result of the careful experiments of Van Hall et al (1995) and Struder et al (1996b), his original idea that 5-HT has a role to play in the genesis of central fatigue still excites considerable interest (see section 1.2.1.3 below)

5-HT is not the only neurotransmitter that is of interest in central fatigue. A possible role of dopamine (DA), which is heavily involved in motor control, has recently arisen and there is increasing evidence to support this idea.

To establish a clear role of 5-HT/DA in a fatigue of a central nature during prolonged exercise a number of tests might be applied:

(i)   Do brain 5-HT/DA concentrations and activity change during exercise in such a way that they could produce a central fatigue?

(ii)   Do manipulations of brain 5-HT/DA activity alter exercise performance?

(iii)   Do differences in brain 5-HT/DA activity that occur between individuals reflect differences in exercise ability?

1.2.1 The serotonergic system and exercise

Interest in 5-hydorxytryptamine (serotonin) as a neurotransmitter dates from 1953 when Gaddum (1953) found that lysergic acid diethylamide (LSD) acted on 5-HT receptors in peripheral tissues and suggested that its central effects may likewise be due to a serotonergic action. 5-HT is known to have a multitude of actions within the brain. It is involved in the control of arousal, sleepiness and mood (Jacobs & Fornal, 1993) and might therefore be linked to fatigue during prolonged exercise, possibly through an inhibitory influence on motor neurones. An increase in voluntary drive necessary to overcome this inhibition would result in an increased perception of effort. Conversely, there is evidence that an increase in serotonin in certain areas of the brain is beneficial to exercise (Jacobs & Fornal, 1993). It is also interesting to note that there is a substantial evidence to suggest that 5-HT also plays a role in the control of thermoregulation (see section 1.4).

1.2.1.1 Central serotonergic pathways

Serotonergic neurones are widely distributed throughout the brain. The earliest maps of serotonergic neurones and fibres were produced using the formaldehyde-induced fluorescence method and, together with newer methods such as autoradiography, have given a good understanding of the extent of the serotonergic projections within the brain. In their pioneering studies, Dahlstrom and Fuxe (1964) described nine 5-HT containing cell groups in the rat CNS, which they designated B1-B9. The largest collection of 5-HT neurones is found in the dorsal raphe nuclei in the lower brain stem and in humans is estimated to contain about 165,000 cells which have projections to all areas of the brain (Figure 1.3). 5-HT neurons concentrated in the midline raphe nuclei in the pons and medulla project diffusely to the cortex, limbic system, hypothalamus and spinal cord.
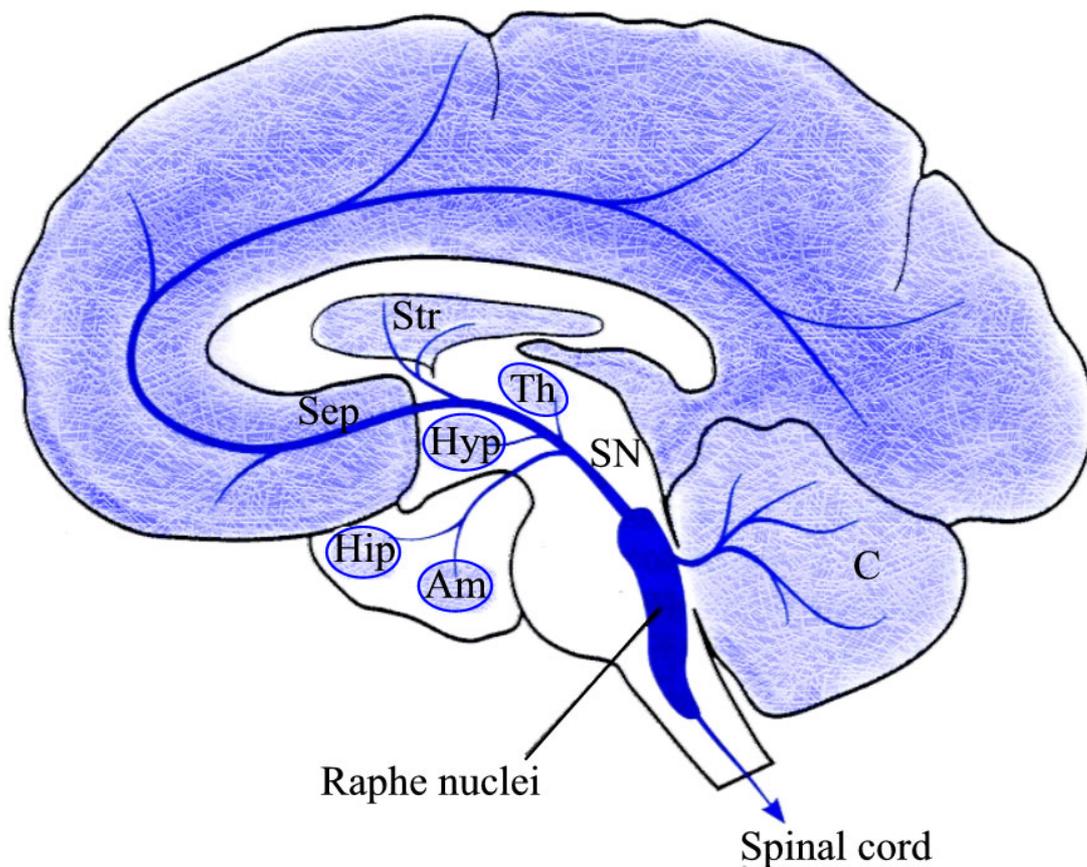
Figure 1.3 5-HT neurones and major pathways in the brain. The location of the main groups of cells bodies and fibre tracts is in solid black. Light shaded areas show the location of serotonergic terminals. (Am – Amygaloid nucleus; C – cerebellum; Hip – hippocampus; Hyp – hypothalamus; Sep – septum; SN – substantia nigra; Str – striatum; Th – thalamus.)

There are a number of receptor subtypes that mediate the central and peripheral actions of 5-HT. There are seven types of receptor ($5\text{-HT}_{1\text{-}7}$) with further subtypes (A-D) of 5HT_1 and 5HT_2. 5-HT_1 receptors occur mainly in the CNS and, in the case of 5-HT_{1D}, in some blood vessels (see Table 1.1). The general actions of the 5-HT_1 receptor group are neural inhibition and vasoconstriction. 5-HT_2 and 5-HT_3 receptors occur in greater number outside the CNS than do the 5-HT_1 group and the effects of these groups are generally excitatory (see Table 1.1).

Receptor	Location	Main Effects
1A	CNS	Neuronal inhibition Behavioural effects: sleep, feeding, thermoregulation, anxiety
1B	CNS	Presynaptic inhibition Behavioural effects
1D	CNS Blood vessels	Cerebral vasoconstriction Behavioural effects: locomotion
2A	CNS PNS Smooth Muscle Platelets	Neuronal excitation Behavioural effects Platelet aggregation Smooth muscle contraction Vasoconstriction / vasodilatation
2B	Gastric Fundus	Contraction
2C	CNS Choroid plexus	CSF secretion
3	PNS (GI tract) CNS	Neuronal excitation Emesis Behavioural Effects: Anxiety

Table 1.1 5-HT$_{1/2/3}$ receptor subtypes and their main effects. For more detail see (Barnes & Sharp, 1999).

The precise localisation of the 5-HT neurons in the brain has allowed their electrical activity to be studies in detail. From this it has been found that in vertebrates certain behavioural and physiological functions relate to 5-HT pathways, namely: various behavioural responses; control of mood and emotion; control of sleep/wakefulness; control of sensory transmission; control of body temperature, (Rang *et al.*, 1999).

1.1.1.2 Control of 5-HT synthesis and release

Serotonin is an indolealkylamine whose chemical designation is 3-(2-aminoethyl)indol-5-ol, but usually goes under the name 5-hydroxytryptamine (5-HT). The chemical structure of 5-HT closely resembles that of its precursor the amino acid tryptophan (TYP). 5-HT is synthesised from TYP in a two step process catalysed by the enzymes tryptophan hydroxylase and aromatic L-amino acid decarboxylase (Figure 1.4)

A number of control mechanisms regulate 5-HT synthesis and some involve alterations to the active state of tryptophan hydroxylase. There is considerable

evidence that an increase in the rate of firing of serotonergic neurones leads to an increase in the activity of this enzyme. Conversely a suppression in firing rate through the action of somatodendritic $5\text{-}HT_{1A}$ autoreceptors has been shown to be consistent with a reduction in the V_{max} of tryptophan hydroxylase (Fernstrom *et al.*, 1990). There is no real evidence of a direct inhibitory action of 5-HT itself on the enzyme.

$$\text{L-Tryptophan} + O_2 \xrightarrow[\substack{\text{Tryptophan} \\ \text{hydroxylase}}]{\text{Tetrahydrobiopterin}} \text{L-5-Hydroxytryptophan (5-HTP)}$$

| Pyridoxal phosphate | Aromatic L-amino acid decarboxylase |

5-Hydroxytryptamine
(5-HT; serotonin) $+ CO_2$

| Monoamine oxidase (MAO) | Flavin adenine dinucleotide (FAD) |

5-Hydroxyindoleacetaldehyde

| H_2O, NAD^+ | Aldehyde dehydrogenase |

5-Hydroxyindoleacetic acid (5-HIAA
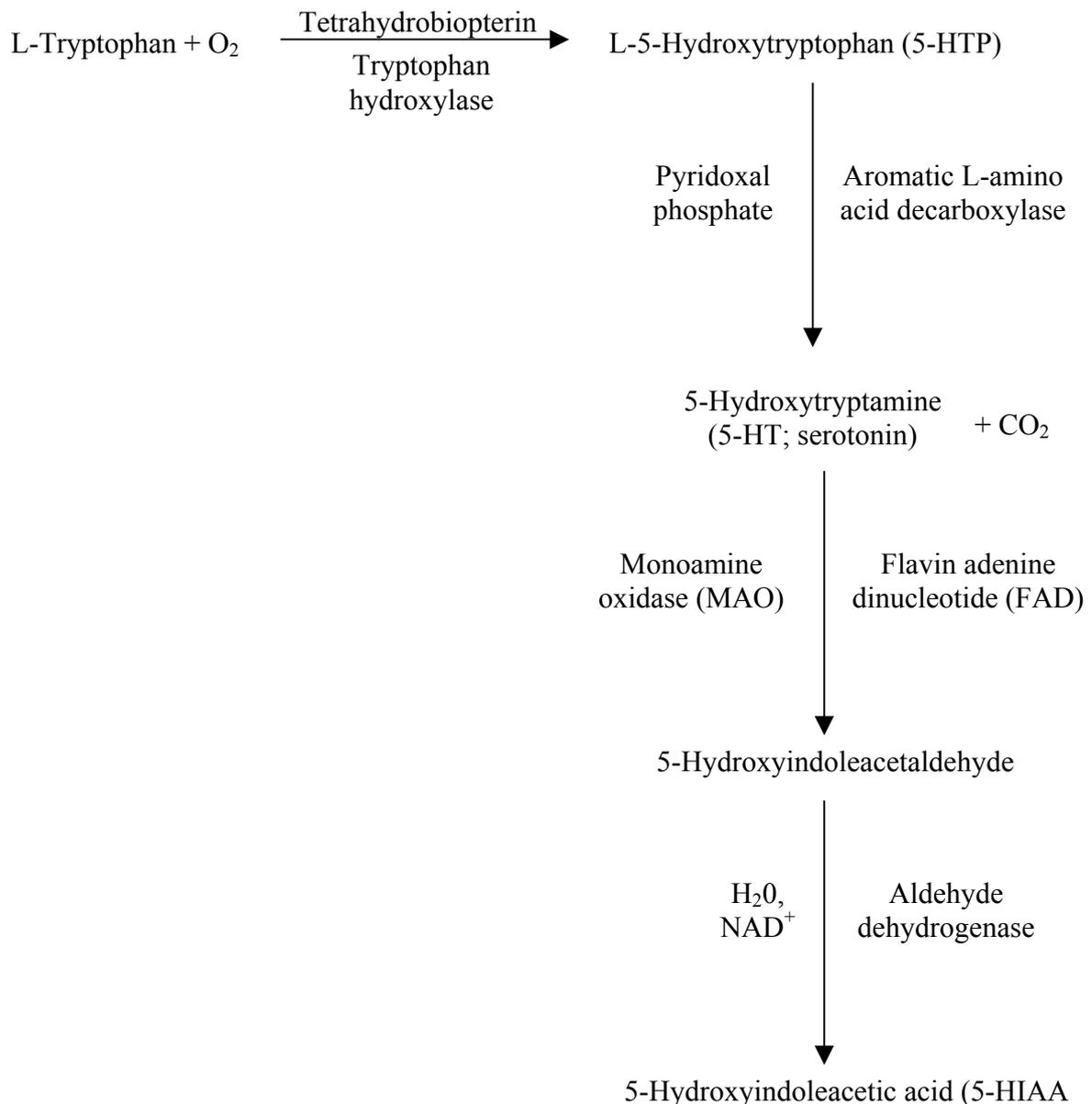
Figure 1.4 Synthesis and catabolism of serotonin. Serotonin (5-HT) is synthesised from tryptophan in two enzyme-catalysed steps by the enzymes tryptophan hydroxylase and aromatic L-amino acid decarboxylase. Serotonin is initially catabolised by the enzyme monoamine oxidase to 5-hydroxyindoleacetaldehyde, which is rapidly converted to 5-hydroxyindolacetic acid by the enzyme Aldehyde dehydrogenase. The cofactors for each enzyme are also shown.

The regulation of 5-HT release by inhibitory autoreceptors is similar to that which occurs with many neurotransmitters. 5-HT neurones possess both somatodendritic and presynaptic autoreceptors. Somatodendritic autoreceptors suppress cell firing, probably through hyperpolarization, and are also thought to have a role in collateral inhibition of 5-HT neurones (Aghajanian, 1981). This leads to reductions in 5-HT synthesis and release in the areas to which the neurones project. Presynaptic autoreceptors are not thought to influence cell firing but do inhibit 5-HT release (Sawada & Nagatsu, 1986), probably by altering some aspect of the neurotransmitter stimulus-secretion coupling. Whether activation of presynaptic autoreceptors inhibits 5-HT synthesis is still uncertain (Sawada & Nagatsu, 1986).

5-HT autoreceptors seem to respond to chronic changes in extracellular 5-HT. Repeated administration of 5-HT reuptake inhibitors has been shown to cause a functional desensitisation of both autoreceptor types (Chaput *et al.*, 1986; Moret & Briley, 1990).

Under resting conditions there may be little role for these autoreceptors since 5-HT is rapidly removed by the synaptic reuptake system (Wolf & Kuhn, 1990). However the autoreceptor system may well come into play with the behavioural and physiological changes that occur during exercise and other challenges.

Termination of the effects of 5-HT release is mainly through an active reuptake process. This process is saturable, Na^+ dependent and requires ATP (Graham & Langer, 1992), it is specific for 5-HT selectively and can therefore be inhibited by a number of drugs such as the reuptake inhibitors used in the treatment of depression.

In the nervous tissue 5-HT is broken down to 5-hydroxyindoleacetic acid (5-HIAA) that is considered to be the primary metabolite of 5-HT. In this two-step process 5-HT is first converted to 5-Hydroxyindoleacetaldehyde through oxidative deamination and then oxidised (Figure 1.4).

An obvious rate-limiting step in the formation of any compound is the availability of its precursors. Studies in rats have shown that under normal conditions tryptophan hydroxylase is not saturated with TYP (Fernstrom & Wurtman, 1971). This is strongly supported by studies that have shown that increasing TYP availability increases both the synthesis and release of 5-HT both in animals (Lookingland *et al.*, 1986; Diksic *et al.*, 1991; Westerink & De Vries, 1991) and humans (Gillman *et al.*, 1981).

1.2.1.3   5-HT and exercise

Brain serotonergic activity changes with exercise. *In vivo* electrophysiological studies of serotonergic neurones have found that firing rates of the dorsal raphe neurones in animals are regular and highest when they are awake and walking on a treadmill and almost completely abolished during REM sleep (Jacobs & Fornal, 1993).

Newsholme and colleagues (1987) first suggested that during prolonged exercise the increased brain serotonergic activity might produce lethargy and a loss of drive and motivation to exercise, thereby producing a central fatigue (discussed in greater detail below, section 1.2.1.3). This idea is supported by the observations that serotonergic pathways project into the brain limbic system which is associated with motivation and fatigue (Jacobs & Azmitha, 1992) and increases in brain 5-HT can have effects on arousal, lethargy, sleepiness and mood linked to altered perceptions of effort (Young, 1986). The original mechanism that Newsholme and colleagues (1987) proposed related to changes in peripheral amino acid concentrations affecting brain TYP concentration and subsequently 5-HT synthesis (see section 1.2.1.3b) and since this time there has been a growing interest in the role of 5-HT in central fatigue.

1.2.1.3a   Changes in brain 5-HT with exercise

To support a role for 5-HT in fatigue during prolonged exercise it is necessary to observe increases in brain 5-HT activity and concentrations and this can only be carried out directly in animals. Numerous studies in rats have shown exercise-induced increases in brain 5-HT at fatigue (see Chaouloff, 1989; Meeusen & De Meirleir, 1995) and there is growing (albeit indirect) evidence to suggest that

increases in brain 5-HT activity occurs in humans during prolonged exercise (Struder & Weicker, 2001b).

5-HT is a prominent excitatory neurotransmitter for PRL release (Struder & Weicker, 2001a) and changes in PRL levels in the blood have therefore been used as a marker for changes in central 5-HT activity. Data from a range of human and animal studies suggests that the serotonergic neurones of the dorsal raphe nuclei project to hypothalamic sites to stimulate prolactin (PRL) secretion (see Figure 1.5) through activation of 5-HT receptors (Van de Kar *et al.*, 1996). However PRL release is also under the control of dopaminergic neurones (Figure 1.5) and so care must be taken when interpreting changes in PRL as an indicator of changes in central 5-HT activity.

There have been many studies of the effects of exercise on blood PRL concentrations in humans (Frewin *et al.*, 1976; Brisson *et al.*, 1981; Christensen *et al.*, 1985; De Meirleir *et al.*, 1985b; Smallridge *et al.*, 1985; Brisson *et al.*, 1986; Brisson *et al.*, 1987; Luger *et al.*, 1988; Melin *et al.*, 1988; Brisson *et al.*, 1989; Knudtzon *et al.*, 1989; Saini *et al.*, 1990; Brisson *et al.*, 1991; Ferrari *et al.*, 1991; Luger *et al.*, 1992; Boisvert *et al.*, 1993; Kraemer *et al.*, 1993; Struder *et al.*, 1996a; Struder *et al.*, 1996b; Elias *et al.*, 1997; Struder *et al.*, 1997; Pitsiladis *et al.*, 1998; Bridge *et al.*, 1999; Struder *et al.*, 1999; de Vries *et al.*, 2000; Vigas *et al.*, 2000). The common finding being that blood PRL concentrations increase with prolonged exercise, although this increase is, to some extent, dependent on the intensity of the exercise.

Hypothalamus

5-HT and DA neurones

Median eminence

Primary capillary plexus

Superior hypophyseal artery

Neurones projecting to the posterior pituitary

Pituitary stalk

Long portal veins

Posterior pituitary

Anterior pituitary

Lactotrophs

Inferior hypophyseal artery

Prolactin

Secondary capillary plexus
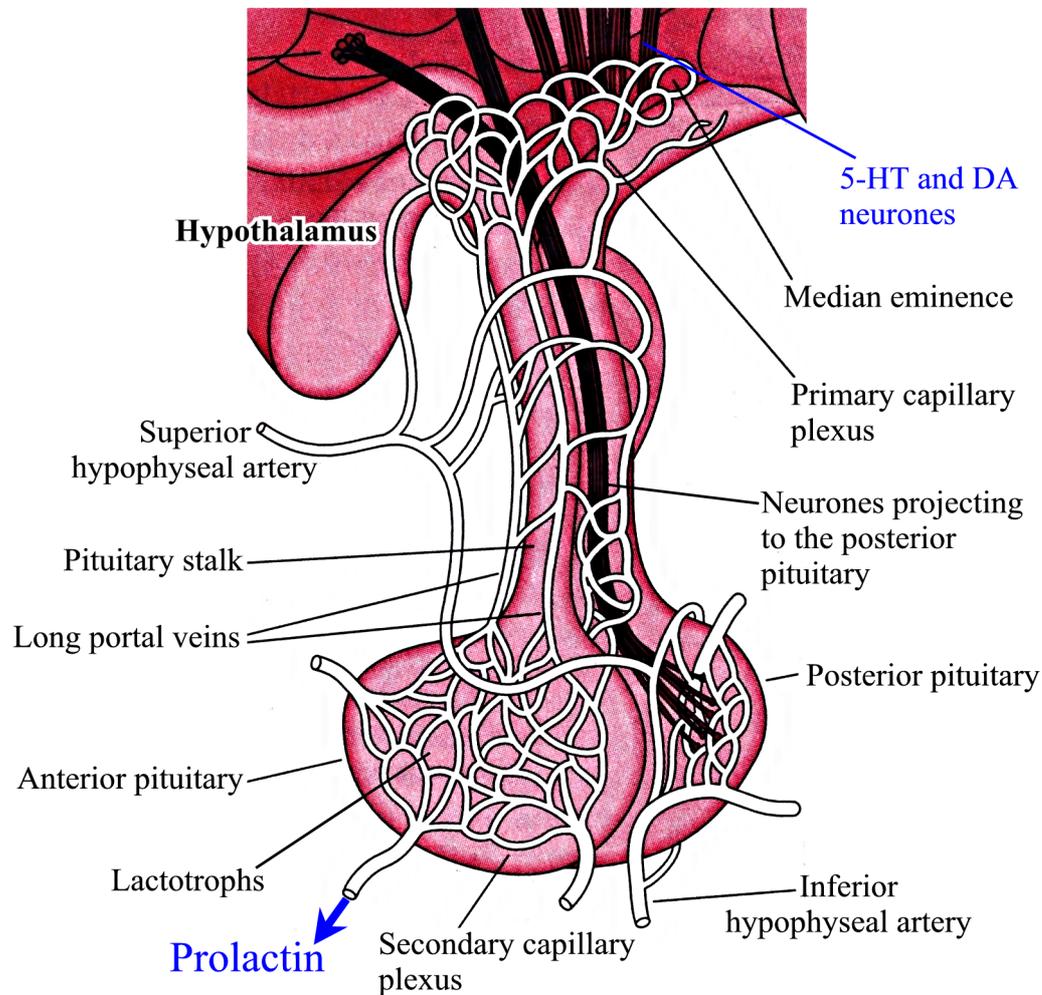
Figure 1.5 Control of prolactin release from the anterior pituitary gland (after Feldman *et al.*, 1997). Serotonergic pathway activation exerts a stimulatory effect on release, whereas dopaminergic tuberohypophyseal pathway activation inhibits release.

5-HT neurones project from the raphe nuclei to central hypothalamic areas and the hypothalamus, thereby inducing 5-HT dependent prolactin release through activation of 5-HT_{1A} and/or $5\text{-HT}_{2A/2C}$ receptors (Van de Kar *et al.*, 1996). Receptor activation results in the secretion of prolactin releasing factors (PRF) into the perivascular spaces of the primary capillary plexus of the hypothalamic-hypophyseal portal system. These PRF then travel to the anterior lobe of the pituitary and act on lactotrophs to stimulate prolactin secretion.

DA is also released from the axons of the tuberohypophyseal pathway into the perivascular spaces of the primary capillary plexus and travels to the anterior pituitary gland where to acts on lactotrophs to inhibit PRL secretion. Numerous other substances also affect prolactin release to a lesser degree (e.g. oestrogen, thyroid hormones, vitamin D and glucocorticoids).

Brisson and colleagues (1981) found exercise at 55% maximal oxygen uptake to reduce serum PRL concentrations whilst bouts of exercise at 70% and 85% of maximal oxygen uptake both increased serum PRL. This is in agreement with the findings of Luger and colleagues (1988; 1992) who found no increase in serum PRL at 50%, a slight increase at 70% and a large increase at 90% maximal oxygen uptake. It is interesting to note that the apparent threshold of around 70-75% maximal oxygen uptake is similar to that considered necessary to increase the concentration of TYP in the brain (Struder et al., 1997).

It has been suggested that PRL release is related to anaerobiosis and lactate formation (De Meirleir et al., 1985a; Luger et al., 1992). The first of these studies (De Meirleir et al., 1985a) reported that one-hour of sub-maximal cycle ergometry at a workload at which blood lactic acid remained below 4mmol·l^{-1} was accompanied by a decrease in plasma PRL levels. However PRL levels were shown to increase promptly and significantly when the anaerobic threshold was reached in a maximal exercise test (De Meirleir et al., 1985a). The exercise intensity resulting in a blood lactate level below 4mmol·l^{-1} corresponded to an oxygen uptake of 44-55% of maximal so in view of previous reports (Brisson et al., 1981; Luger et al., 1988; Luger et al., 1992) it is not surprising that PRL was not released at this intensity. Luger and colleagues (1992) have also looked at the role of lactic acid accumulation in PRL release during exercise. Exercise-induced levels of PRL were not achieved by L-lactate infusion at doses resulting in similar blood lactate concentrations to those seen at 70 and 90% maximal oxygen uptake. The authors therefore suggested that while lactate cannot be totally excluded it does not appear to play a major role.

1.2.1.3b   Manipulating brain 5-HT during exercise through diet

Tryptophan, the precursor of 5-HT, is an essential amino acid that must be supplied in the diet. Since the administration of TYP has been shown to alter both the synthesis and release of 5-HT, it might be expected that manipulation of TYP in the diet would lead to changes in 5-HT metabolism. However when fasted rats were given a meal that increased the level of TYP in the plasma, brain tryptophan and 5-HT concentrations did not rise (Fernstrom & Wurtman, 1972). Further experiments found this to be due to competition for a blood-brain barrier transporter (the L-system) that

TYP shares with a group of amino acids called the large neutral amino acids (LNAA) including the branched chain (BCAA) amino acids, valine, leucine and isoleucine. The rate-limiting step in the synthesis of 5-HT may be the transport of TYP across the blood-brain barrier and when TYP is given in combination with other amino acids competition prevents the extra TYP from entering the brain. Hence it is not only the concentration of TYP in the blood stream that is important, but also the BCAA, which make up roughly 75% of the LNAA, that determines TYP transport into the brain (Figure 1.6).

Another important factor regulating the transport of TYP into the brain is that TYP is the only amino acid that binds to plasma albumin and an equilibrium between bound and free TYP (f-TYP) exists (Figure 1.6). At rest only about 10% of TYP is free in the plasma and it is thought that the concentration of f-TYP governs the rate of uptake into the brain. This idea is supported by studies in animals that have shown a relationship between f-TYP and the concentration of TYP in the brain, whilst no such relationship has been shown for total plasma TYP concentration.

REST

A TYP

A TYP

A FFA

f-TYP

A TYP

BCAA

BCAA

f-TYP

BCAA

f-TYP

Neurone

TYP →→→ 5-HT

PROLONGED EXERCISE

A FFA

A FFA

A FFA

f-TYP

A TYP

f-TYP

f-TYP

BCAA

FFA

f-TYP

f-TYP

f-TYP

Neurone

TYP →→→ 5-HT

5-HT

5-HT
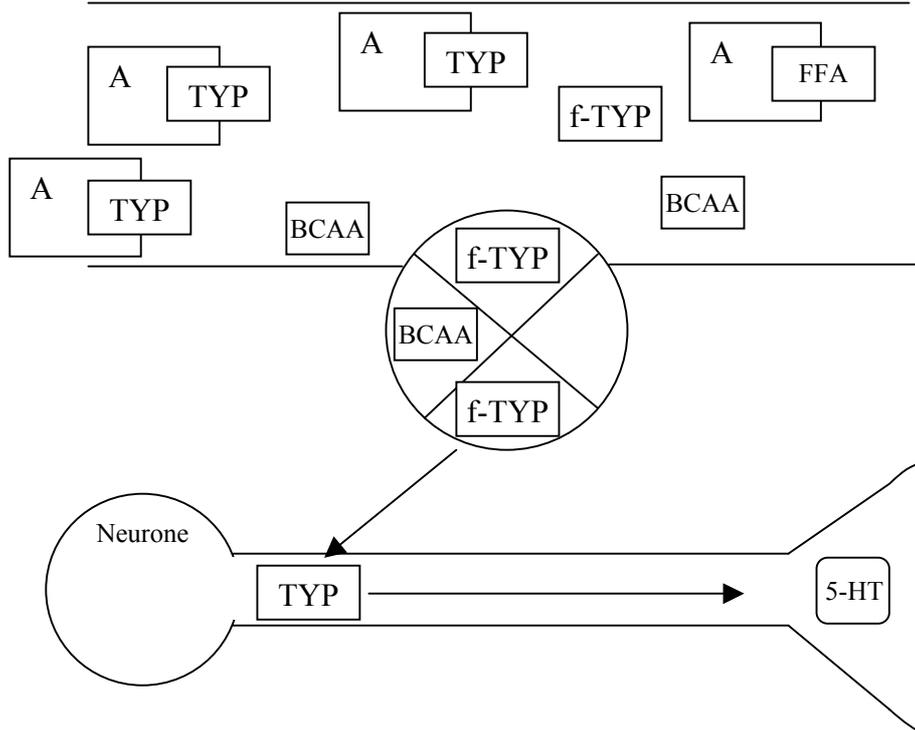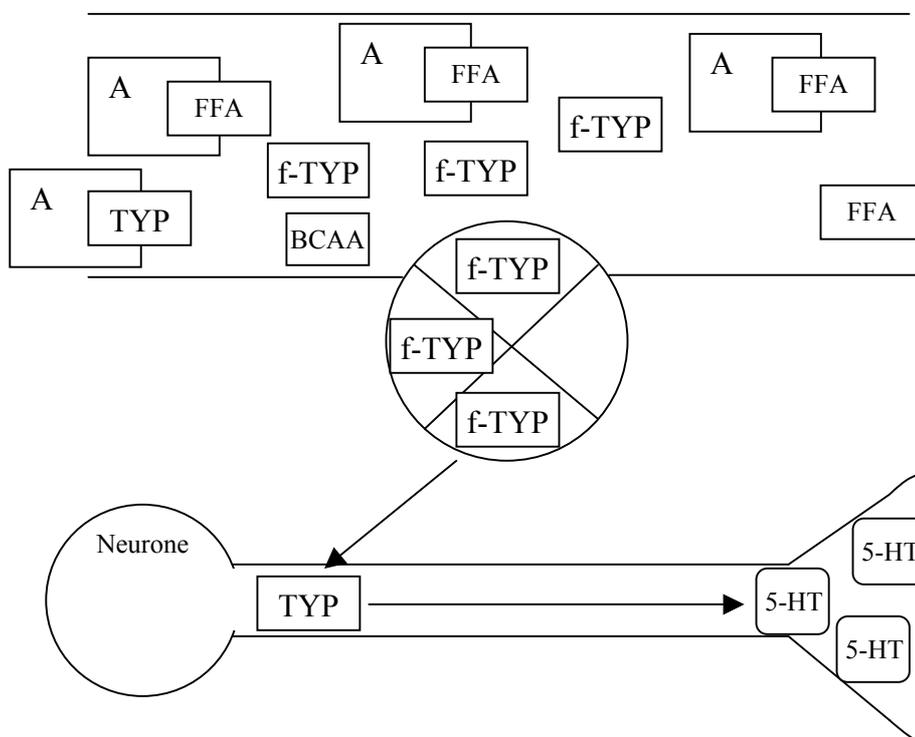
Figure 1.6 Effects of prolonged exercise on plasma concentrations of free tryptophan (f-TYP) free fatty acids (FFA) and branched chain amino acids (BCAA). TYP binds to Albumin (A) in competition with FFA. Increases in plasma FFA with prolonged exercise results in FFA binding to albumin (A) displacing TYP and increasing the plasma f-TYP concentration. This along with reductions in plasma BCAA results in an increased f-TYP/BCAA ration and increases in blood brain TYP transport and 5-HT synthesis (after Davis & Bailey, 1997).

The concentration of f-TYP in the plasma during exercise is mainly influenced by changes in plasma free fatty acids (FFA) which are also transported bound to albumin and compete for the same binding site. A release of FFA from adipose tissue during exercise results in an increase in plasma FFA which can displace TYP from albumin thereby elevating plasma f-TYP concentration (Figure 1.6). Several factors such as hormonal levels, a low blood glucose and reduced muscle glycogen content at the start of exercise are known to stimulate the release of FFA from adipose tissue. As a result of these changes, and a reduction in plasma BCAA due to muscle BCAA uptake (van Hall *et al.*, 1996), an increase in the f-TYP/BCAA ratio is found after exercise and a recent study by Strüder et al (1997) suggests that increases in the ratio during prolonged (~5 hour) exercise may be dependent upon exercise intensity. An increase in the ratio was found during the final hours of exercise at 75% of maximal oxygen uptake whereas no significant elevation in the ratio was found at 50% of maximal oxygen uptake.

If the mechanism described above does regulate TYP entry into the brain and thus the synthesis of 5-HT then manipulation of the f-TYP/BCAA ratio during exercise should influence the onset of fatigue (Figure 1.6). Several studies have looked at the effects of feeding BCAA during exercise to maintain a low f-TYP/BCAA ratio and have claimed improvements in performance (Blomstrand *et al.*, 1991; Blomstrand *et al.*, 1997; Mittleman *et al.*, 1998) although there are doubts about the experimental design in some cases. Blomstrand *et al.*, (1991) looked at two groups of male subjects running a marathon. One group was given 16g of BCAA in four separate aliquots during the race whilst the control group received only water. In addition to this the subjects were allowed any other drink provided during the race (including carbohydrate drinks). No difference was found in marathon times between groups unless the subjects were divided into groups of fast and slow runners. This resulted in a small but significant reduction in marathon time in slow runners. Problems that arise with this study are: (i) the BCAA and control groups were not matched for marathon performance; (ii) the carbohydrate intake and nutritional status should have been controlled and should have been the same in each group; (iii) the arbitrary division of runners in to fast and slow groups based upon a set marathon time is not an acceptable statistical procedure. Also in the same study no effect of BCAA supplementation was found in cross-country runners participating in a 30km race. In the other study by

Blomstrand et al (1997) seven cyclists were given a either a BCAA drink containing 90mg·kg body weight^{-1} (6-8g) of BCAA or a flavoured placebo during one hour of cycle ergometry at 70% of maximal oxygen uptake followed by 20 minutes at maximum. The evening before the ride the subjects performed a bout of exercise to reduced muscle glycogen levels. This was to achieve amore rapid rise in f-TYP during the test the following morning. The authors found that ratings of perceived exertion and mental fatigue were lower during exercise with BCAA supplementation when compared to placebo. However the authors did not look at the effects of the BCAA supplementation on exercise time to fatigue and used only the amount of work done in the 20 minutes of maximal exercise as a measure of performance and found no differences between trials. Additionally, lowering muscle glycogen levels the previous day may have affected muscle metabolism so that BCAA could have been used as an additional fuel (Wagenmakers *et al.*, 1989; Weicker & Stobel, 1994).

The third of these studies (Mittleman *et al.*, 1998) was carried out during exercise in heat. The authors found that time to exhaustion increased from 137 to 153 minutes with BCAA supplementation. However the exercise intensity used in this study was very low (40% maximal oxygen uptake) whereas it has been suggested that intensities near 75% maximal oxygen uptake are where significant increases in the f-TYP/BCAA ratio occur (Struder *et al.*, 1997).

In contrast to the studies discussed above, others have found no positive effects on performance (Wagenmakers & van Hall, 1995; Struder & Weicker, 2001b). In one such study attempts were made to both increase and decrease TYP entry into the brain (Struder *et al.*, 1996b). To increase the f-TYP, an oral soy oil solution was given to elevate plasma triglycerides, followed by an infusion of heparin during exercise which liberates FFA, increasing f-TYP and thus the f-TYP/TYP ratio. To decrease TYP entry LNAA were infused to prevent the decline in plasma LNAA, usually seen with exercise and thus maintain the plasma f-TYP/LNAA (BCAA) ratio compared to the placebo trial. However, in neither instance was there any significant difference between trials in perceived exertion or plasma PRL and subjects completed the 90 minute exercise period in both trials.

It is interesting to note in this study that in the early stages of the exercise significant increases in plasma FFA, f-TYP and f-TYP/LNAA ratio did not increase plasma PRL concentration. However at the end of exercise when athletes were beginning to fatigue (90 minutes running) plasma FFA, f-TYP and f-TYP/TYP ratio were similar to those at the start and yet plasma PRL was significantly increased. These results indicate that whatever role TYP uptake has to play, some other mechanism must play the major part in the regulation of PRL release.

Another strategy used to manipulate the f-TYP/BCAA ratio during exercise is to feed BCAA together with carbohydrate. It is well known that the ingestion of carbohydrates during exercise can delay fatigue, probably by maintaining blood glucose levels and the supply of energy when muscle glycogen levels are low. However, it is possible that carbohydrates may also affect central fatigue by altering the f-TYP/BCAA ratio. The intake of carbohydrates before or during exercise reduces that exercise-induced increase in plasma FFA (Jeukendrup *et al.*, 1999) probably by stimulating insulin secretion, which is known to inhibit lipolysis (Snow *et al.*, 2000). Additionally, carbohydrate ingestion delays the rise in plasma FFA and the rise in f-TYP during sustained exercise although, if exercise continues for longer than 2-3 hours, there is an increase in the plasma concentration of FFA and f-TYP whether or not carbohydrates are consumed (Davis *et al.*, 1992; McConell *et al.*, 1999). Several studies have investigated the effect of BCAA and carbohydrate on exercise and found no difference in performance between the ingestion of BCAA with carbohydrates and carbohydrates alone (Blomstrand *et al.*, 1995; Davis *et al.*, 1999; van Hall *et al.*, 1995). The latter authors also question whether f-TYP is as important to the transport process as is generally supposed and point out that the ammonia production associated with the metabolism of large doses of BCAA may itself cause problems since ammonia is known to be very toxic.

The final way to alter the f-TYP/BCAA ratio is through the ingestion of TYP which would be expected to reduced performance. Van Hall et al (1995) found no effect of TYP ingestion on exercise to exhaustion at 70-75% maximal oxygen uptake, although the reproducibility of the exercise times to exhaustion in this study was very large. Other studies that have given TYP before exercise have found contrasting results (Segura & Ventura, 1988; Stensrud *et al.*, 1992). In the first of these studies twelve

male subjects ran to exhaustion at 80% maximal oxygen uptake either with or without TYP supplementation (Segura & Ventura, 1988). Subjects were given 300mg of TYP the night before, at breakfast, lunch and one hour before the test to increase brain TYP levels. Unexpectedly exercise time to fatigue was increased by 49.4% after TYP administration and this was accompanied with lower ratings of perceived exertion. In the second study (Stensrud *et al.*, 1992) twenty-four male subjects ran to exhaustion at 100% maximal oxygen uptake. In the TYP trial they were given 240mg the night before the test, 360mg in the morning, 240mg at lunch and 360mg one hour prior to the test. However no change in time to exhaustion was found between trials.

One practical difficulty with experiments attempting to manipulate brain 5-HT synthesis by diet or infusion, is that there seems to be a reduced control of central 5-HT synthesis by TYP during exercise (Chaouloff *et al.*, 1987). A second conceptual problem is to assume that a global increase in TYP or 5-HT will have a single behavioural outcome. As emphasised before, 5-HT pathways are widely distributed throughout the brain and whilst some may be involved with inhibition, others may well be beneficial in facilitating motor output during exercise (Jacobs & Fornal, 1993).

1.2.1.3c   Effects of Drugs

There are a wide range of drugs which affect 5-HT metabolism or action. Some of the best known are the monoamine oxidase inhibitors which were the first pharmacological treatment to be developed for depression. As their name implies they inhibit the first step in 5-HT breakdown and thus, presumably, increase brain levels. More recently popular interest has centred on drugs such as *Prozac*, selective 5-HT re-uptake inhibitors (SSRI), which are the newer drugs used to treat depression. In general, drugs that work presynaptically alter the synthesis, metabolism or reuptake of the transmitter while post-synaptic actions can be as full agonists, partial agonists or antagonists.

5-HT re-uptake inhibitors, given acutely, have been shown to increase fatigue and/or perceived exertion during exercise in humans (Wilson & Maughan, 1992; Davis *et al.*, 1993; Struder *et al.*, 1998). Blockade of 5-HT reuptake by SSRI treatment increases

synaptic 5-HT concentrations and thereby stimulates many behavioural and physiological adaptations mediated by the transmitter.

Wilson & Maughan (1992) were the first to use the SSRI paroxetine and reported a decrease in time to fatigue which they attributed to an increase in serotonergic activity. They did not measure PRL which is thought to be indicative of hypothalamic 5-HT activity. A more recent study, using time to fatigue as the end-point, involved ten male cyclists with, and without, administration of 20mg of paroxetine. Time to exhaustion was significantly reduced after paroxetine administration (Struder *et al.*, 1998) but surprisingly there was no difference in PRL release between placebo and paroxetine treatments. It is possible that a single low dose (20mg paroxetine) of the SSRI used in both these studies may have had a 5-HT antagonist effect through activation of inhibitory 5-HT$_{1A}$ autoreceptors (Artigas *et al.*, 1996) of the pathway controlling PRL release.

Two studies have used 5-HT agonists or antagonists to alter brain serotonergic activity during exercise (Marvin *et al.*, 1997; Meeusen *et al.*, 1997). Meeusen and colleagues (Meeusen *et al.*, 1997) used the 5-HT$_{2A/2C}$ receptor antagonist ritanserin during exercise to fatigue at 65% of the maximal aerobic power output. The authors gave 0.3mg$^{.}$kg^{-1} of ritanserin or a placebo twenty-four hours, and immediately, before the test but no differences were found in time to fatigue between trials and prolactin was not measured. The other study to pharmacologically alter brain 5-HT in humans during exercise used the 5-HT$_{1A}$ receptor agonist buspirone (Marvin *et al.*, 1997). Subjects exercised to fatigue at 80% of maximal oxygen uptake after ingesting buspirone (45mg) or a placebo one hour prior to exercise. Ratings of perceived exertion and plasma PRL during exercise were increased after buspirone ingestion whilst time to fatigue was reduced. This study provides the only clear evidence so far, in humans, that alteration in central 5-HT activity, as shown by change in plasma PRL, result in changes in exercise tolerance.

Whilst the human experiments in this area are rather limited, there is a large volume of work carried out on animals which shows that the pharmacological modulation of central 5-HT activity influences fatigue during endurance exercise. For example, application of quipazine dimaleate (5-HT agonist), m-chlorophenylpiperazine (5-

HT$_{2/2C}$ receptor agonist) and LY-53,857 (5-HT antagonist) respectively reduced and increased endurance, while xylamidene tosylate, a 5-HT antagonist with only a peripheral action, had no effect (Bailey *et al.*, 1993a, b; Davis, 1995).

1.2.1.3d   Effects of training status

Several studies have reported differences in measures of 5-HT function between trained and sedentary individuals. Reduced resting PRL concentrations have been found in male distance runners and endurance athletes compared to sedentary controls (Wheeler *et al.*, 1984; Weicker & Struder, 2001). Six weeks of endurance training led to a reduced resting PRL (Marvin *et al.*, 1999a) and other authors have found reduced resting PRL after 3 weeks of moderate training (Weicker & Struder, 2001). In contrast, other studies have not found changes in resting prolactin levels after training (Hurley *et al.*, 1990; Pitsiladis *et al.*, 1999; Dwyer & Flynn, 2002) or between trained and sedentary individuals (Pitsiladis *et al.*, 1999).

The azapirone derivative, buspirone, has a high affinity for 5-HT$_{1A}$ receptors (Eison & Temple, 1986) and has been widely used to probe serotonergic function in humans, acting as a neuroendocrine challenge (Meltzer *et al.*, 1983; Anderson & Cowen, 1992; Bakheit *et al.*, 1992; Jakeman *et al.*, 1994; Sharpe *et al.*, 1996; Bridge *et al.*, 2001). The technique involves giving the agonist and following the appearance of PRL in the blood over the next 2-3 hours.

Jakeman and colleagues (1994) found elite endurance athletes to have a decreased sensitivity (reduced PRL response) to a 5-HT$_{1A}$ agonist challenge with buspirone which they suggested might be a training adaptation associated with increased exercise tolerance in these individuals. This was not, however, found by Pitsiladis and colleagues using the same 5-HT$_{1A}$ agonist (Pitsiladis *et al.*, 1999). Contrasting findings to those of Jakeman (1994) have also been shown in a study that looked at postsynaptic 5-HT$_2$ function using d-fenfluramine (Strachan & Maughan, 1999). These authors found no difference in the PRL response to drug administration between trained and untrained subjects. A recent study by Marvin and colleagues (1999a) found that the PRL response to buspirone was not altered after training, and Dwyer and Flynn, (2002) came to a similar conclusion.

Although the evidence seems to be against a training-induced effect on the neuroendocrine response to a buspirone challenge the major reservation about all these studies is that although significant improvements in aerobic fitness were seen the levels reached were well below those of the elite endurance athletes studied by Jakeman and colleagues (1994) (maximal oxygen uptake 43±5 (Pitsiladis *et al.*, 1999) ; 50±8 (Marvin *et al.*, 1999a) & 45±3 (Dwyer & Flynn, 2002) compared with 74±1 ml˙kg˙min^{-1} (Jakeman *et al.*, 1994)). It may be that it requires far longer and more rigorous training to modify the serotonergic pathways involved in PRL release.

Strachan and Maughan (1998) hypothesised that one way in which the synaptic concentration of 5-HT could be reduced through training is through an increase in number or activity of neuronal 5-HT transporters. They measured platelet 5-HT transporter density in red blood cells and found the density was greater in endurance trained males compared to sedentary controls. Whether this peripheral measure reflects central 5-HT transport density and whether such differences in transporter density would be sufficient to affect synaptic 5-HT clearance remains to be seen.

At the opposite end of the spectrum to elite athletes are patients suffering from chronic fatigue syndrome, which includes post viral fatigue syndrome, and seriously affects aerobic exercise capacity (Maffulli *et al.*, 1993). Such patients have been found to have a greater sensitivity to buspirone than healthy controls (Bakheit *et al.*, 1992; Sharpe *et al.*, 1996; Sharpe *et al.*, 1997). A recent single case study of a patient suffering from CFS who substantially recovered over a period of 18 months found that as exercise tolerance increased, and subjective measures of fatigue and anxiety improved, the response to buspirone returned to the normal range, having been very high when first examined (Sharma *et al.*, 2001).

1.2.1 The dopaminergic system and exercise

It was not until the late 1950s that dopamine (DA) was shown to be present in the brain. DA is both a precursor for adrenaline and noradrenaline as well as having its own explicit functions within the body and is thought to constitute as much as 80% of the total brain catecholamine content. In rats an intimate relationship has been shown between DA production and all aspects of motor behaviour, speed, and posture (Freed & Yamamoto, 1986) and an influence of a decrease or increase in brain DA function can be correlated with exercise capacity in rats (Heyes *et al.*, 1985). Changes in brain DA with exercise should also be considered in relation to DA linked behavioural responses such as motivation and emotion (Scheel-Kruger & Willner, 1991).

1.2.2.1 Central dopaminergic pathways

In their classic mapping of the brain Dahlström and Fuxe (1964) designated noradrenaline and dopamine cell groups with the letter A and the current nomemclature spans A8-A15. Dopamine containing cells are found in relatively rostral parts of the brain (i.e., the midbrain, hypothalamus, and olfactory bulbs; Figure 1.7).
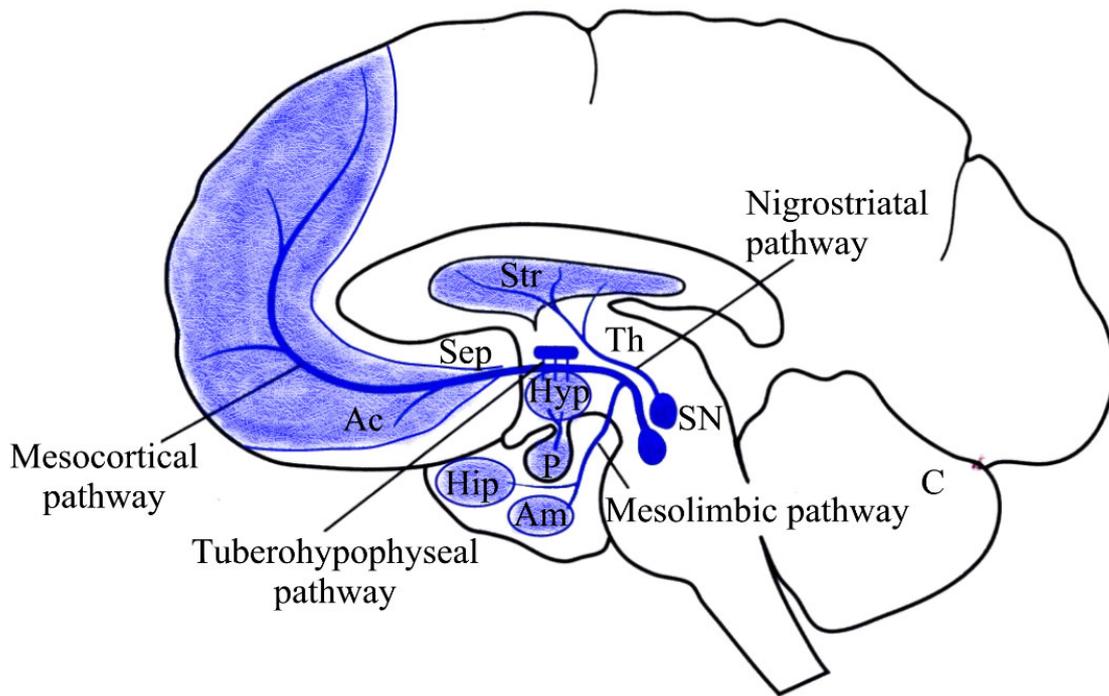
Figure 1.7 DA neurones and major pathways in the brain. The location of the main groups of cells bodies and fibre tracts is in solid black. Light shaded areas show the location of serotonergic terminals. (Ac – Nucleus accumbens; Am – Amygaloid nucleus; C – cerebellum; Hip – hippocampus; Hyp – hypothalamus; P – pituitary gland; Sep – septum; SN – substantia nigra; Str – striatum; Th – thalamus.)

Dopamine neurones form three main systems within the brain (Figure 1.7). These are; the nigrostriatal pathway, with the cell bodies in the substantia nigra and the axons terminating in the corpus striatum; the mesolimbic/ mesocortical pathway whose cell bodies occur in groups in the midbrain and project to parts of the limbic system, nucleus accumbens, the amygdaloid nucleus and to the cortex; and, finally, the tuberohypophyseal system which is a group of neurones with axons that run from the hypothalamus to the pituitary gland, the secretions of which regulate PRL.

Two types of dopamine receptor (D_1 and D_2) were originally distinguished by pharmacological and biochemical techniques and further subtypes (D_1-D_5) have been revealed by gene cloning. The distribution and function of these receptors is shown in Table 1.2. The receptors are grouped into two main families D_1 and D_2 which have their actions linked to stimulation and inhibition of adenylate cyclase respectively.

29

		D₁ type		D₂ type		
		D_1	D_5	D_2	D_3	D_4
Distribution	**Functional Role**					
Cortex	Arousal, mood	++	-	++	-	-
Limbic system	Emotion, stereotypic behaviour	+++	-	+++	+	+
Basal ganglia	Motor control	++	+	+++	+	+
Hypothalamus	Autonomic and endocrine control	++	+	-	-	-
Pituitary gland	Endocrine control	-	-	+++	-	-
Action		Mainly postsynaptic inhibition		Pre- and postsynaptic inhibition Stimulation/inhibition of hormone release		

Table 1.2 DA receptor subtypes and the functions, + indicates degree of importance (Rang *et al.*, 1999).

The functions of the dopamine pathways divide broadly into three categories; motor control (nigrostriatal system), behavioural effects (mesolimbic and mesocortical systems), endocrine control (tuberohypophyseal system)

Tyrosine $\xrightarrow[\text{Tyrosine hydroxylase}]{\text{Tetrahydrobiopterin, } O_2, Fe^{2+}}$ L-DOPA

Pyridoxal phosphate | Aromatic L-amino acid decarboxylase

Dopamine
(DA)

MAO

FAD

SAM
Mg^{2+}

COMT

3,4-Dihydroxphenylacetaldehyde
(DHPA)

3-Methoxytyramine
(3-MT)

Aldehyde dehydrogenase

FAD | MAO

3,4-Dihydroxphenylacetic acid
(DOPAC)

3-Methoxy-4-hydrophenylacetaldehyde
(MHPA)

SAM
Mg^{2+}

COMT

Aldehyde dehydrogenase

Homovanillic acid
(HVA)

Figure 1.8 Synthesis and metabolism of dopamine. Dopamine (DA) is synthesised from tyrosine in two enzyme catalysed steps by the enzymes tyrosine hydroxylase and aromatic L-amino acid decarboxylase. The cofactors for each, tetrahodrobiopterin, Fe^{2+}, O_2 and pyridoxal phosphate, are also shown. Dopamine is metabolised to homovanillic acid (HVA) through oxidative deamination with monoamine oxidase (MAO) or with *O*-methylation by catechol-*O*-methyltransferase (COMT). The cofactors fro each enzyme are also shown flavin adenine dinucleotide (FAD), *S*-adenosyl-methionine (SAM), and Mg^{2+}.

1.2.2.2   Control of DA synthesis and release

The rate-limiting step in the biosynthesis of dopamine is the hydroxylation of tyrosine (TYR) to dihydroxyphenylalanine (DOPA) by the enzyme TYR hydroxylase (Figure 1.8).   Activity of this enzyme can be inhibited by catecholamines suggesting a feedback inhibitory mechanism. DOPA is decarboxylated to dopamine by the enzyme dopa-decarboxylase.   In normal physiological conditions dopamine is first metabolised to 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase and aldehyde oxidase. DOPAC is further metabolised into homovanillic acid by catechol-*O*-methyltransferase (see Figure 1.8).

As in the serotonergic system, there are presynaptic dopaminergic autoreceptors, activation of which inhibits both dopamine synthesis and release (Feldman *et al.*, 1997). The somatodendritic dopamine autoreceptors cause a reduction in cell firing which in turn causes a reduction in release in terminal areas (Santiago & Westerink, 1991).

1.2.2.3   DA and exercise

1.2.2.3a   Changes in brain DA with exercise

Recent advancements in positron emission tomography (PET) scanning are starting to allow the measurement of changes in brain neurotransmitters in humans pre and post exercise. One such study found no change in brain DA concentration 5-10 minutes after 30 minutes of treadmill exercise (Wang *et al.*, 2000) although it would have been more interesting had the exercise been continued to fatigue.

DA neurones are critical components of the motor system, there are a number of studies of the effects of exercise upon the dopamine synthesis and metabolism in animals (see Meeusen & De Meirleir, 1995). Most studies find an increase in brain DA and/or DOPAC levels during exercise but when animals are taken to fatigue decreased or unchanged brain DA/DOPAC levels were found in the hypothalamus (Meeusen & De Meirleir, 1995). Hoffmann et al (1994) found decreased DA and

DOPAC levels in the limbic forebrain at exhaustion suggesting a decreased level with no change in turnover.

Bailey *et al*., (1993a) found elevated levels of brain DA and DOPAC in rats after one hour of exercise but which then decreased, returning to resting levels at fatigue. Administration of quipazine dimaleate, a general 5-HT agonist, blocked the increase after one hour and LY53857, a 5-HT antagonist, prevented DA and DOPAC decrease at fatigue. The authors point out that there is clearly an interaction between serotonergic and dopaminergic pathways during exercise.

Bailey *et al* (1992) have argued that the effects of 5-HT activity on fatigue may be through the impairment of DA synthesis and this may be mediated by increased levels of 5-HT. This idea is based on the finding that the $5-HT_{1C}$ agonist m-chlorophenylpiperazine (m-cpp) reduced brain DA and DOPAC in rats exercising to fatigue. Support for this idea comes from the work of Spaminato et al (1985) who also showed that the 5-HT agonist m-cpp reduces DA synthesis in rats.

Heyes et al (1988) suggested that time to exhaustion in rats is influenced by the activity of nigrostriatal dopaminergic neurones, and that central dopaminergic activity modulates performance. A possible dopaminergic pathway linking the limbic cortex and pre-motor areas, through the limbic striatum and sensorimotor striatum has been suggested by Smith and Bolam (1990). They envisage that the limbic brain may 'gate' information through the sensorimotor striatum, linking emotion and motivation with motor control. The existence of such a pathway suggests that DA may play a role in central fatigue through a coupling of motivation and motor control.

1.2.2.3b   Effects of drugs

Heyes *et al*., (1985) administered the dopamine agonist apomorphine to rats with 6-hydroxydopamine (destroys DA nerve terminals) induced lesions of the central dopaminergic pathways to determine the influence of central dopaminergic activity on exercise. Lesioned rats, without apomorphine administration, ran for a shorter time before exhaustion than control rats, while administration of apomorphine resulted in

lesioned rats running significantly longer than control rats. The authors suggest that increasing central dopaminergic activity increases time to fatigue.

Increases in brain TYR have been shown to affect TYR hydroxylase activity (Carlson & Lindquist, 1978) and augmented neuronal dopaminergic responses to TYR have been shown to occur in previously activated neurones (Sved, 1983). Strüder *et al.,* (1998) administered 10mg of L-TYR, the precursor of dopamine, to endurance trained cyclists before exercise to exhaustion at a workload that resulted in a blood lactate concentration of 2mmol$^.$l^{-1}. No changes were found in endurance times between trials although plasma PRL was significantly elevated above resting and placebo levels after 30 minutes of exercise following TYR administration. It might have been expected that an increase in brain DA would lead to a reduction in blood PRL concentration through increased inhibition of secretion. A reduction in PRL has previously been seen in rats injected with TYR (Sved *et al.*, 1979) however this only occurred after prior activation of DA neurones by reserpine administration (reserpine blocks neuronal DA reuptake and storage) and other studies support the idea that TYR administration only enhances DA release from activated neurones (Sved, 1983). Since PRL release was enhanced after DA administration in the experiments of Strüder *et al.*, (1998) other factors may have reduced dopaminergic neural activity during exercise. One factor could have been 5-HT as there is a suggestion that 5-HT can influence fatigue through inhibition of the DA system during exercise (Bailey *et al.*, 1993a).

Intracerebral infusion of the DA precursor L-DOPA has been shown to enhance motor activity in rats (Vogt, 1973) and is well known for its beneficial effects on motor activity in Parkinson's patients. Meeusen *et al.*, (1997) administered L-DOPA to male subjects before exercise to fatigue at 65% of the workload corresponding to maximal oxygen uptake. L-DOPA was given in combination with carbidopa (4mg$^.$kg^{-1}) which prevents peripheral decarboxylation of L-DOPA. Time to exhaustion did not differ from that of the placebo trial. The dose used had previously been shown to increase plasma and brain DA level in rats (Sarre *et al.*, 1992) although whether this also applies to humans is unknown. There is some evidence that although L-DOPA was given in conjunction with a peripheral decarboxylation inhibitor in this study, at least some partial peripheral metabolism of L-DOPA occurred since there were higher

plasma DA levels after L-DOPA administration. Whether there was a significant effect of administration on central DA levels is unclear.

More direct manipulation of brain DA can occur through administration of amphetamines. Amphetamines have been shown to be potent brain DA releasers (Adams *et al.*, 2002) and re-uptake inhibitors (Azzaro *et al.*, 1974) and increase exercise capacity in humans (Wyndham *et al.*, 1971; Chandler & Blair, 1980; Laties & Weiss, 1981). It is possible that there may be an additional peripheral effect of amphetamines, however no differences in maximal oxygen uptake or heart rate have been found in humans (Wyndham *et al.*, 1971).

Caffeine is well known to improve exercise capacity in humans (Graham & Spriet, 1995). Though the mechanisms for this improvement are not clear. It was originally thought that caffeine led to a shift in substrate metabolism away from carbohydrate towards fat oxidation and glycogen sparing, but this seems largely to have been disproved (Graham *et al.*, 2000). There is the possibility that as caffeine acts as a calcium releasing substance in muscle *in vitro* may have a similar action on muscle *in vivo*. (Wiles *et al.*, 1983) found no evidence for an action of aminophylline, a more potent methyl xanthine, affecting either the contractile properties or fatigability of human muscle *in vivo*. There have, however, been reports of caffeine improving stimulated muscle performance in tetraplegics patients which supports a peripheral action (Mohr *et al.*, 1998). Caffeine is known to cross the blood brain barrier and the possibility exists that its action may have a large central component. Caffeine has been shown to have a major protective effect against the development of dopamine depletion related Parkinson's symptoms in human populations (Ross *et al.*, 2000a; Ross *et al.*, 2000b; Ascherio *et al.*, 2001; Ross & Petrovitch, 2001) and in experimental animal models (Ross & Petrovitch, 2001). In trials that have shown improvements in exercise capacity and performance with caffeine ingestion it may well be that this is the result of an enhanced brain DA activity.

1.2.2.3c   Effects of disease and training

It has been suggested that the predominance of African middle and long distance runners is related to differences in their DA systems compared to Caucasians (Gilbert, 1995) although the evidence for this is sketchy at best. More direct evidence for DA being important in motor control and performance comes from Parkinsonian patients. In the early 1960s investigators started to find that there was a depletion of DA neurones in post mortem brain tissues of patients with Parkinson's disease (see Hornykiewicz & Kish, 1986 for review). These large losses (>80%) were found to be in all DA cell groups in the brain but particularly in the substantia nigra pars compacta (Feldman *et al.*, 1997). The classic symptoms of the disease include resting tremor, bradykinesia, rigidity and postural disturbances that are largely attributed to the damage to DA innervation in the neostriatum, which forms a complex loop between the basal ganglia and the neocortex (Feldman *et al.*, 1997). Although the basal ganglia itself does not initiate motor commands it plays a critical role in co-ordinating and synchronising such commands and is important for the execution of smooth, co-ordinated and repetitive movements. Parkinson's patients report increased sense of effort or fatigue during motor tasks (Ziv *et al.*, 1998) and it has been suggested that this is a consequence of patients having to use other pathways to effect complex motor commands, a little like learning a new skill, every time a routine movement is carried out (Berardelli *et al.*, 2001). Administration of the DA precursor levodopa (L-DOPA) produces a reduction in symptoms (Shan *et al.*, 2001) and about 20% of patients are restored to normal motor function, at least in the early years of the disorder.

1.2.3   Interaction between 5-HT and DA in fatigue and during exercise

In the work reviewed so far concerning serotonergic and dopaminergic systems there have been a number of suggestions that they may interact in regulating motor activity, and there are numerous studies which have addressed this point. In general it appears brain 5-HT increases and DA decreases at fatigue (Meeusen & De Meirleir, 1995) and it has been suggested that this is more than coincidence and implies some reciprocal control and interaction of the two systems (Spampinato *et al.*, 1985; Meltzer, 1992; Ferre *et al.*, 1994; Huttunen, 1995; Wong *et al.*, 1995; Iyer & Bradberry, 1996; Kapur & Remington, 1996; Howell *et al.*, 1997; Thorre *et al.*, 1998). It has been suggested that a major site of these interactions is the serotonergic dorsal raphe nuclei (Thorre *et al.*, 1998).

Davis & Bailey, (1997) have hypothesised that central fatigue is the result of changes in the ratio of 5-HT and DA. They suggest that a low 5-HT/DA ratio favours improved performance, through increased arousal, motivation and optimal muscular coordination, whilst increased in the 5-HT/DA ratio results in decreased performance. This latter mechanism would constitute a central fatigue.

1.3   Fatigue during exercise in the heat

Many studies have shown that high ambient temperatures reduce exercise capacity in man and increase ratings of perceived exertion (e.g. Febbraio *et al.*, 1996; Galloway & Maughan, 1997; Nybo & Nielsen, 2001c). Except when carried out at low intensity or in cold conditions, endurance exercise is associated with a steady increase in core temperature that can be detrimental to performance. To combat this increase in temperature the body activates several thermoregulatory responses to dissipate the heat.

A redistribution of blood to the skin occurs to facilitate heat loss and it is possible that this may divert blood away from the working muscles (Fink *et al.*, 1975). However, there is both indirect and direct evidence that this is not a serious problem. If blood supply to working muscles was restricted they would be working under potential anaerobic conditions and this should be evident as a high blood lactate, but there is no such evidence of metabolic crisis. Blood lactate, although tending to be higher than when working in cool conditions, does not show any large or rapid increase as subjects fatigue in the heat (Galloway & Maughan, 1997).

It is possible that excessive sweating coupled with inadequate fluid intake may lead to dehydration and reduced blood flow (Gonzalez-Alonso *et al.*, 1998). However, the reduction in muscle blood flow that is seen does not cause fatigue by compromising muscle metabolism or oxygen delivery to the active muscle (Gonzalez-Alonso *et al.*, 1999a) since the oxygen extraction by working muscles increases to compensate for the reduced flow.

The influence of temperature on enzymic rates (Q_{10} effect) is well known and it is possible that exercise in the heat may increase metabolic rate and the rate of carbohydrate depletion. Fink *et al.*, (1975) were the first authors to demonstrate an effect of temperature on substrate utilisation. They found increased glycogen usage during one hour of intermittent exercise at 41°C compared to the same exercise at 9°C. Since this time other authors have found that exercise in the heat results in increased intramuscular carbohydrate oxidation compared to cooler environments

(Febbraio *et al.*, 1994a; Febbraio *et al.*, 1994b; Hargreaves *et al.*, 1996; Galloway & Maughan, 1997). This increase in oxidation appears only to be present during submaximal exercise and if a marked increase in body temperature occurs (see Febbraio, 2000). Increased carbohydrate oxidation and glycogen depletion might lead to earlier fatigue but, Galloway & Maughan, (1997) calculated the total carbohydrate oxidation during exercise to fatigue at different ambient temperatures and found the total was lowest (90±6g) at fatigue during exercise at 31°C compared to trials at 4°C (168±20g), 11°C (166±19g) and 21°C (149±11g). Clearly depletion of endogenous carbohydrate stores cannot be the explanation of the more rapid fatigue seen in the heat.

Whilst some effect of redistribution of blood and increased carbohydrate oxidation cannot be ruled out it has been suggested that the major factor limiting exercise in the heat is body temperature itself (Bruck & Olschewski, 1987). An upper limit to core temperature has been described at which athletes fatigue (Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; Gonzalez-Alonso *et al.*, 1999b) for reasons that are not related to metabolic or cardiovascular changes. This suggestion arises from the observation that during exercise in the heat, fatigue occurs at a relatively fixed core temperature and the duration of exercise can be prolonged or shortened by respectively reducing or raising core temperature before the start of exercise. The time to fatigue is thereby determined by the time it takes for core temperature to reach the critical temperature (Lee & Haymes, 1995; Teller *et al.*, 1998; Gonzalez-Alonso *et al.*, 1999b).

Recently, Nybo and Nielsen, (2001a) have shown that subjects made hyperthermic by exercise in the heat were unable to fully activate their quadriceps and that this effect was accompanied by changes in their electroencephalogram. These observations add weight to the suggestion that raised core temperature gives rise to central fatigue rather than affecting peripheral muscle function (Nielsen *et al.*, 1997; Nybo *et al.*, 2001; Nybo & Nielsen, 2001b, a, c).

Nybo and Nielsen, (2001b) found reductions in middle cerebral artery blood flow with hyperthermia and fatigue in the heat. The same authors (Nybo & Nielsen, 2001c) also

looked at brain EEG activity during exercise in the heat and found a strong correlation between core temperature, brain EEG activity and perceived exertion.

Hirata *et al.*, (1987) found that ratings of perceived exertion during handgrip exercise in hyperthermic conditions were reduced by facial fanning and this also increased time to fatigue. The fanning had no effect on oesophageal temperature while tympanic temperature was reduced. It is most unlikely that fanning had any effect on peripheral muscle metabolism, suggesting a central action. Further support for a central temperature-related component of fatigue in the heat comes from a study by Marvin *et al.*, (1999b). They found that cooling the head by fanning and spraying water reduced the perception of exertion and increased time to fatigue, during cycle ergometry at 31°C suggesting that core temperature may not be the only aspect of body temperature that is important.

There is, therefore, strong evidence to suggest that the cause of fatigue in the heat is related to high core temperatures leading to changes in brain activity and increases in perceived exertion. It is of particular interest to see how this mechanism may be related to the changes in serotonergic and dopaminergic pathways discussed in previous sections.

1.3.1 Neuroendocrine responses to exercise and heat

There are few studies that have looked at the direct effects of hyperthermia on 5-HT and DA activity. Hyperthermia is generally found to increase rat brain 5-HT concentrations (Mohamed & Rahman, 1982; Sharma & Dey, 1987; Sharma *et al.*, 1992; Dey *et al.*, 1993) and, likewise DA has been found to increase in the rat striatum (Zhao *et al.*, 2001) and hypothalamus (Kao *et al.*, 1994; Hasegawa *et al.*, 2000).

It is possible to assess human brain activity in response to hyperthermia by indirect means, such as following the release of neuroendocrine hormones. Passive heating has been shown to stimulate PRL release (Mills & Robertshaw, 1981; Christensen *et al.*, 1985) and blood PRL concentrations have been reported to be linearly related to

core temperature during exercise (Brisson *et al.*, 1986; Melin *et al.*, 1988). PRL levels remain low and relatively unchanged during exercise at around 70% maximal oxygen uptake at cool ambient temperatures, however blood levels rise 4-5 fold towards the point of volitional fatigue during exercise in the heat (around 35°C), which typically occurs between 30 and 60 minutes in reasonably fit young subjects (Pitsiladis *et al.*, 1998). It has been suggested that one of the main stimuli for increases in PRL release during exercise is a thermic stress (Brisson *et al.*, 1986) and PRL release has been shown to be more clearly related to heat stimuli than changes in other possible releasing factors such as β-endorphin, vasoactive intestinal peptide or thyrotropin (Brisson *et al.*, 1991). The greater release of PRL in hot environments is indicative of changes in central hypothalamic activity responding to raised circulating blood temperature. However core temperature may not be the only factor controlling PRL release since skin temperature rises in hot environments and cooling the head and face during exercise has been found to attenuate PRL responses as wells as ratings of perceived exertion (Brisson *et al.*, 1989; Marvin *et al.*, 1999b).

Increases in blood PRL concentration with passive exposure and exercise in the heat may be due to changes in either, or both, 5-HT and DA. Changes in brain levels of both have been reported (see above) but there is a conundrum in that whilst increases in 5-HT activity is known to stimulate PRL release, increased DA is generally regarded as being inhibitory and there are clearly complex interactions between the pathways controlling thermoregulatory responses and those involved in PRL release and, possibly, central fatigue.

1.4 Thermoregulatory control

Animals have two basic strategies for dealing with excessive heat loads, the first is behavioural thermoregulation in which the animal seeks to minimise or avoid the source of the heat. This is a fundamental response and can seen at every level of evolution from unicellular organisms moving away from heat, to amphibians and fish seeking cooler water, to mammals seeking shade and cool water to drink. The neural networks involved probably evolved long before autonomic thermoregulation. Evidence of behavioural thermoregulation can be seen in experiments in which the POAH is lesioned. These lesions have been shown to impair autonomic thermoregulation but do not prevent an animal from performing an operant response to maintain a constant body temperature. Rats with lesions of the POAH that are placed in a cold chamber will press a bar to turn on a heat lamp (Carlisle, 1969) and those in a warm environment will turn on a fan (Satinoff & Rutstein, 1971). It must also be remembered that an important source of heat is physical exercise and, while muscle may be more efficient than many mechanical devices, still about 75% of the energy appears as heat. Reducing the level of physical activity is an important component of behavioural thermoregulation.

The second form of thermoregulation, autonomic thermoregulation, is largely under the control of pathways located in the preoptic area and anterior hypothalamus (POAH). This involves the activation of thermoregulatory heat loss and heat gain mechanisms (e.g. sweating, vasodilatation, shivering) in response to changes in body temperature. The POAH is at least one area in the brain that integrates behavioural and autonomic thermoregulation.

1.4.1 The preoptic area and anterior hypothalamus

The importance of the POAH in thermoregulation has been demonstrated in many studies largely carried out on animals. Satinoff, (1964) showed that rats would press a bar to provide heat when the POAH is cooled directly by an implanted thermal probe. Conversely, if hypothalamic temperature is raised rats have also been shown to turn on a fan to provide cooling or to press a bar to cool the temperature of water perfusing a hypothalamic thermonode (Corbit, 1969). These studies support the notion that

hypothalamic temperature is a most important factor in subjective thermal comfort. A recent study in humans using PET scanning has shown that in response to whole body heating a significant increase in hypothalamic metabolism occurs (Nunneley *et al.*, 2002), providing some indirect evidence of an increased neuronal activity.

The POAH contains many thermosensitive neurones that detect changes in internal temperature and initiate the appropriate thermoregulatory responses. Preoptic warming of anaesthetised animals has been shown to induce panting (Hellstrom & Hammel, 1967; Jacobson & Squires, 1970; Boulant & Gonzalez, 1977), sweating (Schonung *et al.*, 1971), increase skin blood flow (Jacobson & Squires, 1970; Schonung *et al.*, 1971) and, in non anaesthetised animals, it elicits behavioural changes that increase heat loss (Adair, 1977). These responses, and those that are related to heat conservation and production mechanisms, are subject to a classic error-system feedback control in which the temperature of the POAH is compared to a set point and appropriate adjustment of heat loss and heat gain are made in response to the error signal.

The POAH is populated by temperature sensitive neurones. The response of these neurones to changes in POAH temperature and skin temperature has been studied in individual neurones using microelectrodes to record changes in firing rate activity. The hypothalamic sensitivity of thermoregulatory responses is the change in response for each degree change in POAH temperature away from the set point (Boulant, 1981, Figure 1.9a). The hypothalamic thermosensitivity of a single neurone is the slope of the change in POAH temperature against the impulses per second that a neurone fires (Figure 1.9b).
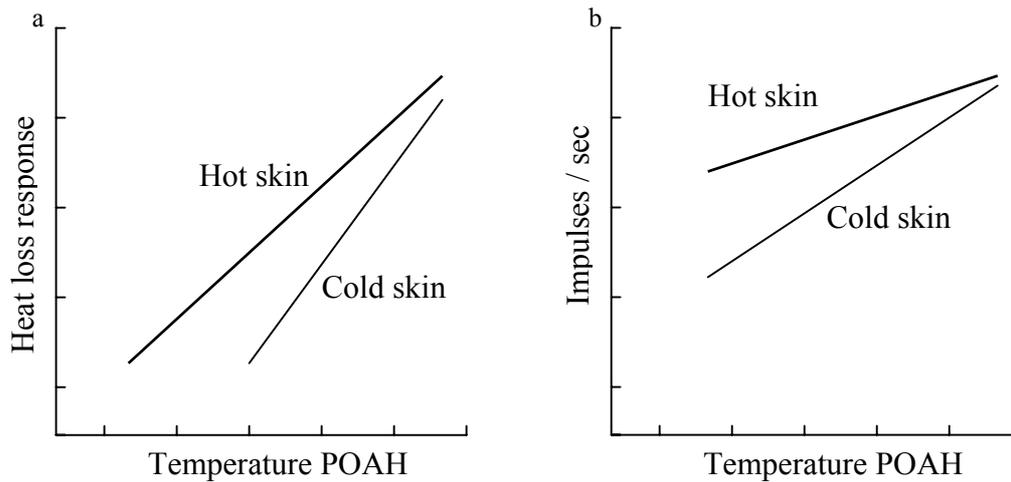
Figure 1.9 a) Hypothalamic sensitivity of a thermoregulatory heat loss response or the change in that response for every degree change in POAH temperature above the set point. b) Hypothalamic thermosensitivity of a warm-sensitive neurone. The different lines indicate different skin temperatures and how they affect the response.

There are two types of temperature sensitive neurone located in the POAH; warm-sensitive and cold-sensitive. About 30% of POAH neurones are sensitive to warm temperatures; they increase their firing rates in response to increases in T_{POAH} and decrease when T_{POAH} decreases. Generally a hypothalamic neurone must have a sensitivity of at least 0.8 impulses per second per degree Celsius to be considered warm-sensitive (Boulant, 1981). Many warm-sensitive neurones have a threshold, or set point temperature, and are only thermosensitive over a certain temperature range. A very small (often <5%) proportion of POAH neurones are classified as cold-sensitive (Boulant, 2000). These cold-sensitive neurones increase their firing rate as POAH temperature falls and decrease as temperature rises. A hypothalamic neurone must have a negative thermosensitivity of at least –0.6 impulses per second per degree Celsius to be considered cold-sensitive (Boulant, 1981). In addition to these temperature sensitive neurones there are other neurones that are temperature insensitive and these make up the majority of hypothalamic neurones (>60%). The temperature insensitive neurones tend to fire at a very slow rate and show little change in their firing rate in response to changes in T_{POAH}. The majority of local excitatory and inhibitory inputs within the POAH come from these temperature insensitive neurones which may provide tonic synaptic input to 'interneurones' such as the cold-sensitive neurones shown in Figure 1.10. Such 'interneurones' may compare synaptic inputs from temperature insensitive neurones and inputs from temperature-sensitive neurones and in this way may appear to be warm or cold-sensitive.

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Single-unit studies have shown that the temperature sensitive neurones of the POAH receive a large afferent input from cutaneous thermoreceptors and from deep body thermoreceptors, such as those in the spinal cord (Boulant & Hardy, 1974). Cutaneous thermoreceptors are stimulated when the skin is warmed or cooled and this afferent information ascends through the anterolateral tract of the somatosensory system. A major pathway in this system is the lateral spinothalamic tract which conveys information to various nuclei in the brain stem reticular formation. From here this somatosensory information is relayed to the thermosensitive neurones of the POAH. In this way the rostral hypothalamus serves as an integrator of thermal information comparing peripheral and central temperature information. The integration of thermal information from the cutaneous thermoreceptors is an important factor determining subjective comfort in humans (Frank *et al.*, 1999). In one study that recorded thermal comfort while core and skin temperatures were altered independently, it was found that core and skin temperature contributed equally to thermal comfort whilst core temperature predominated in the autonomic regulation of thermoregulatory responses of vasodilation and reduced heat production (Frank *et al.*, 1999).

Despite the evident importance of the hypothalamus there is some evidence from clinical studies of patients with hypothalamic lesions that reflex thermoregulatory pathways can function independently of the hypothalamus (Johnson *et al*, 1990) and this is in keeping with suggestions that the hypothalamus may not receive peripheral temperature information (Berner & Heller, 1998). These authors concluded that consideration should be given to integration of skin temperature lower in the neuroaxis. Their idea is supported by studies in patients with spinal cord injuries who have the ability to respond to changes in skin and core temperature even though there is no afferent feedback to the brain (Downey *et al.*, 1976; Huckaba *et al.*, 1976; Attia & Engel, 1983) although the control of body temperature involved was not as precise as in intact subjects.

Areas other than the hypothalamus have also been found to be thermosensitive. One of these is the midbrain through which peripheral thermoreceptors send afferent information to the POAH via the reticular formation. The neurones of the midbrain have been shown to be sensitive to changes in temperature (Hori & Harada, 1976;

Sato, 1984).   Many years of studies using brain lesions led Boulant, (1996) to conclude that no single neural area acts as the thermoregulatory control centre. Instead there seems to be a hierarchy of structures extending through the hypothalamus, brain stem and spinal cord. The brain stem and spinal cord are capable of crudely sensing body temperature changes and implementing some thermoregulatory responses but when the POAH is included, temperature is regulated more precisely and the CNS is more sensitive to changes in central and peripheral temperature (Boulant, 2000). Interestingly, neurones in the midbrain can respond to changes in hypothalamic temperature, suggesting that the POAH can send information back to the brain stem (Sato, 1984). This pathway through the median forebrain bundle to the lower brain stem has been shown to be important in transmitting effector signals to areas controlling changes in skin blood flow (Kanosue *et al.*, 1994).

These observations have lead to the suggestion that thermal information is integrated at various levels of the neuroaxis in a hierarchically organised fashion; spinal cord, brain stem and hypothalamus. Presumably an integrator exists at each of these levels receiving input and transmitting output signals. The hypothalamus is the highest of these centres and the question arises as to how it organises the various thermal inputs it receives in order to activate the thermoregulatory responses to heat and cold.

Numerous neuronal models have been proposed to explain how the CNS can maintain a balance between heat production and heat loss. The first model was presented by Hammel, (1965) who suggested that it is the comparison of excitatory and inhibitory synaptic inputs from warm-sensitive and temperature insensitive neurones that provides the basis for responses to changes in body temperature. Our current understanding is based on the model of Boulant (Figure 1.10, Boulant, 1996).
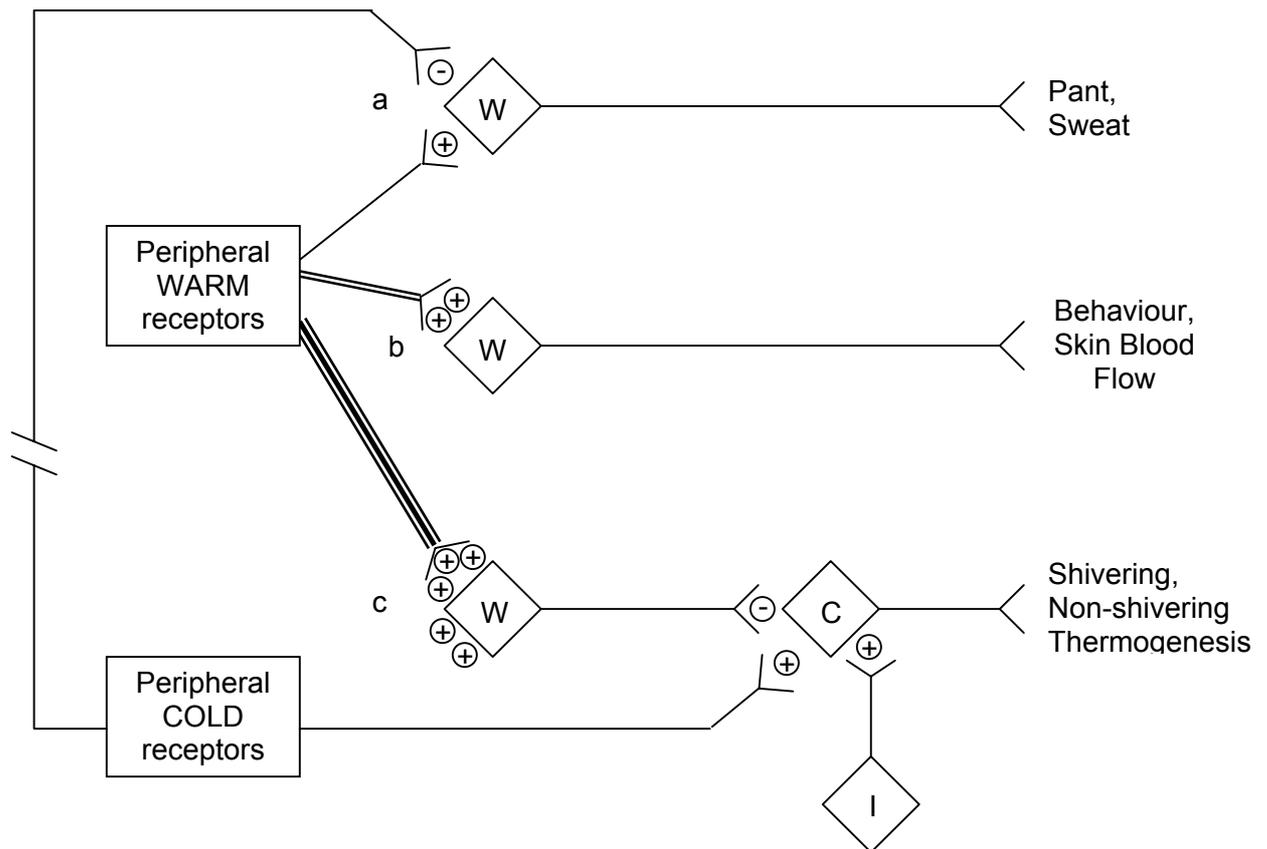
Figure 1.10 The neuronal model of thermoregulation proposed by Boulant (Boulant, 1996) in which 4 types of thermosensitive neurones are depicted: warm (W) neurones (a, b and c) and cold (C) neurons and in addition temperature insensitive neurones (I).

The model consists of 4 different types of thermosensitive neurones; three are warm-sensitive (W) and one is cold-sensitive (C), together with temperature insensitive neurones (I). Warm sensitive neurone 'a' has a low spontaneous firing rate and responds only when the animal is hyperthermic. It is postulated that these neurones are most likely to control heat loss (sweating or panting). The low firing rate is attributed to inhibition from peripheral cold-receptors and/or limited input from peripheral warm-receptors. Warm-receptors labelled 'b' vary their firing frequency in a range around normal hypothalamic temperature and are most likely to control skin blood flow and behavioural thermoregulatory responses. These neurones receive moderate inputs not only from ascending sensory pathways but also have links to the limbic system. This linkage is potentially very important for understanding central fatigue as it provides a link between the central processing of thermal information and the higher centres controlling emotions, behaviour and learning. Interestingly it also provides a pathway linking skin blood flow to emotional state (e.g. "hot and bothered" and flushing under emotional stress). Warm-sensitive 'c' neurones have the highest

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firing rates, attributed to the fact that they receive the largest afferent excitatory input and may have a greater sensitivity to endogenous factors including testosterone and estradiol, osmolality, decreased glucose etc. Because they have such a high firing rate, it is unlikely that they would increase their firing rate during hypothalamic warming and Boulant (1996) suggests that these neurones act primarily to inhibit cold-sensitive neurones, and therefore inhibit shivering and non-shivering thermogenesis.

The major part of the peripheral input to the POAH impinges on neurones ultimately controlling heat production rather than heat loss. Cold-sensitive neurones receive direct input from cutaneous cold-receptors and indirect input from cutaneous warm-receptors through warm-sensitive neurones (c). The latter neurones inhibit POAH cold-sensitive neurones. Thus with a cold skin, cold-sensitive neurones are doubly activated because of positive input from cutaneous cold-receptors and the removal of negative input from cutaneous warm-receptors. This model fits well with studies of animals and humans showing that changes in skin temperature are 2 to 3 times more effective in activating heat production than heat loss (Benzinger & Kitzenger, 1963; Hellstrom & Hammel, 1967).

Most of the local excitatory and inhibitory inputs within the POAH come from temperature insensitive neurones and their function is not at all clear. The temperature sensitive neurones may not fire spontaneously, but could be driven by the temperature insensitive neurones, one of which is shown in Figure 1.10 innervating a cold-sensitive neurone. The output neurones (a, b, c plus the cold sensitive neurone in Figure 1.10) would thus be driven tonically by temperature insensitive neurones and their output modulated by hypothalamic temperature and direct and indirect inputs from peripheral warm and cold receptors.

If the hypothalamus controls thermoregulatory responses as suggested by Boulant, (1996) and illustrated in Figure 1.10, then it would be expected that changing the temperature of the hypothalamus and/or skin would not only affect thermoregulatory responses but also the ability or desire to exercise, as part of the behavioural response.

Isolated heating and cooling of the head have been shown to affect sweating rates (McCaffrey *et al.*, 1975). Subjects sat in an environmental chamber maintained at an ambient temperature of 40°C and their heads were then exposed to heating or cooling (70°C or 29°C) for 30 min. Head heating resulted in increases in head skin, tympanic and oral temperatures whilst rectal temperature remained stable and oesophageal temperature rose slower than tympanic and oral temperature. Mean sweating rate increased rapidly as head and skin temperature increased and continued to increase in parallel with tympanic and oral temperatures after head skin temperature had stabilised. During head cooling, mean sweating rate fell whilst rectal and oesophageal temperatures were unchanged but tympanic and oral temperatures declined. It was assumed by the authors that head heating and cooling altered the temperature of the hypothalamus and they concluded that brain temperature is the most important stimulus for thermoregulatory responses which are primarily designed to maintain brain temperature (McCaffrey *et al.*, 1975). Head cooling has been shown to increase core temperature during exercise whilst brain temperature remains precisely regulated and constant (Cabanac & Caputa, 1979b) and the preliminary findings of Marvin *et al.*, (1999b) showed a similar trend.

Cabanac and Caputa, (1979a) sat subjects in a warm bath at 38.6-38.7°C who reported their ratings of body temperature. Oesophageal and tympanic temperatures rose to a similar extent and subjects found the warm water immersion to be unpleasant. Facial fanning was described as a pleasant stimulus and was accompanied by a reduction in tympanic temperature whilst oesophageal temperature continued to rise. The authors conclude that brain temperature is an important factor determining temperature sensation and comfort.

In the studies just described it is generally assumed that tympanic temperature gives a good indication of brain and, probably, hypothalamic temperature. However whether selective cerebral cooling occurs in humans is still a matter of considerable contention (see Brengelmann, 1993; Cabanac, 1993 for review). Tympanic temperature is a difficult measure to make and can quite easily be contaminated by cold skin temperature in and around the ear. In addition, measurements of conduction velocity

in the auditory nerve, which should give a direct indication of the temperature of the brain, failed to show any change with head cooling (Nielsen & Jessen, 1992)

Changing head temperature certainly has marked effects on thermoregulation and thermal comfort and if this is not the result of cooling the brain then cooling a relatively small area of skin on the head must have a considerable influence on central brain activity.

1.4.2   Neuroendocrine control of thermoregulation

Since the mid-1960s there have been numerous studies in which a wide variety of substances have been injected peripherally or directly into the brain and changes in body temperature observed (Clark, 1979). The monoamine theory of thermoregulation, proposed by Feldberg and Myers (1964) proposed that the two main neurotransmitters governing heat loss and heat gain in the hypothalamus were norepinephrine and 5-hydroxytryptamine respectively. Since that time there has been a large volume of work, almost exclusively in animals, that has looked at the role of various putative neurotransmitters in the control of thermoregulation in the hypothalamus. This search illustrates a common tendency to equate a single function, e.g. heat loss, to a single pathway and transmitter. However it is now widely appreciated that most, if not all, functions are coded by multiple neurotransmitters, hormones, neuromodulators, and the interaction of diverse neural circuits (Brezina & Weiss, 1997; Segovia *et al.*, 1997).

In this vein, Feldberg and Myers, (1964) proposed that a balanced release between serotonin and noradrenaline could serve as the neurochemical basis for the control of temperature. Shortly thereafter, acetylcholine was proposed as an inhibitory neurotransmitter mediating heat gain or heat loss (see Cox & Lomax, 1977 for review), and peptides were suggested as neuromodulators (see Ruwe *et al.*, 1983 for review). Today a long list of neurotransmitters and neuromodulators has been reported to change body temperature when injected into the cerebral ventricles or directly into the brain. However, evidence supporting a role for any of these substances transmitting specific thermal information among neurones in the CNS is equivocal (Blatteis, 1981; Myers & Lee, 1989). Differences in methodology, animal

species, route of drug administration, dose, anaesthetics, restraint and ambient temperature have seriously complicated the interpretation of the data collected. Furthermore, in the majority of studies changes in body temperature alone were measured which could simply be a secondary response to activation of another autonomic physiological system. For serious progress to be made, direct measurements are required of thermoregulatory effector mechanisms (e.g., sweating, vasodilation, panting) in response to endogenous release of putative neurotransmitters.

Since the late 1960s the most consistent thermoregulatory responses have been elicited by central stimulation with 5-HT and DA. However, even these responses vary between species.

1.4.2.1 5-HT and thermoregulatory control

Since 1960 it has been known the raphe nuclei receive collateral input from the ventral spinal tract, where information about skin temperature and pain travels upwards and it has been suggested that the ascending raphe nuclei have a role in thermoregulatory function (Hillegaart, 1991). Furthermore, the raphe nuclei are the only source of serotonin-containing neurones in the brain and these serotonin neurones project widely in a rostral direction to areas that include the preoptic area of the hypothalamus (Fuxe, 1965). It has also been known for sometime that microinjections of 5-HT into the preoptic area can cause changes in the body temperature of an animal, although the evidence about the direction of these changes has been conflicting. The extensive microinjection study of Komiskey & Rudy, (1977) showed there are two distinct hypothalamic regions sensitive to serotonin: an anterior one that drives heat loss and a posterior one that drives heat gain.

Clearly one possibility that would fit these observations is that raphe nuclei receive input from cutaneous warm and cold receptors and relay this information to the preoptic area, with serotonin as the likely transmitter (Hellon, 1981). Dickenson, (1977) found that changing the skin temperature of the rat could specifically drive a large proportion of the neurones in the raphe nuclei as shown by microelectrode recordings. Injections of LSD, a specific serotonin blocker, caused a reversible inhibition of these cells, which also lost their sensitivity to skin temperature changes

(Hellon, 1981). It is possible that afferent neurones of skin thermoreceptors may also project directly to the preoptic area of the hypothalamus but this is not proven.

5-HT innervation of the hypothalamus comes from fibres arising from cell bodies located in the raphe dorsalis, raphe magnus and raphe centralis superior, or groups B7-B9, to use the original terminology of Dahlstrom and Fuxe, (1964). Many pharmacological experiments have shown that 5-HT injected ICV or directly into the POAH at certain doses produces hyperthermia. However at other doses it produces hypothermia. Myers (1980), using push-pull perfusion found an increase in 5-HT in perfusates from the POAH when the animals were cooled, but not when they were heated suggesting a role of 5-HT in heat conservation and production.

Single unit recordings have also reported contradictory results: 5-HT seems to produce non-specific effects. In fact Watanabe *et al.*, (1986) reported that of the total number of warm-sensitive neurones recorded in the hypothalamus, 91% were activated by 5-HT. 37% of hypothalamic neurones that were thermally insensitive were also excited by 5-HT, presenting a confused picture of 5-HT's role in thermoregulation.

Woolf *et al.*, (1975) have suggested the 5-HT increases inhibitory input from central thermosensors to effector neurones leading to an increase in vasoconstriction and thermoregulatory heat production in the rabbit. White *et al.*, (1985) have also suggested a role for 5-HT in heat conservation but this time through excitation of effector neurones leading to vasoconstriction. In contrast Bruck and Zeisberger, (1990) looked at changes in the guinea pig and rat and found increased heat loss and reduced heat production as the result of activating the 5-HT system. To confuse the issue further, in the anterior hypothalamus of the cat, most of the warm-sensitive, cold-sensitive and thermoinsensitive neurons are inhibited by 5-HT (Jell, 1974).

Obviously one of the major problems with the discussion above is that there are considerable differences between species. Additionally it is unlikely that 5-HT is the sole mediator of thermal information in hypothalamic pathways. It seems very likely that thermal information does reach the hypothalamus via the mid brain and this

involves 5-HT, but the precise role that 5-HT plays in thermoregulatory control by the POAH remains unclear.

1.4.2.2   DA and thermoregulatory control

DA is another prominent amine thought to participate in hypothalamic temperature regulation. Lee *et al.*, (1985) reviewed the literature in this area and concluded that in rats, rabbits and other species, including humans, DA plays a role in the thermoregulatory pathways mediating heat loss. Microinjections of DA in both ventricles and POAH reduce body temperature in the rat (Lee *et al.*, 1985). There are a few electrophysiological studies of the effects of DA on firing rates of the temperature sensitive neurones of the hypothalamus. In the cat Sweatman and Jell, (1977) showed that DA microiontophoretically applied to cold-sensitive neurones decreased their spontaneous firing rate, suggesting that the effects of DA may be through an inhibition of heat production mechanisms. This hypothermic effect is further supported by *in vitro* electrophysiological recordings from tissue slices obtained from the hypothalamus of the rat. Perfusing the preparations with DA reduced the activity of cold-sensitive neurones but increased that of the warm sensitive neurones in the hypothalamus (Scott & Boulant, 1984). The results of this study rule out the possibility that DA may depress the firing of all hypothalamic temperature sensitive neurones in a non specific manner whilst at the same time strengthening the evidence for the role of DA in thermoregulation by mediating heat dissipation

The evidence discussed so far has suggested that there is a role for DA in thermoregulation, although most of the studies discussed have introduced DA into the brain through injections. Strong evidence for endogenous DA in the normal control of body temperature comes from studies by Cox *et al.*, (1978) and Cox and Lee (1980). In these studies rats were placed under a heat lamp to stimulate heat dissipation mechanisms consisting of vasodilatation of blood vessels in the tail. In experimental rats treated with either a DA antagonist or 6-OHDA, the vasodilation was significantly reduced and the rats were less able to control their core temperature, indicating that DA initiates heat loss mechanisms. Similar conclusions can be drawn from the work of Ohara *et al.*, (1972) who reported that hypothalamic DA content

declines when rats are placed under heat stress. An experiment, in cats, in which the endogenous stores of DA and NE were labelled within the anterior hypothalamic pre-optic area showed that DA but not NE was released at catecholamine sensitive sites of the POAH when ambient temperature was increased to the point at which heat loss mechanisms were activated (Ruwe & Myers, 1978). At sites outside the POAH only NE was released showing a specificity of DA activity when heat loss mechanisms are activated (Myers, 1980).

DA microinjected into other areas of the brain, particularly in areas involved in motor behaviour, also affects temperature regulation. For example, injecting DA into the caudate putamen produced hypothermia that can be antagonised by DA-receptor blockers (Lee *et al.*, 1985). In the substantia nigra, another area of motor control, microinjections or apomorphine (DA receptor agonist) produced a dose-dependent hypothermia that was also antagonised by a specific DA receptor blocker (Lee *et al.*, 1985). This hypothermia was associated with increased heat loss and decreased heat production, and it was independent of ambient temperature. Furthermore, the same dose of apomorphine injected into the POAH produced similar hypothermic effects (Lee *et al.*, 1985).

The significance of the basal ganglia affecting hypothalamic function is not clear but it could be an example of integration of function, an anticipatory activation of thermoregulatory control mechanisms in advance of changes in body temperature during exercise.

Support for a role of the nigrostriatal pathway is also obtained from clinical reports in humans. Parkinson's patients suffer from degeneration of the nigrostriatal pathways and exhibit abnormalities in heat dissipation and thus become heat intolerant. In a series of experiments between 1981 and 1985 Lin and colleagues presented evidence for the nigrostriatal dopaminergic pathway being involved in thermoregulation in rats. Lesions of DA neurons within the substantia nigra produced by 6-OHDA were found to interfere with thermoregulation (Lin *et al.*, 1981). Intrastriatal administration of apomorphine decreased metabolism and resulted in hypothermia, whereas haloperidol produce the opposite effects in rats in the cold (Lin *et al.*, 1982). It was also found that cold sensitive neurones in the striatal area were inhibited by apomorphine but

excited by haloperidol (Lin & Tsay, 1985). A few years after these experiments the same authors found that stimulating the substantia nigra could inhibit both heat production and heat loss mechanisms in the rat (Lin *et al.*, 1992) depending on the ambient temperature. Below an ambient temperature of 22°C, stimulation led to a decrease in core temperature, whereas at an ambient temperature of 30°C stimulation lead to a hyperthermia and cutaneous vasoconstriction a finding that seems almost counter-intuitive.

The above series of experiments, although confusing, demonstrate an involvement of the nigrostriatal DA pathways in thermoregulation although the precise role is still not fully understood.

Lee et al (1985) have proposed a scheme depicting possible components of central dopaminergic systems that interact to influence thermoregulation (Figure 1.11)
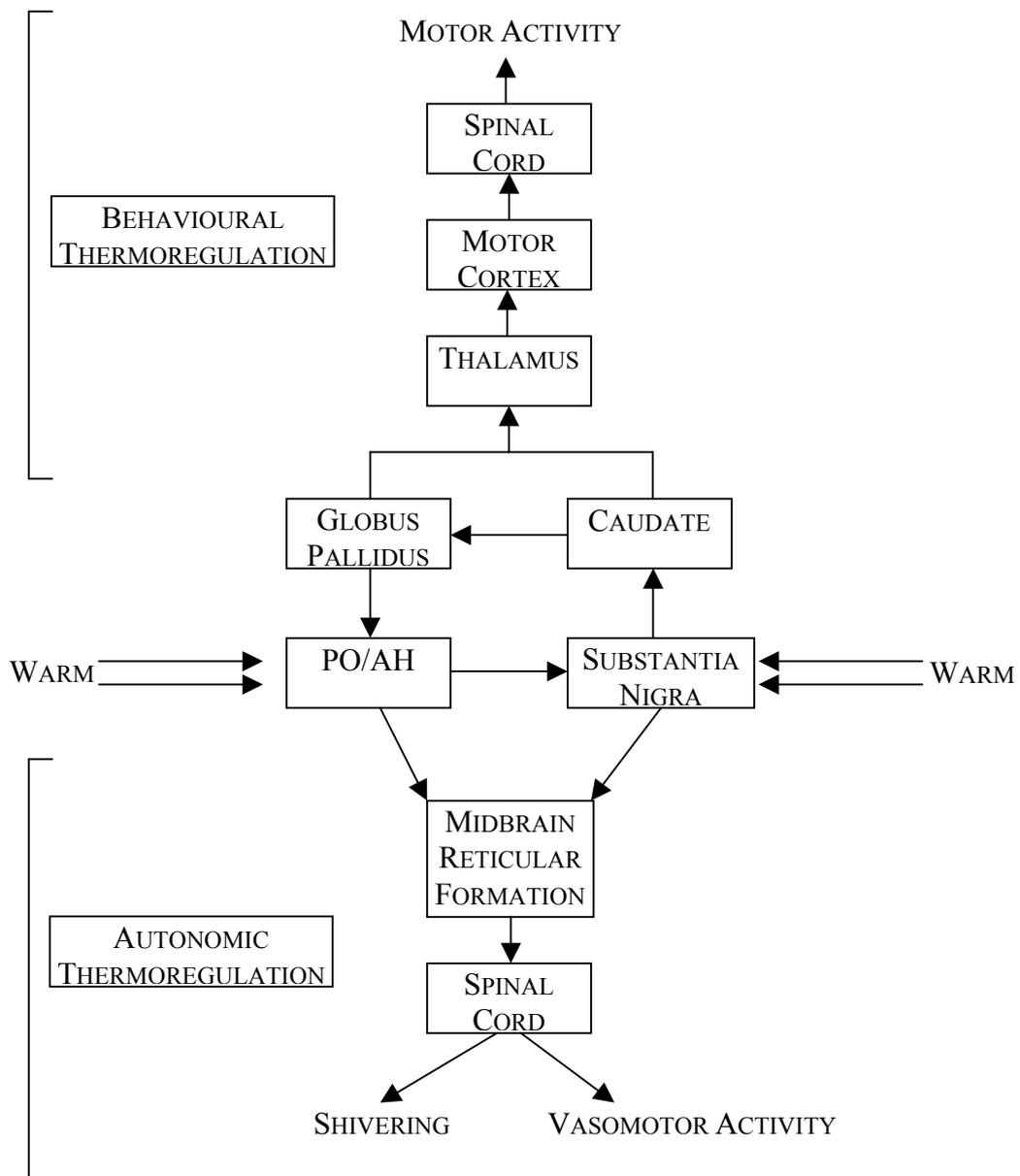
Figure 1.11. Hypothetical scheme of components of central dopaminergic systems interacting to influence heat loss, adapted from Lee et al (Lee *et al.*, 1985).

This scheme was proposed from a review of the evidence pointing to a role for DA in thermoregulation. The authors have proposed a link between the substantia nigra and the preoptic area of the anterior hypothalamus but whether this pathway actually exists within the brain is uncertain. There is certainly some evidence to support its existence, although it may be that the substantia nigra functions independently of the preoptic area receiving thermal signals through a thermosensitive structure which is currently unknown (Lee *et al.*, 1985).

1.5 Central fatigue as part of an integrated response to exercise in the heat: outline of the work presented in subsequent chapters.

The experimental approach described in the following chapters has been stimulated by previous work in four main areas.

The first concerns the role of serotonin in limiting endurance exercise, largely initiated by Newsholme and continued with important work in animals demonstrating the involvement of serotonergic and dopaminergic systems. This was pioneering work which not only drew attention to the reality of central fatigue but also gave a theoretical framework that stimulated intense interest in the actions of central pathways and their influence on exercise. The difficulty with much of the work is that it is basically a biochemical approach and it treats the brain as a homogeneous tissue, regulated by levels of different hormones. Often the conclusions are somewhat simplistic i.e. increases in serotonin are bad for continued exercise. One way forward in this area is to try and identify the central pathways involved and in this respect measurement of pituitary hormones provide an indication of hypothalamic activity and much of the work described in this thesis uses prolactin for this purpose.

The second major influence has been the work of Nielsen and colleagues in the last ten years, who have shown that increases in body temperature and central fatigue is a major limitation for exercise in the heat. In the absence of a simple test for central fatigue during dynamic exercise, Nielsen and co-workers have systematically demonstrated that peripheral explanations for fatigue, such as reductions in blood flow to working muscles, do not explain the loss of performance. More recently this group has begun to provide more direct evidence in terms of EEG changes and assessment of their subjects' ability to activate limb muscles under hyperthermic conditions. The one area that this group has not explored is the neuropharmacology of the fatigue processes they have been studying.

In attempting to understand the behavioural, endocrine and thermoregulatory responses to exercise it is essential to have some broad theoretical model of the interaction of neural pathways against which to test ideas and design experiments. The

work of Boulant has been especially important in defining these interactions in the hypothalamus and demonstrating how central and peripheral thermoreceptors can combine to provide multiple outputs that cover behavioural changes (which could be construed as central fatigue) as well as the more obvious thermoregulatory responses of sweating and shivering.

Finally, the work of Brisson has been very influential in developing many of the ideas explored in this thesis. Although not primarily concerned with questions of fatigue, Brisson and colleagues provided much stimulating and important information about the control of hypothalamic function by core and skin temperature. The implications of this work for fatigue mechanisms have been elaborated in the present series of experiments. Chapters 2 and 3 are concerned with these points and describe how core and skin temperature interact to modify the perception of exertion, endurance and prolactin secretion (Chapter 2) and how other stress hormones (GH and Cortisol) are affected by body temperature.

When using prolactin as a marker of hypothalamic function it is important to know how its release is controlled, since both increased 5-HT and decreased DA are known to be potential mechanisms. In Chapter 4 the specific 5-HT_{1A} antagonist pindolol has been used to separate out these two mechanisms when prolactin release is stimulated with Buspirone, a drug that has been used as a neuroendocrine challenge to test the sensitivity of putative serotonergic pathways involved in fatigue mechanisms. The results show that it is possible to use buspirone in combination with pindolol to assess the sensitivity of both serotonergic and presumed dopaminergic pathways in the hypothalamus.

This modified neuroendocrine challenge was used in the experiments described in Chapter 5 to look for correlates between the sensitivity of hypothalamic serotonergic and dopaminergic pathways and exercise tolerance in the heat.

It would be most interesting to know how prolactin release during exercise is controlled, whether it is primarily by serotonergic stimulation or the withdrawal of dopaminergic inhibition. In theory, exercise in the presence of pindolol should answer this question, but in practice the beta antagonist properties of pindolol mean

that it is impossible to exercise at a sufficient intensity to release prolactin. However, much of the work described here, and elsewhere, indicates that during exercise it is a rise in temperature, rather than other factors such as acidosis, that releases prolactin. Consequently, the work described in Chapter 6 is based on the premise that passive heating can mimic the effects of exercise, at least as far as prolactin is concerned. It is quite feasible to combine passive heating with pindolol blockade and thus determine the extent of serotonergic and dopaminergic control of heat-induced prolactin release.

While body temperature is clearly very important in regulating exercise, it is not necessarily the only central influence. A range of legal and illegal substances appear to affect performance, of which caffeine is the most accessible drug to use for experimentation. The approach used in the work described in Chapter 7 was to assume that caffeine interacts with the hypothalamic pathways involved in temperature regulation and, if this were the case, it would be expected to influence prolactin release. This was found not to be the case and this finding opens up a new area, suggesting additional central pathways involved in fatigue, possibly involving the nucleus accumbens or the basal ganglia.

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Chapter 2

THE INFLUENCE OF AMBIENT TEMPERATURE ON PERCEPTION OF EXERTION AND EXERCISE-INDUCED PROLACTIN RELEASE

2.1 INTRODUCTION

High ambient temperatures are well known to reduce exercise capacity and increase ratings of perceived exertion (e.g. Galloway & Maughan, 1997) but there are no simple explanations for this behaviour at the level of skeletal muscle metabolism or function. Heat exposure and exercise in the heat are associated with a host of responses by the regulatory systems of the body in an attempt to maintain homeostasis. The most obvious response to exercise in the heat is to increase heat loss by vasodilation and increased sweating but there are also behavioural changes that reduce heat gain together with a substantial release of pituitary hormones.

Exercise in the heat leads to a redistribution of blood to the skin to facilitate heat loss and this may divert blood away from the working muscles (Fink *et al.*, 1975) but during exercise at around 70%VO$_2$max there is no evidence of a reduction in leg blood flow (Gonzalez-Alonso *et al.*, 1998) or a metabolic limitation in the working muscles. Consequently it has been suggested that increasing body temperature may play a major role in the development of fatigue (Bruck & Olschewski, 1987) and it has been shown that there is an oesophageal temperature at which subjects fatigue that is not related to metabolic or cardiovascular changes affecting the skeletal muscles (Nielsen *et al.*, 1997). Thus the duration of exercise can be prolonged or shortened by manipulating core temperature before exercise so that it takes more, or less, time to reach the critical temperature (Lee & Haymes, 1995; Teller *et al.*, 1998). In these circumstances the perception of exertion is related to the initial temperature and rate of heat storage.

Prolonged fatiguing exercise is associated with the appearance in the circulation of a number of hormones originating from the pituitary gland under the control of the hypothalamus. Among these is prolactin (PRL), the circulating level of which is known to increase during fatiguing exercise (Pitsiladis *et al.*, 1998) and to be sensitive to changes in body temperature (Brisson *et al.*, 1991). Passive heating has been shown to stimulate PRL release at rest (Mills & Robertshaw, 1981; Christensen *et al.*, 1985) and PRL concentrations have been found to be linearly related to rectal temperature during exercise (Brisson *et al.*, 1986; Melin *et al.*, 1988). PRL levels remain low and relatively unchanged during exercise at around 70% VO$_{2max}$ in cool ambient

temperatures, however blood PRL levels rise 4-5 fold towards the point of volitional fatigue during exercise in the heat, which typically occurs between 30 and 60 minutes in reasonably fit young subjects (Pitsiladis *et al.*, 1998). It has been suggested that one of the main stimuli for increases in PRL release during exercise is a thermal stress (Brisson *et al.*, 1986) and a close relationship has been shown between rectal temperature and PRL release in response to exercise (Brisson *et al.*, 1991). While body temperature is clearly important, the relationship between rectal temperature and PRL release is modified by cooling the head and face during exercise (Brisson *et al.*, 1989; Marvin *et al.*, 1999). It remains a matter of considerable debate as to the mechanisms involved, whether it is due to direct cooling of the brain, of the blood reaching the hypothalamus or a reflex arising from skin afferents. The stimulus for prolactin release provided by deep body temperature, at least when assessed by rectal temperature, is modulated by some other factor that must differ between exercise in hot and cool conditions.

In a similar way, perception of exertion is modulated by a number of factors. The original 15-point Borg scale is based on the relationship between heart rate and perceived exertion during exercise and the increased heart rate seen during exercise in the heat is an obvious candidate for the increase in perceived exertion. It is also likely that increases in body temperature contribute to perceived exertion and changes in blood lactate and glucose concentrations could also play a role.

It is clear that environmental temperature during exercise has major effects on perception of exertion and development of fatigue and that it also affects the hormonal responses. Since the hormonal response to exercise is largely under the control of the hypothalamus it is of interest to know whether there is any association between changes in perception of exertion and the hormonal responses that could indicate a common underlying mechanism. To date there have been no studies in which changes in perception and pituitary hormones have been directly compared and, consequently, this was the primary purpose of the work described in the present chapter.

2.2   METHODS

General Design

Subjects performed two 40 minute exercise tests on a cycle ergometer at an ambient temperature of 15°C or 35°C during which blood samples were collected every 10 minutes and rectal and skin temperature measured every 5 minutes. Tests were randomly assigned and balanced for order.

Subjects

Eleven trained male cyclists participated in the study. Their age, body weight and maximal oxygen consumption (VO_{2max}) were 22.5±3.3 y (Mean±SD), 71.9±5.3 kg, and 4.55±0.50 $l.min^{-1}$, respectively. The study was approved by the Local Research Ethics Committee and subjects gave their informed consent in writing.

Experimental Design

Visit 1

Subjects completed an incremental exercise test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and VO_{2max}. Workload was increased by 35 Watts every 3 minutes until volitional exhaustion. Expiratory gases were analysed and averaged over a 10 second period using a computerised on-line system (Oxycon Alpha, Jaeger, Bunnik, The Netherlands). W_{max} was calculated using the method of (Kuipers *et al.*, 1985):

$$W_{max} = W_{final} + (t \times W) / T$$

Where W_{final} is the power of the last completed stage, t is the time reached in the final uncompleted stage, T is the duration of each stage and W is the workload increment of each stage.

Visits 2 & 3

On the day before visit 2, subjects recorded their diet and were asked to adhere to the same diet on the day before visit 3. On the day of a test subjects arrived at the laboratory at 8am, having fasted from midnight. A cannula was inserted into an

antecubital vein to obtain blood samples and was kept patent by flushing with 3ml saline every 10 minutes. To ensure that subjects began each trial euhydrated they were given a bolus of water ($8ml^.kgbw^{-1}$) to consume during the 45 minutes between cannulation and the start of exercise, which was spent resting at an ambient temperature of around 18^oC. A resting blood sample was taken and subjects then moved into the thermal chamber and began to exercise on a stationary electrically braked cycle ergometer (Lode Excalibur Sport, Lode, Groningen, Netherlands). Exercise continued for 40 minutes at a constant work rate of 65% W_{max} at an ambient temperature of either 15°C (50% relative humidity) or 35°C (30% relative humidity). Subjects were allowed fluid *ad libitum* during exercise and were asked to drink a minimum of $3ml^.kgbw^{-1}$ water every 15 minutes to maintain hydration. Heart rate was continuously recorded (Polar Vantage NV, Polar OY, Finland). Venous blood samples (8ml) were taken every 10 minutes during exercise for determination of haematocrit, haemoglobin, lactate, glucose and PRL. Whole body ratings of perceived exertion (RPE, Borg, 1975) were obtained every 10 minutes during exercise.

Temperature measurements

Rectal and skin temperatures were recorded every 5 minutes (Squirrel Meter Logger, Grant Instruments, Cambridge, UK), the latter using the four site formula of (Nielsen & Nielsen, 1984).

Blood analysis

Haematocrit was measured in triplicate by centrifugation. Blood glucose and lactate were measured on plasma samples using enzyme-linked assays (Sigma Diagnostics, Poole UK); haemoglobin was measured using the cyanomethaemoglobin method (Sigma Diagnostics, Poole UK). PRL was measured by radioimmunoassay (Skybio Ltd., UK).

Data and Statistical Analysis

Total hormone release was measured from the area under the curve of hormone level with time (AUC) calculated using the trapezoid method. Changes in plasma volume were calculated using the method of Dill & Costill, (1974). All data were tested for

their approximation to a normal distribution. Significant differences within trials, where data were normally distributed, were determined using repeated measures ANOVA with Bonferroni corrections for multiple comparisons. Where data were not normally distributed a Friedman test was used. Between trials, where data were normally distributed, significances were determined at matched time points using Student's paired t-test. For data that were not normally distributed (PRL), significances were determined using Wilcoxon non-parametric tests. Data are given as mean ± standard error of the mean, unless otherwise stated.

2.3   RESULTS

All subjects managed to complete both trials although they reported that exercise in the heat was much more arduous.

Perceived Exertion

Ratings of perceived exertion (RPE) increased during exercise in both conditions and were significantly higher throughout the exercise in the hot compared to the cool condition (Final values 13.2±0.5 at 15°C and 15.7±0.9 at 35°C, Figure 2.1, $p<0.05$).
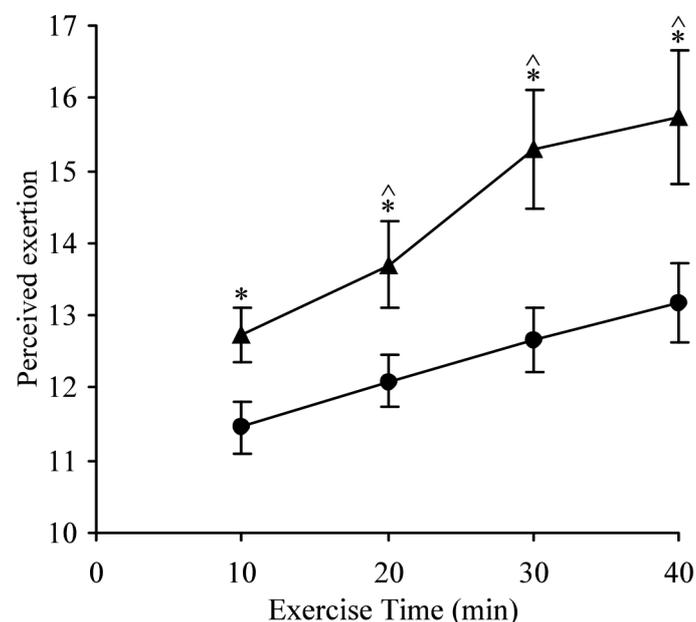

Figure 2.1 Ratings of perceived exertion (Borg scale). ● 15°C ▲35°C. Data are Mean±SEM. * indicates a significant difference between trials; ^ indicates a significant difference from 10 minute value in both trials ($P<0.05$).

Cardiovascular and respiratory changes

Heart rate (Figure 2.2) increased during the course of each trial ($p<0.01$) and was significantly higher during exercise in the heat (Final values 150±4 beats·min^{-1} at 15°C and 164±4 beats·min^{-1} at 35°C, $p<0.01$). There was a non significant trend for ventilation to be higher during exercise in the heat.

Oxygen uptake during exercise was consistently slightly higher in all subjects in the heat (~0.08 l·min^{-1}) so the percentage of VO_{2max} increased from 72±7% in cool

conditions to 74±6% in the heat (p=0.037). There was no change with time in either exercise condition.
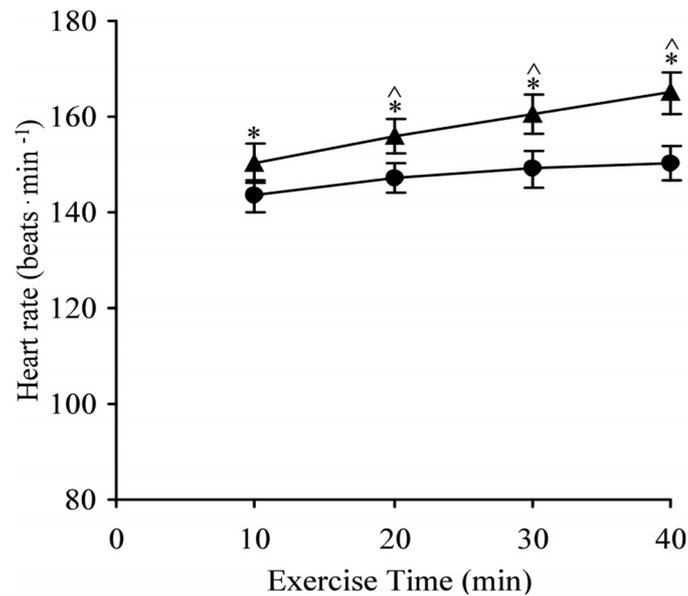
Figure 2.2 Heart rate response to exercise. ● 15°C ▲35°C. Data are Mean±SEM * indicates a significant difference between trials; ^ indicates a significant difference from 10 minute value in both trials (P<0.05).

Body temperature responses

Initial rectal temperatures did not differ significantly between trials (36.9±0.4°C at 15°C and 37.1±0.5°C at 35°C). The temperature increased significantly during both trials (Final values 38.4±0.1°C at 15°C and 38.9±0.2°C at 35°C, Figure 2.3a, p<0.05) and while the rectal temperature in the heat was always slightly greater than in the cool, the difference was only significant at 30 and 40 minutes (Figure 2.3a, p<0.01). In the cool trial, the mean rectal temperature was constant between 30 and 40 minutes, suggesting that a thermal equilibrium had been achieved, whilst during exercise in the heat, rectal temperature continued to increase at this time (p<0.05). Mean skin temperature was significantly higher in the hot conditions. There was a drift downwards in skin temperature during the latter part of the exercise although this was only significant during exercise in the cool (Figure 2.3b, p<0.01).
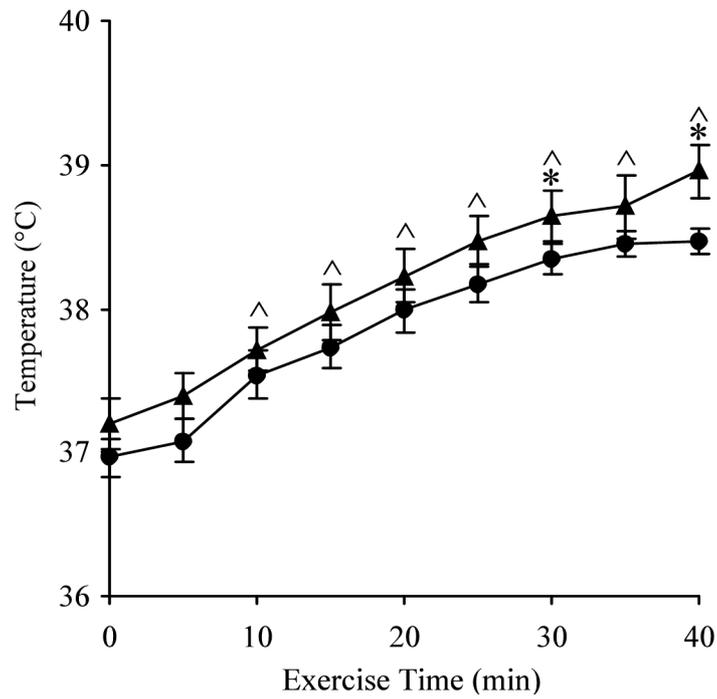
Figure 2.3a Rectal temperature responses to exercise. ●15°C ▲35°C. Data are Mean±SEM. * indicates a significant difference between trials ^ indicates a significant difference from resting value in both trials (P<0.05).
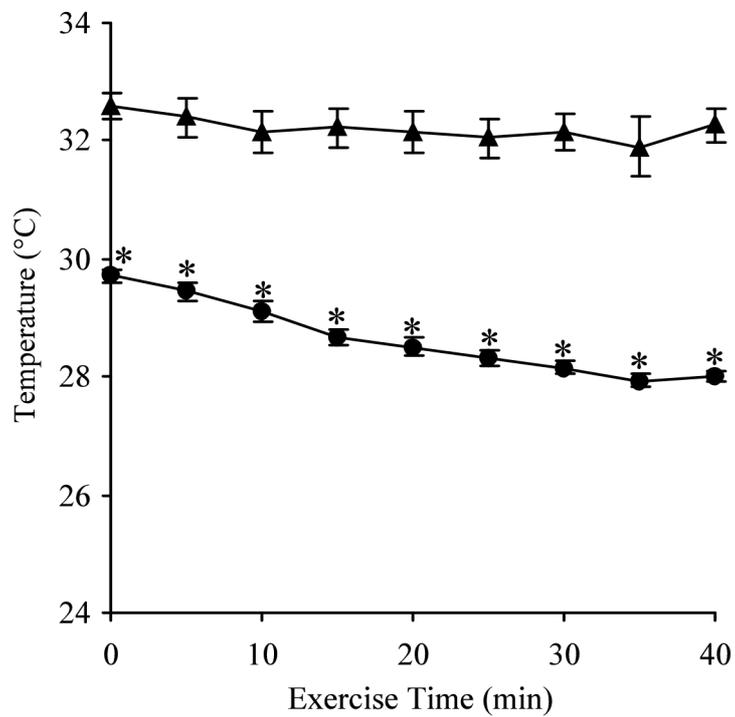
Figure 2.3b Mean skin temperature responses to exercise. ●15°C ▲35°C. Data are Mean±SEM. * indicates a significant difference between trials (P<0.05).
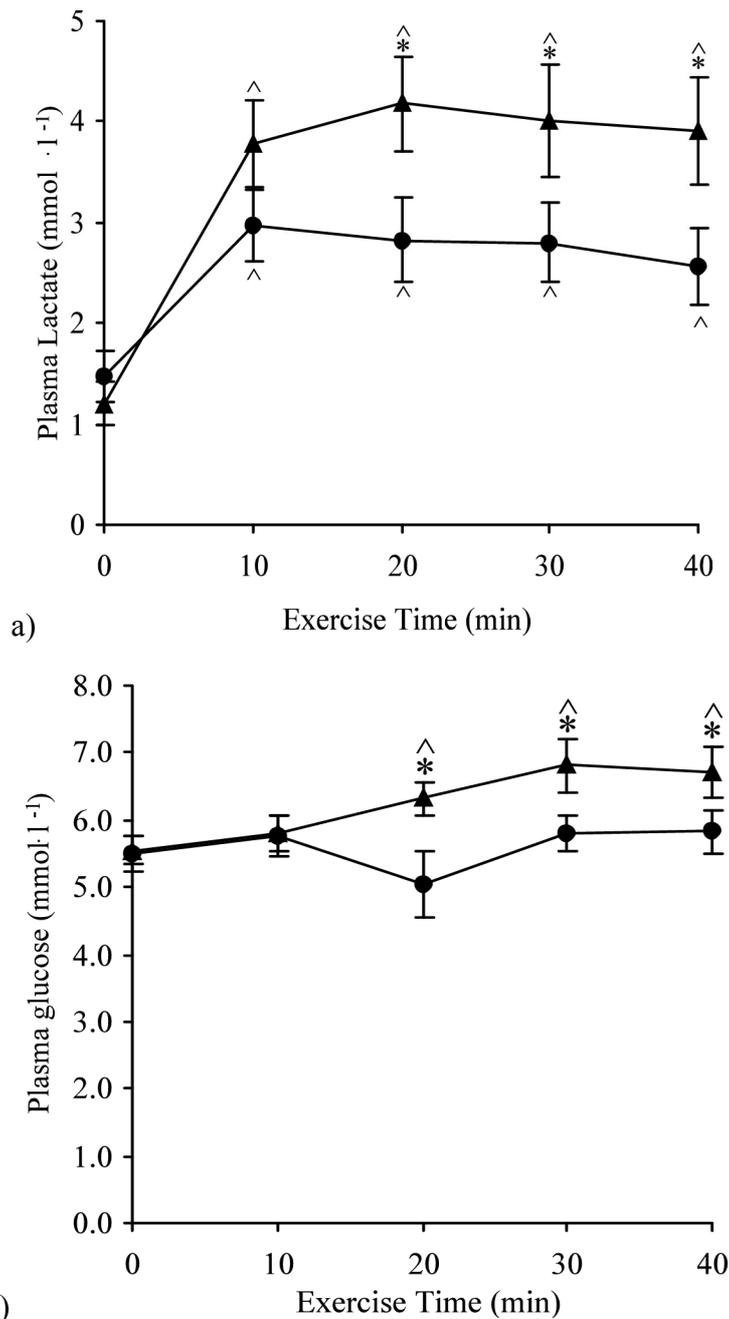
Figure 2.4 Metabolic responses to exercise. a) Plasma Lactate; b) Plasma Glucose; ● 15°C ▲ 35°C. Data are Mean±SEM. * indicates a significant difference between trials; ^ indicates a significant difference from resting value ($P<0.05$) but no difference between marked time points within a trial.

Metabolic responses

Blood lactate increased in the first 10 minutes of both trials and then remained relatively constant in all subjects, although the steady state concentrations varied between individuals (range after 20 minutes of exercise, 1.3mmol·l^{-1} to 5.9mmol·l^{-1} at 15°C and 2.3mmol·l^{-1} to 6.9mmol·l^{-1} at 35°C). After 20 minutes of exercise, lactate was significantly higher during exercise in the heat and remained elevated for the

remaining 20 minutes (final values 2.5±0.4mmol·l^{-1} at 15°C and 3.90±0.5 mmol·l^{-1} at 35°C, Figure 2.4a, p<0.01). No change in blood glucose occurred during exercise in the cool whereas in the heat, values were significantly higher during exercise than at rest and were higher than in the cool condition for the last 20 minutes of exercise (Figure 2.4b, p<0.05). No differences were found between trials in either haematocrit or haemoglobin and calculated changes in plasma volume did not differ between trials (final values −0.6±2.5% at 15°C and −1.3±1.8% at 35°C) indicating that subjects maintained hydration during the exercise and any differences found between the two trials in terms of blood metabolites or PRL was not a consequence of haemoconcentration.

Hormonal Response

There were no differences in resting PRL between trials. PRL increased above resting values after 20 minutes exercise in the cool and after 10 minutes in the heat and continued to increase throughout both trials (Figure 2.5, Wilcoxon, p<0.05). Prolactin values were significantly higher during the trial in hot conditions compared to the cool trial at every time point during exercise (Figure 2.5, Wilcoxon, p<0.05).
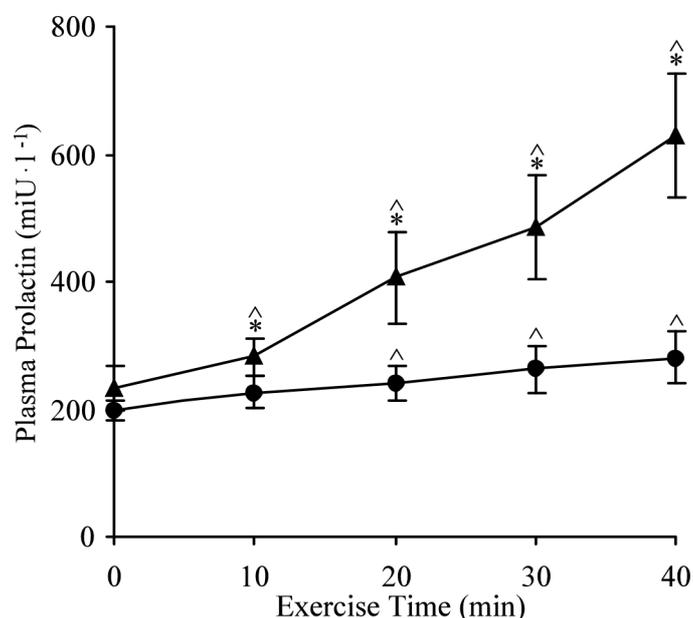
Figure 2.5 Hormonal response to exercise. Plasma prolactin ● 15°C ▲35°C. Data are Mean±SEM. * indicates a significant difference between trials; ^ indicates a significant difference from resting value (P<0.05).

Total PRL release, measured by AUC, was higher in the heat (9465 miU·min·l^{-1} Cool (IQ range, 5871-11390) v 15730 miU·min·l^{-1} Hot (IQ range, 10935-19319), Wilcoxon,

p=0.04). The increase during exercise in the heat was 5 times greater than in the cool (28% (IQ range, 20-38) Cool v 151% (IQ range, 74-228) Hot), expressing the values as a percentage increase during exercise.

2.4   DISCUSSION

The results presented here confirm the common experience that exercise at an elevated ambient temperature results in higher ratings of perceived exertion than when undertaken at cooler temperatures. The effect of elevated temperature on perception was very similar to the changes in PRL, a pituitary hormone that is under the control of the hypothalamus. The similarity between the two responses, especially in the heat, suggests that they may share common underlying neural pathways.

The observation of increased perception of exertion in the heat is consistent with reports that endurance is reduced in the heat (e.g. Galloway & Maughan, 1997), while the greater response of PRL during exercise in the heat is also consistent with previous reports (Pitsiladis *et al.*, 1998).   Combining the data in Figures 2.5 and 2.1 strongly suggests that PRL and perceived exertion are influenced by the same stimuli during exercise and this was most evident for exercise in the heat (Figure 2.6).
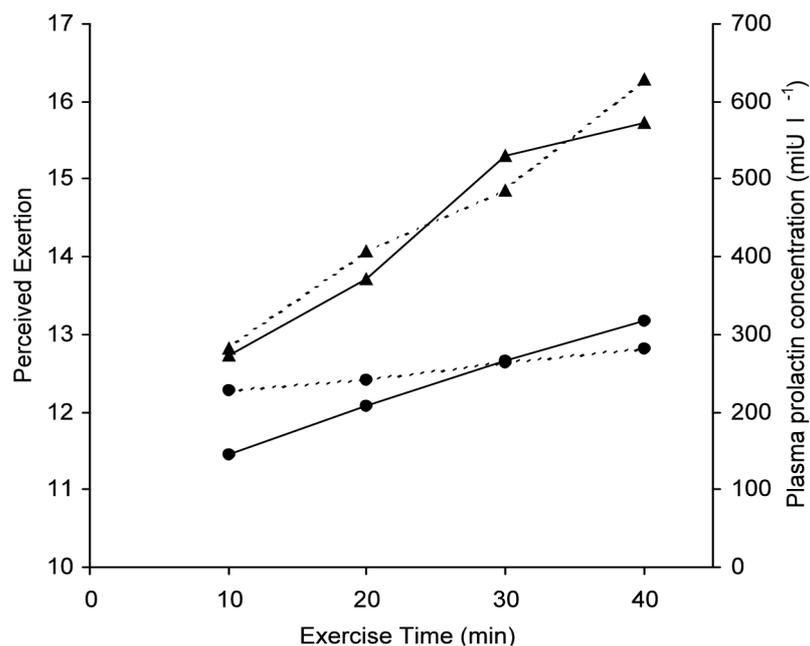
Figure 2.6. Perceived exertion (solid line) and plasma prolactin concentration (dashed line) response to exercise. ● 15°C ▲35°C Data are values from figures 2.1 and 2.5.

Since PRL secretion is controlled by the hypothalamus it would appear likely therefore that similar pathways, at least in the heat, mediate the perception of exertion. Furthermore, heat loss mechanisms are also driven from centres in the hypothalamus and it would appear that the hypothalamus may act to integrate the response to heat

load imposed by exercise (Boulant, 1996); this includes vasodilatation and sweating, to increase heat loss, while increasing feelings of effort and fatigue (indicated by RPE) have the effect of reducing activity and thereby limiting heat gain. The function of PRL release during exercise is not clear although it appears to be part of a general "stress" hormone response that includes growth hormone and ACTH.

The stimulus for both PRL secretion and the increase in rating of perceived exertion during exercise is not entirely certain. To be a likely candidate, the stimulus needs to increase steadily throughout the exercise and, at the same time, to be substantially greater in the heat compared to the cool conditions. None of the factors examined in this study individually fit this profile.

Heart rate: The 15 point Borg scale is based on the heart rate response to exercise and although it is accepted that the scale does not represent a causal relationship between heart rate and perception there could be some awareness of heart rate, either as a result of feed-back or feed-forward, that impinges on consciousness and influences perception of effort. This might also apply to ventilation, which also drifted upwards during exercise in the heat. Heart rate increased significantly throughout exercise and was different in the hot and cold conditions (Figure 2.2) and consequently has some of the attributes required for an effective stimulus. However, the heart rate drift during exercise, even in the heat, was not large, increasing from approximately 150 to 160 bpm, corresponding to one point on the Borg scale, whereas perception of exertion increased by over three points on the scale. It has been suggested that heart rate is not associated with a strong central signal of exertion (Robertson, 1982) and that the relationship between heart rate and RPE is not valid during prolonged, constant load, endurance exercise to fatigue (Garcin *et al.*, 1998). It is also difficult to see how a change in heart rate *per se* could influence PRL secretion.

Plasma lactate: Lactate has been shown to be linearly related to perceived exertion during incremental exercise (Borg *et al.*, 1987). Plasma lactate might itself directly affect perception and the neuroendocrine response to exercise or it could be a marker for metabolic changes in the muscle that stimulate muscle sensory afferents. However in terms of fulfilling the conditions required for the stimulus either for increases in perceived exertion or causing PRL release, blood lactate does not meet all

the necessary requirements. There was a significant difference between exercise in the two conditions but plasma levels were constant, or even falling, after 10 minutes when both PRL and RPE were increasing steadily.

Plasma Glucose: Hypoglycaemia has a major detrimental effect on performance and perception of exertion and infusion of glucose has been shown to reduce RPE, although only after prolonged exercise (~190 min) (Tabata & Kawakami, 1991). Glucose might, therefore, be expected to contribute to the perception of exertion during prolonged exercise. In the present experiments, however, there was no evidence of hypoglycaemia in either exercise condition and, moreover, blood glucose rose significantly in the later stages of exercise in the heat at a time when the perception of effort was increasing.

Skin Temperature: Whilst there were major differences in skin temperature between the two conditions, which fit with the major differences in perception, any suggestion that skin temperature is a sufficient stimulus for changes in perception and PRL secretion must founder on the same arguments that apply to blood lactate. Skin temperature remained constant, or even declined, during the course of the exercise at times when perception and PRL secretion were steadily increasing.

Rectal temperature: As has been discussed above, there are many indications that some aspect of body temperature plays a major role both in determining the perception of exertion and the release of PRL during exercise. Rectal temperature fulfils the criterion of a steadily increasing stimulus during the course of the exercise (especially in the heat) but did not show a significant difference between hot and cool conditions, except in the last 10 minutes of exercise.

It is evident that none of the variables discussed so far can, in isolation, explain the time course of changes in PRL and RPE together with the differences between exercise in hot and cool conditions. One explanation is that a combination of stimuli is responsible and, if it is so, deep body temperature must be a major factor since it is the only variable that continuously increased during exercise in the heat.   This stimulus needs to be modulated by another that is substantially different in the two conditions and blood lactate and mean skin temperature both fulfil this requirement.

Of the two, skin temperature seems a more likely candidate since it is known that skin temperature has a major effect in modulating the action of core temperature on sweating responses (Boulant, 1996).   In this situation, plotting PRL against rectal temperature (Figure 2.7) it would be suggested that the difference between the two relationships is explained by a modulating effect of skin temperature.
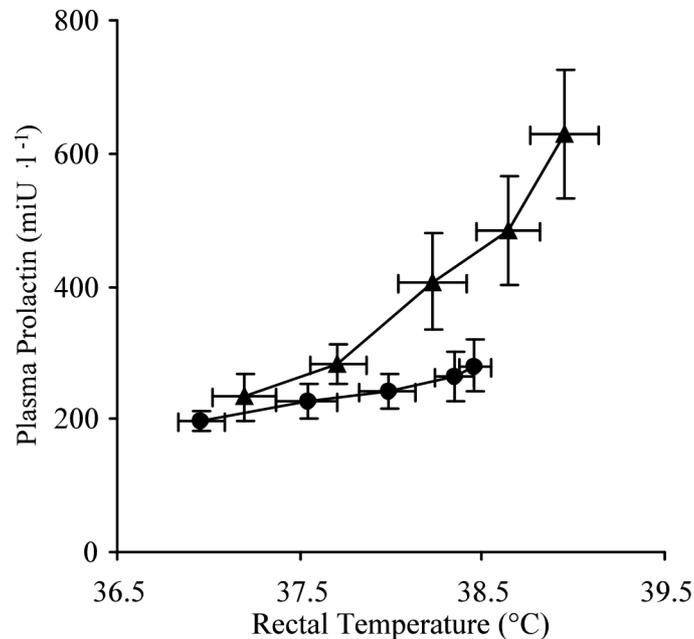
Figure 2.7. Plasma prolactin as a function of rectal temperature ●15°C ▲35°C Data are Mean±SEM

There is, however, an alternative explanation in that the rectal temperature we have measured is not a true representation of the temperature of blood perfusing the brain, which may be the unique stimulus for both RPE and PRL responses to exercise in the heat. It is recognised that there is no one measure of core temperature and it is known that rectal temperature in steady state is generally slightly higher than that measured in the oesophagus. Oesophageal temperature measures the temperature in the vicinity of the heart and thus is closer to the temperature of blood perfusing the brain than rectal temperature. At the start of exercise oesophageal temperature increases faster than rectal and will be higher than rectal but as, exercise continues and the body approaches thermal equilibrium, the two will approach one another with the rectal temperature finally becoming higher than the oesophageal n cool conditions, (Nielsen, 1962). In the heat, however, where thermal equilibrium is not achieved oesophageal temperature may rise faster and remain higher than rectal throughout the exercise (Nielsen, 1976). If this were the case the rectal temperature measured in the cool condition might be overestimating the temperature of blood perfusing the brain, whilst

in the heat, rectal temperature could be an underestimate. The effect of this on the relationship, shown in Figure 2.7 would be to draw the two lines together, possibly into a single relationship suggesting a simple stimulus, that of blood temperature entering the brain.

Further work is clearly required to clarify this last point and experiments might be undertaken with the additional measurement of oesophageal temperature or even of the blood directly perfusing the brain and in this situation it would be of great interest to measure PRL flux across the brain.

In summary, the work described in this chapter has shown a close relationship between the perception of exertion and activity of the hypothalamus, as indicated by the secretion of PRL. It is suggested that the increasing sensations of fatigue and the desire to stop exercising is part of the thermoregulatory response to heat strain, constituting the behavioural response limiting the heat gain side of the equation. The precise stimulus prompting this response remains unclear but must involve some components of body temperature. Whether there is a modulatory effect of skin temperature on the responses evoked by changes in core temperature or whether the responses are directly determined by the temperature of blood perfusing the brain, remains to be elucidated.

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Chapter 3

BODY TEMPERATURE AS A STIMULUS FOR THE PITUITARY HORMONE RESPONSE TO EXERCISE

3.1 INTRODUCTION

Exercise presents a serious challenge to the homeostasis of the body and pituitary hormones have an important part to play in regulating the supply of fuel, modulating inflammatory reactions and prompting repair of damaged tissues. Although much is known about the time course and extent of hormone release in response to exercise, the nature of the releasing signal is far from clear. Obvious candidates, such as increased blood lactate and hypoglycaemia, often show little change and remain constant during prolonged exercise while many hormone levels increase steadily with time.

The pituitary hormone response to such exercise is often described as a "stress response" and, as such, implies a common mechanism to stimulate the release of several different hormones.

Most hormones released during exercise are also increased as the result of heat exposure (Vigas *et al.*, 2000) although the mechanisms of heat induced neuroendocrine activation are not fully understood. The idea of a regulatory role of body temperature in the control of hormone release in exercise has been supported by various studies (Christensen *et al.*, 1984; Brisson *et al.*, 1986; Brisson *et al.*, 1987; Brisson *et al.*, 1991).

Prolactin release from the anterior pituitary gland has been shown to occur in response to raised core body temperature both as a result of exercise and passive heating (Brisson *et al.*, 1991). However core temperature does not seem to offer a complete explanation since the relationship between core temperature and prolactin release is modified by skin temperature, being much reduced for the same core temperature if the skin is relatively cool (Brisson *et al.*, 1986; Brisson *et al.*, 1991; Bridge *et al.*, 1999). This suggests there is a close relationship between prolactin release and thermoregulation and it is of interest to know whether other pituitary hormones are regulated in a similar way.

Growth hormone concentrations increase as a result of exercise and the extent of this release is governed by exercise intensity (Pritzlaff *et al.*, 1999) and is linearly related to core temperature during exercise (Buckler, 1972; Karagiorgos *et al.*, 1979). It has been suggested that this increase is the result of a direct stimulation of core thermoreceptors rather than cutaneous thermoreceptors (Christensen *et al.*, 1984). Growth hormone may in

fact alter skin temperature, rather than respond to it, through the activation of eccrine sweat glands which have growth hormone receptors (Lobie *et al.*, 1990). Additionally, the reduced sweat rate of growth hormone deficient patients returns to normal levels in response to growth hormone administration (Pedersen *et al.*, 1989). Exercise in the heat often results in greater acidosis and higher blood lactate concentrations and it is possible that blood lactate concentration and acidosis may play some part in the exercise induced release of growth hormone (Luger *et al.*, 1992). However, other studies have found no role for lactate in growth hormone response to exercise (Karagiorgos *et al.*, 1979). It is also possible that increased sympathetic nervous activity may be an important mediator of the growth hormone response to exercise (Pritzlaff *et al.*, 1999). The mechanisms behind the increased release of growth hormone in hot environments are therefore not clear.

Cortisol concentrations have been shown to be elevated both in exercise in the heat (Galbo *et al.*, 1979; Hargreaves *et al.*, 1996) and by passive heat exposure (Collins & Few, 1979; Moller *et al.*, 1989) and thermal clamping of body temperature abolishes the exercise induced increase in cortisol concentrations (Cross *et al.*, 1996). Cortisol has been reported to increase during swimming in water of different temperatures only if body temperature increased (Galbo *et al.*, 1979). The possible stimulus for this release is an increased core temperature, however an effect of increased skin temperature and cardiovascular demands during exercise in the heat cannot be ruled out. There is, additionally, an element of psychological stress in the plasma cortisol response to exercise in the heat (Brenner *et al.*, 1997). Cortisol concentrations can increase in the heat as the result of increases in subjective feelings of discomfort (Follenius *et al.*, 1982). It is therefore not clear whether increases in core temperature, an increased skin temperature or increased psychological stress results in the augmented cortisol response to exercise in the heat.

The roles that changes in core and skin temperature play in the augmented hormone secretion during exercise in the heat is not clear. We have, therefore, investigated the possibility that the secretion of prolactin, growth hormone and cortisol have a common release mechanism responding to changes in body temperature.

3.2   METHODS

General Design

Subjects performed two exercise tests to volitional fatigue on a cycle ergometer at ambient temperatures of 20°C or 35°C (relative humidity 30%) during which blood samples were collected every 10 min and rectal and skin temperature measured every 5 minutes. Tests were randomly assigned and balanced for order.

Subjects

Thirteen recreationally active subjects participated in the study. Their mean age, body mass and maximal oxygen consumption (VO_{2max}) were 22.5±3.3 y (Mean±SD), 71.9±5.3 kg, and 4.55±0.50 $l·min^{-1}$, respectively. The study was approved by the Local Research Ethics Committee and subjects gave their informed consent in writing.

Experimental Design

Visit 1 – maximal exercise test

Subjects completed an incremental exercise test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and VO_{2max}. Workload was increased by 35 Watts every 3 minutes until volitional exhaustion. Expiratory gases were collected and averaged over a 10 second period, using a computerised on-line system (Oxycon Alpha, Jaeger, Bunnik, The Netherlands). W_{max} was estimated using the equation of (Kuipers *et al.*, 1985):

$$W_{max} = W_{final} + (t \times W) / T$$

Where W_{final} (W) is the power of the last completed stage, t (s) is the time completed in the final stage, T (s) is the duration of each stage and W (W) is the workload increment.

Visits 2 & 3 – constant load exercise tests

On the day before visit 2, subjects recorded their diet and were then asked to adhere to the same diet on the day before visit 3. On the day of a test subjects arrived at the laboratory at either 8:00 having fasted from midnight. A cannula was inserted into an antecubital vein to obtain blood samples. To ensure that subjects began each trial euhydrated they were given a

bolus of water (8ml·kg body weight (bw)$^{-1}$) to consume during the 45 minute rest period between cannulation and the start of exercise. A resting blood sample was taken and subjects then began to exercise on a stationary electrically braked cycle ergometer. Exercise continued at a constant work rate of 65% of W_{max} and an ambient temperature of 35°C, and 30% relative humidity, until volitional fatigue. Subjects were asked to drink a minimum of 3ml·kg bw^{-1} water every 15 minutes to maintain hydration during the exercise. Heart rate was continuously recorded (Polar Vantage NV, Polar OY, Finland) and V_E, VO_2, and VCO_2 were measured every 15 min. Venous blood samples (8ml) were taken every 10 minutes during exercise for determination of haematocrit, haemoglobin, lactate, glucose, prolactin, growth hormone and cortisol. Whole body ratings of perceived exertion (RPE, Borg, 1975) were obtained every 10 minutes during exercise.

Body temperature measurements

Rectal and mean skin temperatures, the latter using the four site formula of (Ramanathan, 1964), were recorded every 5 minutes (Squirrel Meter Logger, Grant Instruments, Cambridge, UK)

Sweat rate

Subjects were weighed nude immediately before the start of exercise and at the point of fatigue after having first towelled down. Allowances were made for fluid ingested, respiratory water loss (Snellen, 1966), metabolic water loss (Mitchell *et al.*, 1972), and quantity of blood drawn, to arrive at an overall sweat loss which was divided by time to give an average sweat rate over the entire period of exercise.

Blood analysis

Haematocrit was measured in triplicate by centrifugation. Blood glucose and lactate were measured using enzyme-linked assays (Sigma Diagnostics, Poole UK); haemoglobin was measured using the cyanomethaemoglobin method (Sigma Diagnostics, Poole UK). Prolactin and growth hormone were measured using radioimmunoassays (Skybio Ltd, UK). Cortisol was measured by ELISA (DRG Instruments GmbH, Germany). All plasma samples from a single subject were assayed in the same batch.

Statistical Analysis

Changes in plasma volume were calculated from haemoglobin and haematocrit values using the equations of Dill & Costill, (1974). Data were tested for approximation to a normal distribution. Exercise data were analysed up to 40 minutes to include the maximum number of subjects and were analysed using repeated measures ANOVAs (SPSS 10) where data were normally distributed or, otherwise, a Friedman test was used. P values from the ANOVAs were corrected for sphericity using the Huynh-Feldt method, and significant differences between time points within trials were identified using tukey's post-hoc test and between trials with students paired t-tests. Significance was determined using Wilcoxon non-parametric tests if the data were found not to be normally distributed. Total hormone release was measured from the area under the curve of hormone level with time (AUC) calculated using the trapezoid method and corrected for basal values. Data are reported as mean±sem unless otherwise stated.

3.3   RESULTS

Exercise duration

Subjects exercised at the same absolute oxygen uptake in both trials ($3.04\pm0.13^{.}min^{-1}$ at 20°C; $2.99\pm0.13\ l^{.}min^{-1}$ at 35°C) which constituted a relative value of $73\pm2\%VO_{2max}$. Exercise times at 20°C ranged from 45 minutes to just over two hours (mean, 81.2 ± 8.2 min) while at 35°C they ranged from 36 minutes to 98 minutes (mean, 56.8 ± 5.4 min). The time to fatigue in the heat was significantly shorter and, on average, $73\pm5\%$ of the time to fatigue in the cool condition ($p=0.002$).

Body temperature and sweat rate

Rectal temperature increased in a similar fashion for the first 20 minutes when exercising in both the 35°C and 20°C conditions. Thereafter, rectal temperature continued to rise in the hot condition while rectal temperature was significantly lower in the cool condition (Figure 3.1a). Mean rectal temperature at the time of volitional fatigue was $38.4\pm0.2^{o}C$ at 20°C and $38.8\pm0.2^{o}C$ at 35°C ($p=0.008$).

Mean skin temperature at 35°C was higher than at 20°C, by approximately $3\text{-}4^{o}C$, throughout the exercise period ($p=0.001$).   Mean skin temperature in the heat rose significantly in the first 25 minutes of exercise ($p<0.05$, Figure 3.1b) and then remained constant. At 20°C there was an initial small drop followed by a slight rise during the course of the exercise which resulted in a value higher than the start after 30 minutes, values at fatigue were $32.0\pm0.4°C$ at 20°C and $35.2\pm0.2°C$ at 35°C (Figure 3.1b). Sweat rate was significantly higher during exercise at 35°C than at 20°C ($1.32\pm0.08 l^{.}h^{-1}$ at 20°C v $1.58\pm0.12 l^{.}h^{-1}$ at 35°C, $p=0.03$)
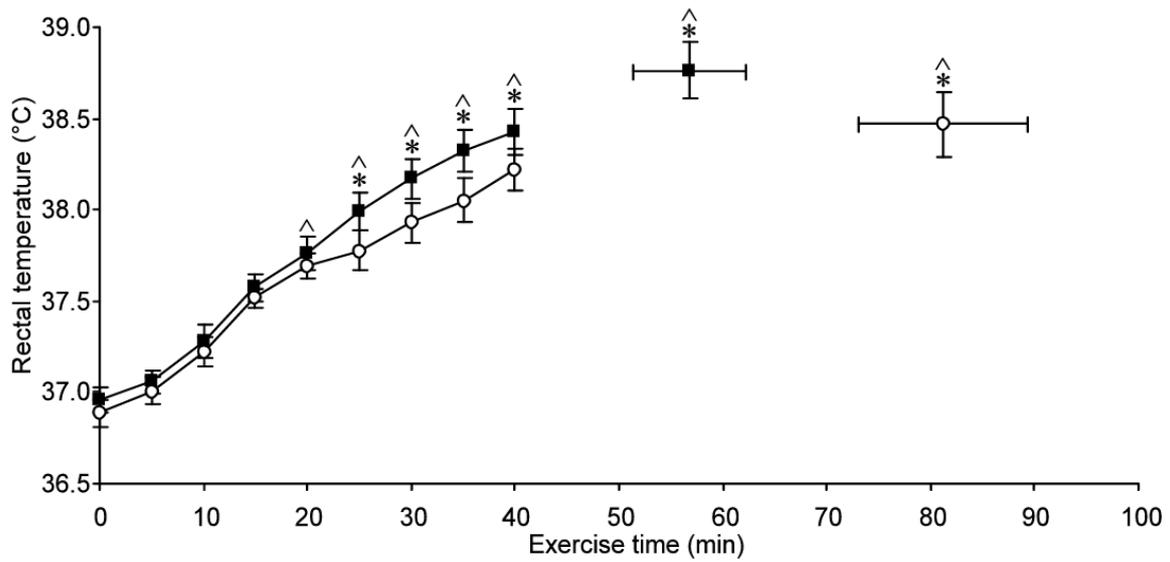
3.1a
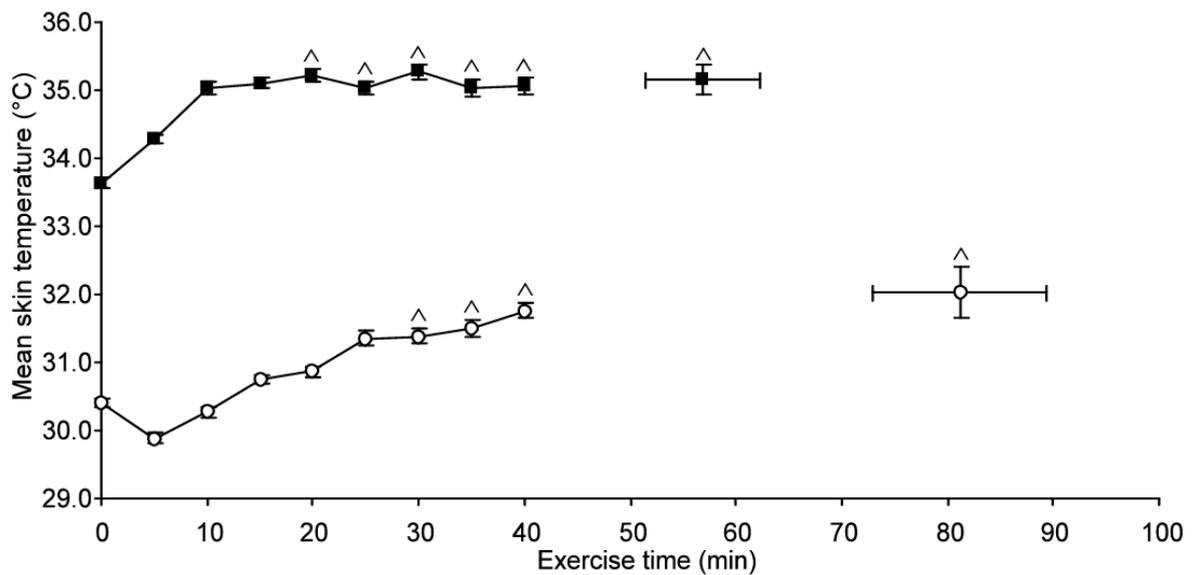
3.1b

Figures 3.1 a & b. Body temperature during exercise at 35°C (solid symbols) and 20°C (open symbols). a. Rectal temperature; b. Mean skin temperature. * indicates difference between trials $p<0.05$; ^ indicates difference from time point 0 minutes within a trial $p<0.05$. At all time points mean skin temperature was higher in the hot trial. Data are mean±SEM.

Metabolic parameters

Blood lactate increased to steady state values after the first 10 minutes of exercise in both conditions (Figure 3.2a) and there were no further significant changes from 10 minutes to the time of volitional fatigue. There was a tendency for steady state values to be slightly higher during exercise at 35°C which was reflected in blood lactate levels at fatigue of

4.3±0.9mM in the 20°C condition and 5.2±0.8mM at 35°C, however this was not a significant difference (p=0.059).
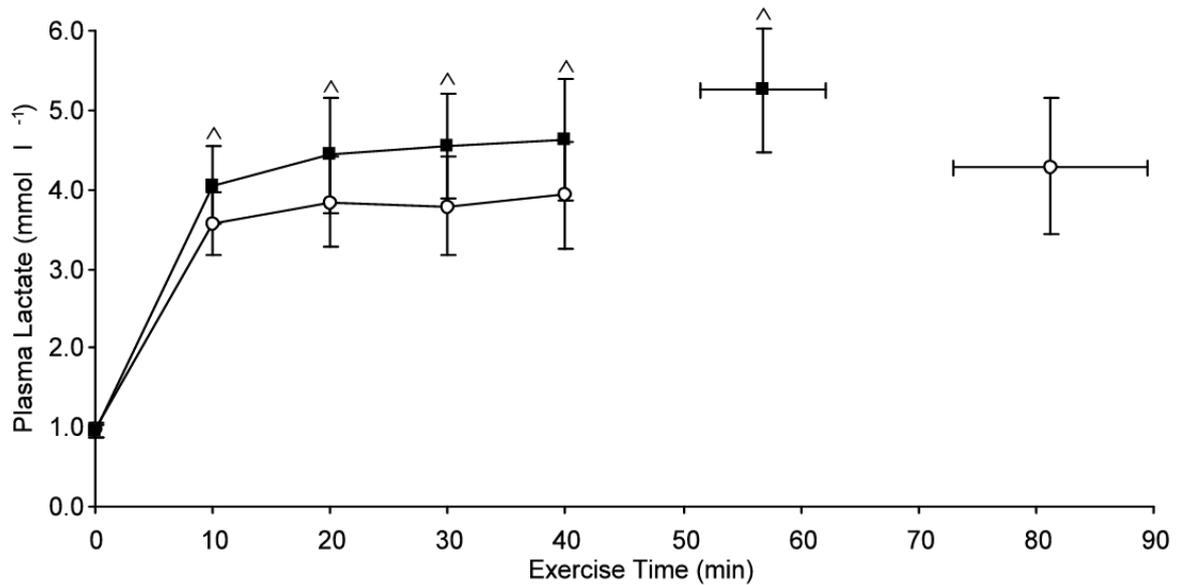
3.2a
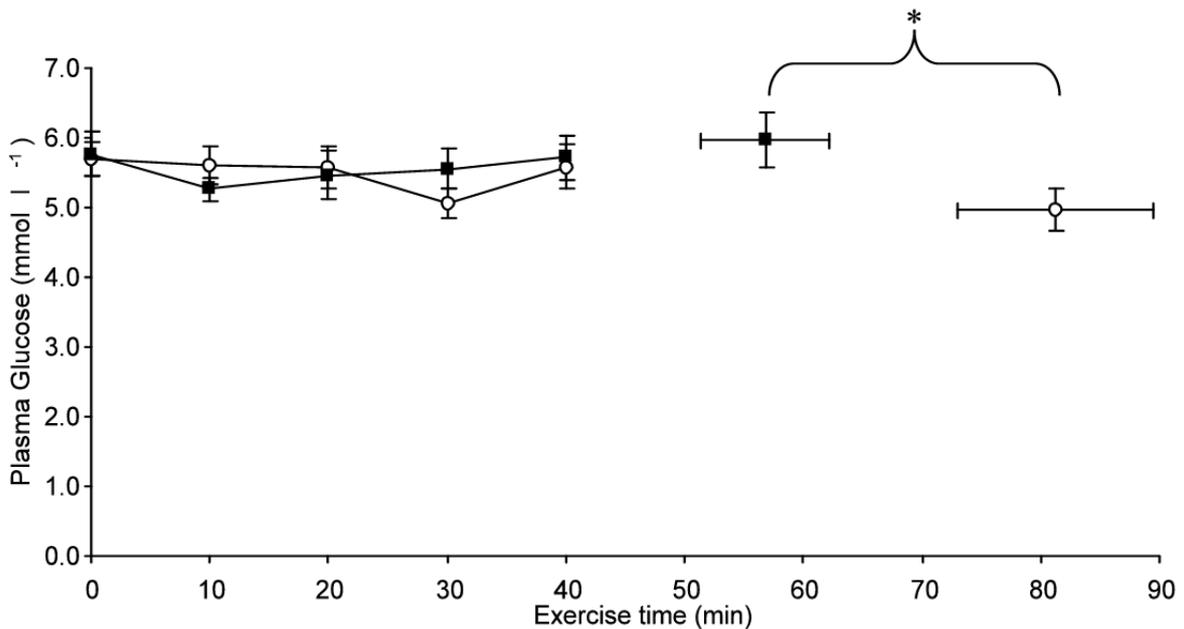
3.2b

Figure 3.2 a & b Blood metabolites during exercise at 35°C (solid symbols) and 20°C (open symbols). a. lactate; b. glucose. Bars labelled F are the values at fatigue (solid bar, hot conditions). * indicates difference between trials $p<0.05$ ^ indicates difference from time point 0 minutes within both trials $p<0.05$. Data are mean±SEM

Blood glucose (Figure 3.2b) did not differ between trials or over time in the first forty minutes of exercise, or between 10 minutes and fatigue. There was a tendency for blood

glucose to fall over the more prolonged periods of exercise at 20°C and this resulted in a significantly lower blood glucose level at fatigue at 20°C, in comparison to exercise at 35°C (4.97±0.30mM at 20°C; 5.97±0.40mM at 35°C, Figure 3.2b, p=0.046).

Cardiovascular and respiratory parameters

Heart rate increased during exercise in both trials and was significantly higher at all times during exercise at 35°C (Figure 3.3a). At 20°C, heart rate after 10 minutes was 147±3 beats·min^{-1}, increasing to 159±3 beats·min^{-1} after 40 minutes. The corresponding figures for exercise at 35°C were 154±3, beats·min^{-1} and 169±4 beats·min^{-1}. Heart rate at fatigue was significantly different between trials (169±3 beats·min^{-1} at 20°C and 174±3 beats·min^{-1} at 35°C, p=0.006) but was less than maximal heart rate determined from the maximal exercise test. No differences in haematocrit were found between trials, haemoglobin and plasma volume change were significantly different between trials only at the 40 minute time point. At fatigue no differences were found between trials in either variable (fatigue values; haematocrit 44.1±1.1% at 20°C, 45.0±0.9% at 35°C; haemoglobin 14.8±0.6 g·dl^{-1} at 20°C and 15.2±0.7 g·dl^{-1} at 35°C; change in plasma volume −2.2±1.8% at 20°C and −3.9±1.0% at 35°C).

Minute ventilation increased significantly during exercise in the 35°C condition (79.2±4.9l·min^{-1} at 15min; 83.2±5.6l·min^{-1} at 30min, p<0.05, Figure 3.3b) but no such increase over time was noted at 20°C. Initial values did not differ between trials, but by 30 minutes values at 35°C were significantly higher than at 20°C (77.9±4.6l·min^{-1} at 20°C; 83.2±5.6l·min^{-1} at 35°C, p<0.05, Figure 3.3b). No changes were seen in respiratory exchange ratio during the course of exercise in either condition and there were no differences between trials, the mean values being 0.97±0.02 at 20°C and 0.98±0.02 at 35°C.
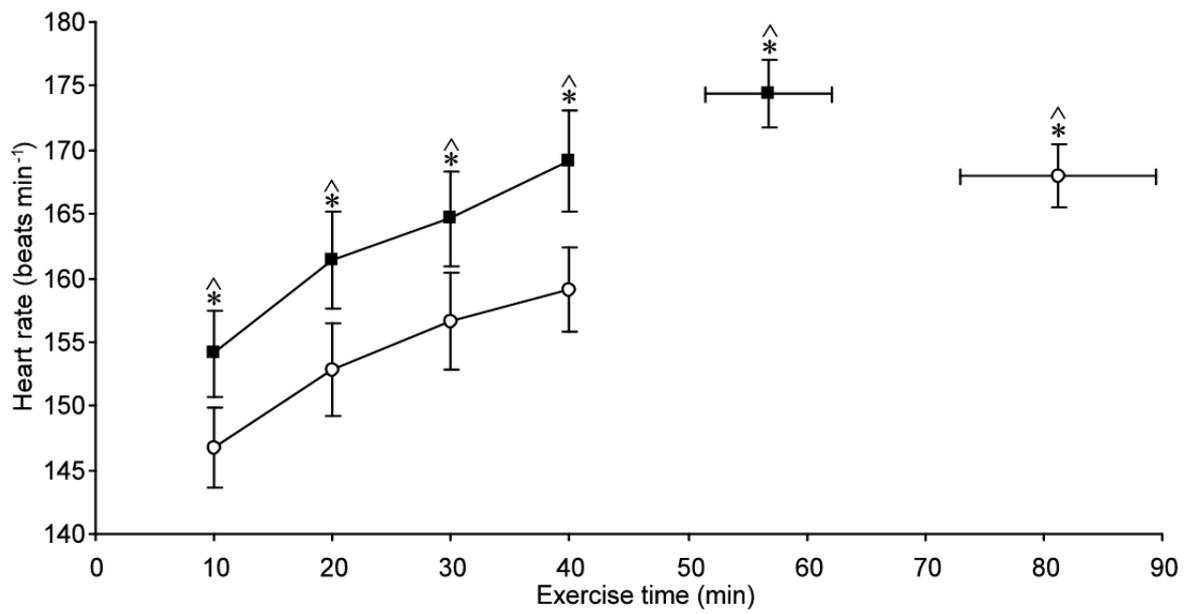
3.3a
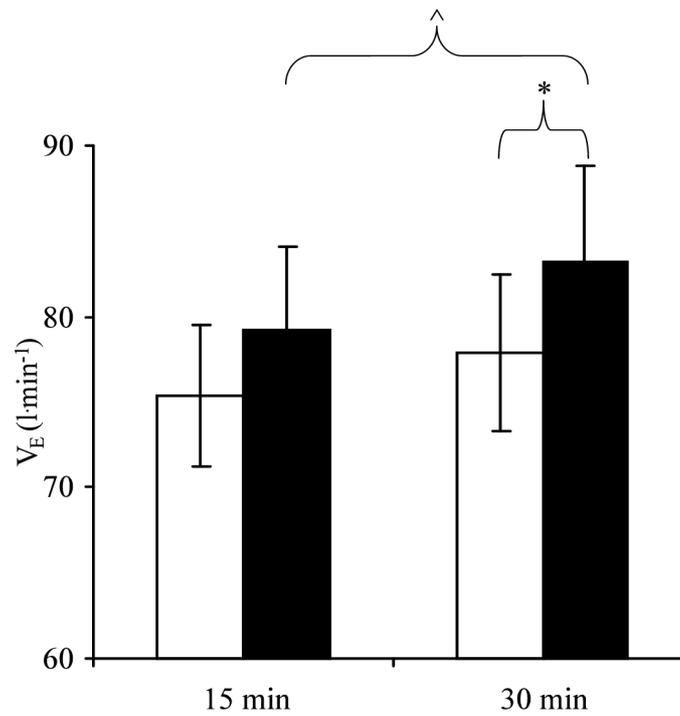
3.3b

Figure 3.3 a & b Heart rate and ventilation responses to exercise at 20°C open circles and clear bars and 35°C closed triangles and solid bars. **a** heart rate, **b** minute ventilation. * indicates difference between trials p<0.05; ^ indicates difference from other time points within trials p<0.05. Data are mean±SEM.

Blood hormone levels.

Prolactin

Changes in circulating prolactin are shown in Figure 3.4a. In the 20°C condition, there was a small increase in prolactin from 30 minutes of exercise with the values at 40 minutes and fatigue being significantly higher than resting levels. At 35°C, prolactin rose progressively and was significantly greater than resting levels after 20 minutes and greater than at 20°C from 10 minutes onwards. The differences in plasma prolactin concentrations were also reflected in the calculated area under the release curve, which was greater after 40 minutes of exercise in the hot condition (Figure 3.4a, $p<0.03$)

Growth hormone

Circulating growth hormone (Figure 3.4b) increased in a linear fashion with duration of exercise up to 30 (35°C) or 40 minutes (20°C) after which the levels begin to plateau. There were no differences between the two conditions either in values at time points during exercise or calculated area under the release curve.

Cortisol

Plasma cortisol (Figure 3.4c) changed relatively little during exercise with no differences between 35°C and 20°C conditions. There was a slight (non-significant) fall in the first 10 minutes followed by a steady, but slow, increase. However this increase was not significant in the first 40 minutes of exercise.

3.4a

3.4b

3.4c
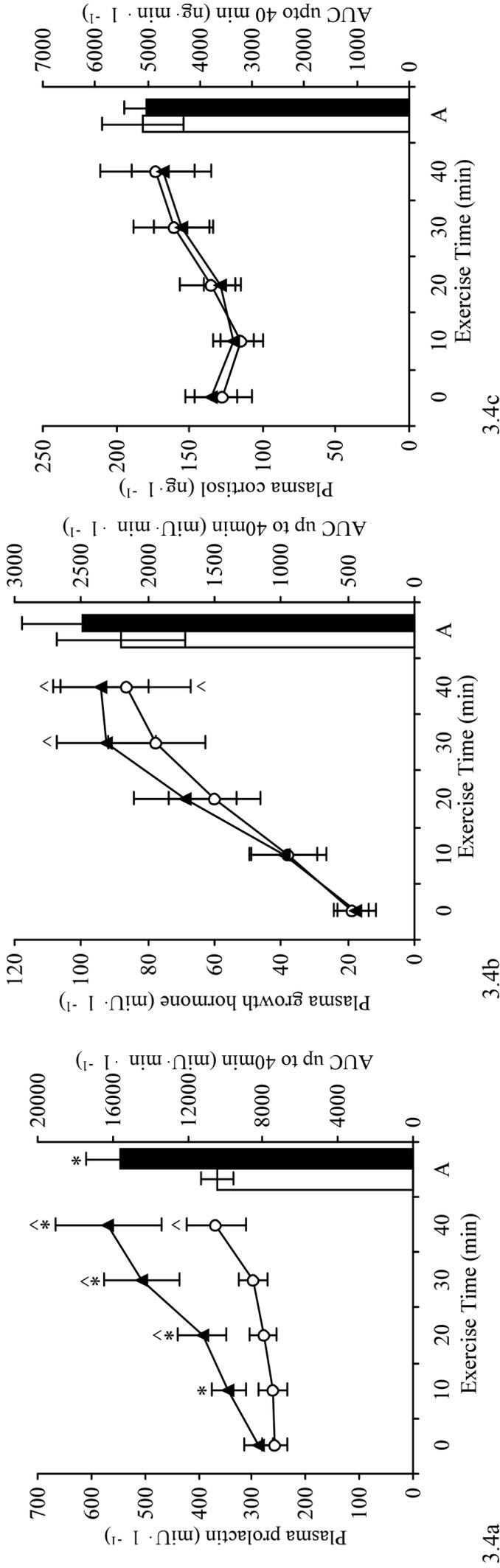
Figure 3.4. a, b & c Hormonal response to exercise at 35°C (closed symbols) and 20°C (open symbols). a. Prolactin, b. Growth hormone, c. Cortisol. Bars to the right indicate the corrected area under the curve for hormone release up to 40 minutes of exercise. * indicates difference between trials $p<0.05$ ^ indicates difference from 0 minutes time point within trial $p<0.05$. Data are mean±SEM.

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3.4   DISCUSSION

The present results are consistent with previous observations concerning the release of prolactin when exercising in hot and cool environments, indicating that ambient temperature has a major effect in modulating the response (Bridge *et al.*, 1999). The new observations are that while growth hormone release during the first 30-40 minutes of exercise shows a close relationship with rising core temperature, unlike prolactin, this relationship is similar in hot and cool conditions where skin temperature is very different. In contrast cortisol release showed very little dependence on either core or skin temperature.

Prolonged exercise above 70%VO_{2max} is demanding and subjectively reported as "stressful". This is especially true when carried out in warm, humid conditions when core temperatures can rise, sometimes, to dangerous levels (>40°C). The pituitary hormone response to such exercise is often described as a "stress response" and, as such, implies a common mechanism to stimulate the release of several different hormones. Possible stimuli arising during exercise include hypoglycaemia, acidosis and increasing body temperature. In the present study it appears most unlikely that there is a direct causal relationship between blood lactate or hypoglycaemia and release of prolactin or growth hormone. Blood glucose hardly changed with exercise duration and lactate, although elevated, remained constant after 10 minutes in both conditions. Glucose feeding studies have shown that plasma cortisol concentration is unlikely to be affected by changes in blood glucose during exercise (Bishop *et al.*, 1999; Bishop *et al.*, 2001) and is probably also unaffected by blood lactate levels.

There have been a number of studies investigating the release of prolactin both during exercise (Brisson *et al.*, 1986; Brisson *et al.*, 1991) and at rest (Brisson *et al.*, 1991) and it is clear that changes in blood osmolality and acidosis are not responsible for stimulating release (Brisson *et al.*, 1986). There is, however, considerable evidence that rising body temperature, as a result of exercise or passive warming, is important and a linear relationship between circulating prolactin and core temperature during low-intensity exercise has been reported (Brisson *et al.*, 1986; Melin *et al.*, 1988). Our own results tend to show a more biphasic relationship between rectal temperature and prolactin release (Figure 3.5a). Skin temperature is also known to influence prolactin release and the present

results confirm our previous observations (Bridge *et al.*, 1999) that ambient temperature has a major influence on prolactin release. Blood lactate was marginally higher when subjects were exercising in the heat but it is unlikely that this accounts for the different responses. Likewise, heart rate and ventilation were higher in the heat but is seems unlikely that an increase of between 7 to 10 bpm should make much difference when, in the cool condition, heart rate had increased 2.5 times above resting with very little prolactin production. Skin temperature appears to be a possible moderator of prolactin release since mean skin temperature was approximately 3-4°C lower when exercising at 20°C. Prolactin secretion is known to be stimulated by serotonergic, and inhibited by dopaminergic activity in the hypothalamus and these two pathways are also involved in temperature regulation (Yamawaki *et al.*, 1983). Heat loss mechanisms are responsive to core temperature, sensed by receptors in the brain stem and preoptic area, but are also modulated by skin temperature with the integration of these signals thought to occur in the preoptic area (Boulant, 1981). It appears, therefore that activation of prolactin release during exercise is, in a large part, driven by the rise in core temperature but that central sensitivity to such changes is altered by signals coming from peripheral thermoreceptors.

However, as mentioned in Chapter 2, there is, an alternative explanation in that the rectal temperature we have measured is not a true representation of the temperature of blood perfusing the brain, which may be the unique stimulus for both RPE and PRL responses to exercise in the heat. It is recognised that there is no one measure of core temperature and it is known that rectal temperature is generally slightly lower than that measured in the oesophagus. Oesophageal temperature measured close to the heart and aorta and thus is closer to the temperature of blood perfusing the brain than rectal temperature. At the start of exercise in cool conditions, oesophageal temperature will rise faster and become higher than rectal but as exercise continues and the body approaches thermal equilibrium the two will approach one another with the rectal temperature becoming higher than the oesophageal in the steady state of thermal equilibrium (Nielsen, 1962). In the heat, however, where thermal equilibrium is not achieved oesophageal temperature may remain higher than rectal throughout the exercise (Nielsen, 1976). If this were the case the rectal temperature measured in the cool condition might be overestimating the temperature of blood perfusing the brain, whilst in the heat, rectal temperature could be an underestimate. The effect of this would be to draw the two lines in figure 3.5a together suggesting a simple common release stimulus.

Further work is required to clarify the relationship between body temperatures and PRL release and experiments might be undertaken in which time dependent differences between rectal and oesophageal temperatures are removed, possibly by passive heating. Additionally measurements could be made of oesophageal temperature or even of the blood directly perfusing the brain and in this situation it would be of great interest to measure PRL flux across the brain.

Growth hormone release from the pituitary is clearly under complex and multiple controls. Release associated with growth and the maintenance of bone and muscle is thought to occur mainly during REM sleep in a characteristic pulsatile fashion (Van Cauter *et al.*, 1998). During exercise there is evidence that such a pulsatile release still occurs and increases in quantity of growth hormone secreted per pulse result in the rising blood concentrations that are seen (Pritzlaff *et al.*, 1999). Like prolactin, growth hormone release can be stimulated by the serotonergic 1A receptor agonist buspirone, implicating serotonergic and/or dopaminergic pathways in the release mechanism (Anderson & Cowen, 1992). The results shown in Figure 3.5b indicate a very clear relationship between growth hormone release and rectal temperature, at least up to 30 or 40 minutes of exercise, this relationship has been shown before by other authors (Buckler, 1972; Karagiorgos *et al.*, 1979; Christensen *et al.*, 1984). Different ambient conditions, however, had no effect on growth hormone release suggesting, that if central thermoreceptors are triggering growth hormone release in response to heat exposure (Christensen *et al.*, 1984), the receptors, or subsequent pathways involved, differ from those controlling prolactin since environmental temperature played no part in modulating the response. This is in line with the suggestion of Christensen *et al.*, (1984) that it is mainly the central thermoreceptors that govern the augmented growth hormone release in the heat.

There was only a modest cortisol response to exercise (Figure 3.3c) and no suggestion that the release was in any way initiated or modulated by body temperature (Figure 3.5c). This is at odds with previous findings that have shown an augmented cortisol release in the heat (Galbo *et al.*, 1979; Cross *et al.*, 1996; Hargreaves *et al.*, 1996) and may be due to the lack of difference in core body temperature during the first 40 minutes of exercise in this study compared with others (38.4±0.1°C after 40 minutes at 35°C v 39.1±0.2°C after 40 minutes at 40°C (Hargreaves *et al.*, 1996)) or the different ambient temperatures used. Cortisol is

only an indirect indicator of hypothalamic function since adrenocorticotropic hormone is the pituitary hormone which causes release of cortisol from the adrenal medulla. The cortisol response is bound to lag behind that of adrenocorticotropic hormone and it is possible therefore that a temperature effect might have been seen at times after 40 minutes. However, core body temperature at fatigue in each condition was different in this study yet no difference in cortisol concentrations were found and a number of subjects exercised for around 2 hours in the cool and 90 minutes in the heat and no effect of ambient temperature was evident in their cortisol responses. Increases in plasma prolactin concentrations are indicative of an increased serotonergic activity and although serotonergic receptor agonists are reported to release adrenocorticotropic hormone (Raap & Van de Kar, 1999), we have no evidence that this raised serotonergic activity results in an increased cortisol release through a raised adrenocorticotropic hormone concentration. Cortisol is known to respond to hypoglycaemia (Nye *et al.*, 2001) and if this is the major stimulus pathway then cortisol will be a relatively delayed response to the stress of exercise.

3.5a

3.5b

3.5c
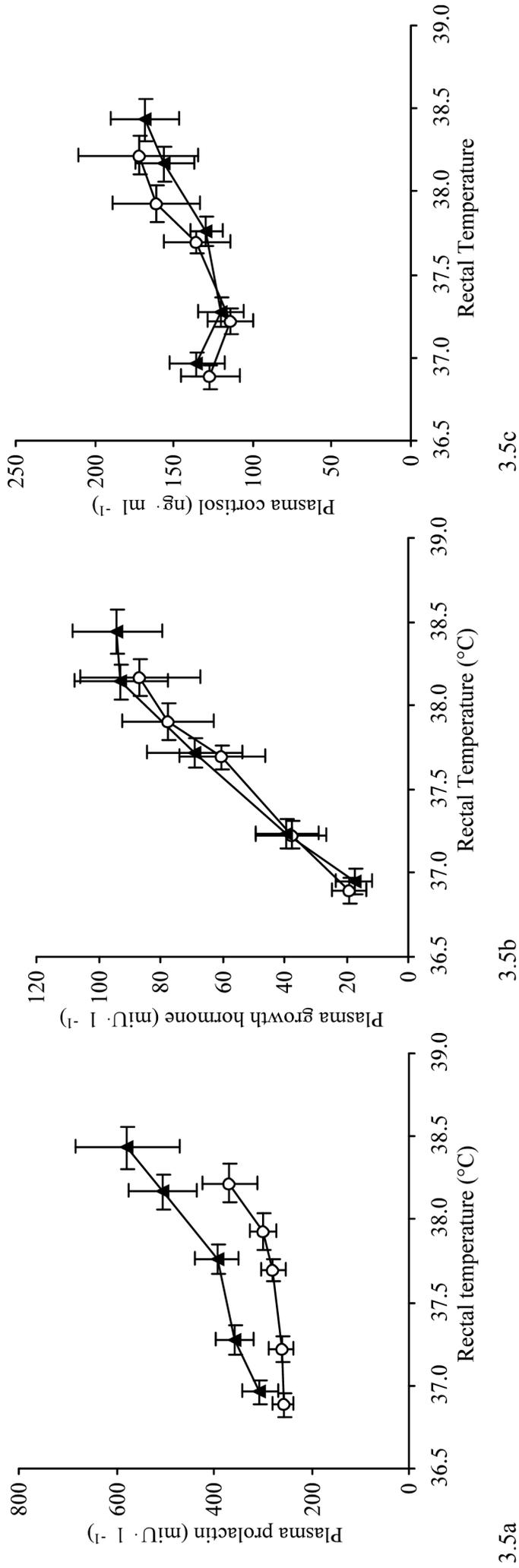
Figure 3.5. a, b & c Hormonal response to exercise in relation to rectal temperature at 35°C (solid symbols) and 20°C (open symbols). a. prolactin, b. growth hormone, c. cortisol. Data are mean±sem

Our original hypothesis was that the higher pituitary hormone response to the stress of exercise in the heat, would have a common stimulus pathway involving the integration of skin and core temperature signals. This is evidently not the case. Although body temperatures probably have an important role to play in the release of both prolactin and growth hormone their pathways must diverge in as much as ambient temperature affects them differently. Our results suggest that cortisol release appears to be controlled in a quite different fashion although the possibility of an effect of core temperature cannot be ruled out.

The conclusion that different mechanisms are responsible for the release of PRL, growth hormone and cortisol is firm regardless of the questions raised of the roles of skin and core temperature in regulating PRL release as discussed in Chapter 2.

ACKNOWLEDGMENT

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Chapter 4

QUANTIFYING THE 5-HT$_{1A}$ AGONIST ACTION OF BUSPIRONE IN MAN

Published

4.1 INTRODUCTION

Alterations in central serotonergic (5-HT) activity are implicated in a wide range of psychiatric disorders as well as in chronic fatigue syndrome. In trying to understand the underlying causes of these disorders, it is valuable to have a measure of the sensitivity of the 5-HT pathways. Multiple 5-HT receptor subtypes are thought to regulate neuroendocrine secretion in man (predominantly $5-HT_{1A}$, $5-HT_{2A}$ and $5-HT_{2C}$; Yatham & Steiner, (1993)) for which few truly selective agonists are available. Consequently a variety of 5-HT agonists of varying degrees of selectivity have been investigated as probes of serotonergic function in man (Mueller *et al.*, 1985; Anderson *et al.*, 1990; Lesch *et al.*, 1990; Kalus *et al.*, 1992; Palazidou *et al.*, 1995). Recently, and mostly for reasons of safety and availability, the partial $5-HT_{1A}$ agonist buspirone (Buspar, Bristol-Myers Squibb) has been used (Coccaro *et al.*, 1990; Bakheit *et al.*, 1992; Jakeman *et al.*, 1994; Meltzer & Maes, 1994; Sharpe *et al.*, 1996) with increases in the plasma concentration of prolactin being taken as the measure of serotonergic activity.

Buspirone is an azapirone derivative with anxiolytic and antidepressant actions (Knapp, 1985; Robinson *et al.*, 1990) that has been shown to have a high affinity for the $5-HT_{1A}$ receptor in animals (Traber & Glaser, 1987) and this action may underlie its therapeutic properties in man (Eison & Temple, 1986). Buspirone can stimulate prolactin release by the activation of post-synaptic hypothalamic $5-HT_{1A}$ receptors (Neuhauser *et al.*, 1988; Yatham *et al.*, 1989; Coccaro *et al.*, 1990) but the prolactinotrophic effects of buspirone are complicated by its dopamine D_2 receptor blocking action (McMillen *et al.*, 1983; Eison & Temple, 1986). Indeed it was originally thought that the prolactin releasing mechanism of buspirone was dopaminergic (Meltzer & Fleming, 1982). The prolactin response to buspirone may, therefore, be due to a combination of hypothalamic $5-HT_{1A}$ stimulation and pituitary D_2 receptor blockade (Meltzer *et al.*, 1983; Meltzer *et al.*, 1991; Maskall *et al.*, 1995).

Several studies have attempted to characterise the relative involvement of serotonin and dopamine receptors in the release of prolactin by buspirone. Pre-treatment with the general 5-HT antagonist compounds, metergoline (Coccaro *et al.*, 1990; Gregory *et al.*, 1990) and methysergide (Neuhauser *et al.*, 1988) abolished the prolactin response to a buspirone challenge suggesting that the action of buspirone is entirely mediated by serotonergic mechanisms. However, both metergoline and methysergide have dopamine agonist actions and so the involvement of pituitary D_2 receptors cannot be ruled out. Indeed a more recent paper by

Maskall *et al.* (1995) in which buspirone was given during maximal dopaminergic blockade found no difference in prolactin release by buspirone or placebo. Possible pituitary prolactin depletion by the dopaminergic blocker was ruled out and the authors concluded that extreme caution must be applied in interpreting the results of studies employing buspirone challenge test in terms of 5-HT sensitivity (Maskall *et al.*, 1995).

Pindolol, best known as a β-adrenoreceptor antagonist, has a high affinity for the 5-HT$_{1A}$ receptor (Hoyer, 1988). It does not appear to possess any activity at dopamine receptors (Hjorth & Carlsson, 1986) and has been shown to attenuate the neuroendocrine and behavioural responses of selective 5-HT$_{1A}$ receptor agonists (Tricklebank *et al.*, 1984; Koenig *et al.*, 1988). Pindolol has been used in man to attenuate the prolactin response to fenfluramine, a general 5-HT releasing agent (Palazidou *et al.*, 1995), and in two studies attempting to characterise the involvement of 5-HT$_{1A}$ receptors in the prolactin response to buspirone. Coccaro *et al.* (1990) reported a suppression of the prolactin response of between 49-90% whereas Anderson & Cowen (1992) found no significant difference in the total prolactin response. Interpretation of these two studies is complicated by the small number of subjects studied, differences in the dosing regimen and the consequences of high and changing baseline prolactin as a result of the stress of intravenous cannulation.

The objective of the present study was to reassess the effects of pindolol pre-treatment on the prolactin response to a buspirone challenge, using a similar dosing protocol to that employed by Anderson & Cowen (1992) whilst taking steps to avoid the complications of shifting baseline values.

4.2 METHODS

Subjects and drug administration

Thirty five healthy male volunteers (mean age 26.1 years, range 18-37 years) gave informed written consent to the study, which was approved by the local Ethics Committee. Subjects were screened with a clinical interview to exclude any previous psychiatric history and had been free of any medication for at least three weeks prior to the study.

The study was of a randomised two-way crossover design that was single blind to the subjects who all made two visits to the laboratory and received on separate occasions placebo or pindolol as a pre-treatment followed by buspirone as the drug treatment. Nine subjects underwent an additional two trials receiving on separate occasions placebo or pindolol as a pre-treatment followed by a placebo. All drugs were administered orally, encased in identical gelatine capsules.

The different trials were;

       I, placebo – buspirone (n=35)

       II, pindolol – buspirone (n=35)

       III, placebo – placebo (n=9)

       IV, pindolol – placebo (n=9)

Visits I and III were preceded by 2 days during which subjects took a placebo and visits II and IV by 2 days in which they took pindolol (10mg twice daily). This dosing regimen was chosen from the literature so that maximal post-synaptic receptor blockade would be achieved. It has previously been shown that a 20mg dose of Pindolol, without a priming dose, results in 46% post-synaptic 5-HT_{1A} receptor occupancy (Rabiner *et al.*, 2000). Whilst this is by no means a total blockade of post-synaptic receptors, the results also show that the occupancy is dose dependant (Rabiner *et al.*, 2000). It is therefore likely that with the priming dose given on the two days before the buspirone challenge and the relatively high dose given an hour before the buspirone (37 ± 5mg, $0.5\text{mg}^{\cdot}\text{kg}^{-1}$ body mass) in this study, that a higher 5-HT_{1A} receptor occupancy has been achieved. Indeed Rabiner *et al.* (2000) suggest that a 30mg dose would be enough to fully block the functional responses to Buspirone.

Neuroendocrine challenge

Subjects arrived at the laboratory at 8am following an overnight fast, although water was allowed *ad libitum* both during the fast and throughout the experiment. Following a 15 min rest a 21G cannula was inserted into a superficial forearm vein and kept patent with saline (Baxter 0.9%). After a further 45 min rest, a baseline blood sample (3ml) was taken and the subject ingested either placebo for treatment I and III, or pindolol (0.5 mg·kgbw^{-1}) for treatments II and IV. Serial blood samples were then taken at 15 min intervals for one hour. Following ingestion of either placebo or buspirone (0.5 mg·kgbw^{-1}; Bristol-Myers Squibb) at this time, further blood samples were withdrawn every 15 min for the next 150min. Subjects were asked to report side-effects on a scale of 1-10 with 5 being normal lower than 5 'less than normal' and higher than 5 'more than normal'. Categories were Light-headed, Nausea, Dizzy, Hot, Cold, Sleepy, Fatigued, Clammy skin, Sweaty and Aches; there was additional space for other comments. Subjects rested in a semi-supine position throughout the experiment but were not allowed to sleep. This protocol was repeated at weekly intervals with subjects receiving a different treatment regimen on each visit. Treatments were randomised and balanced for order. Venous blood samples were left to clot for 20 minutes and serum was separated by centrifugation, stored at -70°C and analysed within 3 months for serum PRL using a radioimmunoassay (Chelsea Kits, Hammersmith Hospital, London). Average inter- and intra-assay coefficients of variation over the whole range were 5.9% and 2.7% respectively. All plasma samples from one subject were assayed in the same batch by one of the authors who was blinded to the study conditions.

Data and Statistical Analysis

The hormone data at each time point approximated a normal distribution and are presented as means and SEM in the figures. The prolactin response was calculated as the area under the curve (AUC) using the trapezoid method with subtraction of the baseline area. The area under the curve was not normally distributed and these data are therefore reported as median and inter-quartile range. Significance was determined using Wilcoxon matched-pairs, signed rank test.

4.3   RESULTS

Effects of pindolol on basal prolactin

In all nine subjects treatment IV pindolol - placebo (10mg twice daily for 2 days, 0.5mg.kgbw^{-1} on test day) resulted in a reduction in the mean serum prolactin compared to treatment III placebo – placebo at all time points after the 0.5 mg·kgbw^{-1} dose was taken (Figure 4.1). The area under the curve reduced from a median of 27798 to 22840 miu·min·l^{-1} which constituted a median reduction of 15% (IQ Range 12-23%, $p<0.01$, two-tail). There was a gradual decline in prolactin over time with both treatments but the rate of decline following pindolol was double that following placebo (26 miu·l^{-1}·hr^{-1} and 13 miu·l^{-1}·hr^{-1} respectively for pindolol and placebo) and this was particularly evident in the first 90 minutes after pindolol was administered.
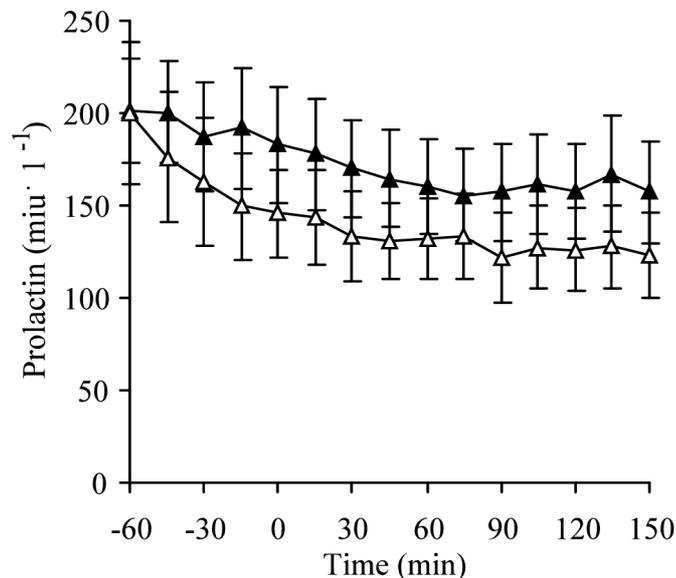
Figure 4.1 The effect of pindolol treatment on tonic prolactin release. Serum prolactin response to treatments III (placebo-placebo, ▲) and IV (pindolol-placebo, △). Pindolol or placebo were given at -60 min and placebo at 0 min.   Data are mean ± s.e.m.

Prolactin response to a buspirone challenge

Treatment I (placebo – buspirone) gave a robust prolactin response (Figure 4.2). The peak prolactin concentration occurred 75 min (60-90 min) after drug administration and reached 317% (IQ Range 213-522%) of the basal level. Prolactin returned to basal levels within about 2.5 hours after buspirone administration. Prolactin area under the curve, following buspirone treatment, and after subtraction of the baseline, was 33267 miu·min·l^{-1} (IQ Range 17228-60690).
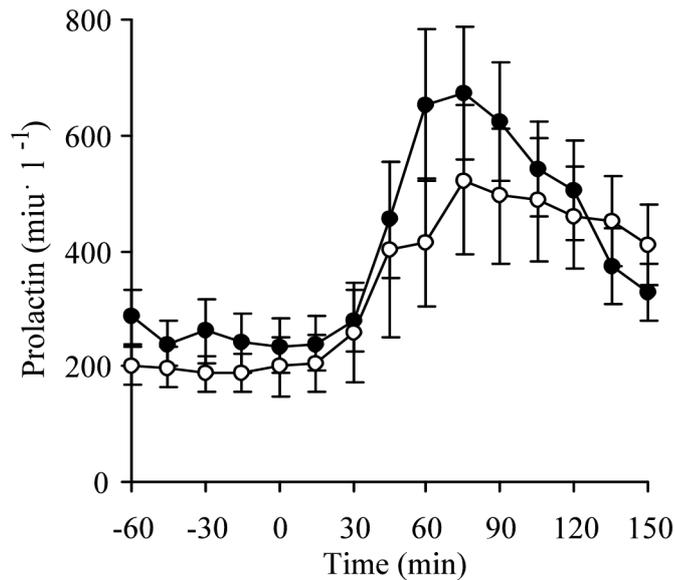
Figure 4.2 The effect of pindolol treatment on the prolactin response to buspirone. Serum Prolactin response to treatment I (placebo-buspirone, ●) and II (pindolol-buspirone, ○). Pindolol or placebo were given at -60 min and buspirone or placebo at 0 min.   Data are given as mean ± s.e.m.
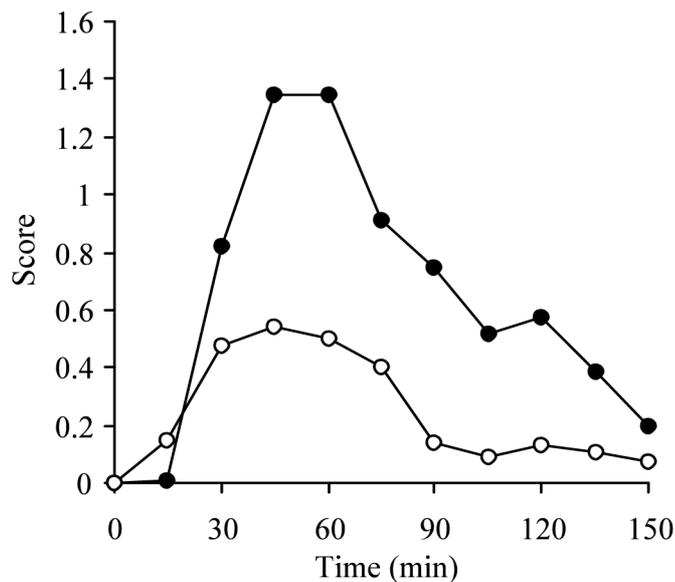
Figure 4.3   Subject side-effects to buspirone. Subject negative side-effects (light-headed, nausea, dizzy, sleepy, fatigued) after correction for basal responses, a positive score reflects a negative feeling. Pindolol – buspirone ○, and placebo - buspirone ●. Data are given as mean.

Oral buspirone was generally well tolerated by the subjects. The most commonly reported side-effect was a light-headed or dizzy feeling typically 45-60 minutes after administration but this was generally not reported as unpleasant. These feelings were often accompanied by nausea. An average of negative side-effects (light-headed, nausea, dizzy, sleepy, fatigued) after correction for basal values is reported in Figure 4.3. When looking at Figures 4.2 and 4.3 together it can be seen that subject reporting of side-effects mirrored the change in prolactin in

the blood. However the magnitude of these side-effects varied from subject to subject and those with the highest blood prolactin levels did not necessarily report the worst side-effects.

Effects of pindolol pre-treatment on the buspirone challenge

Pindolol treatment attenuated the prolactin response to the buspirone challenge (treatment II, pindolol – buspirone, Figure 4.2) in all subjects and to varying extents. Peak values were reduced by about 50% and the area under the curve was reduced by a median value of 52%, with an IQ Range of 22-82%.
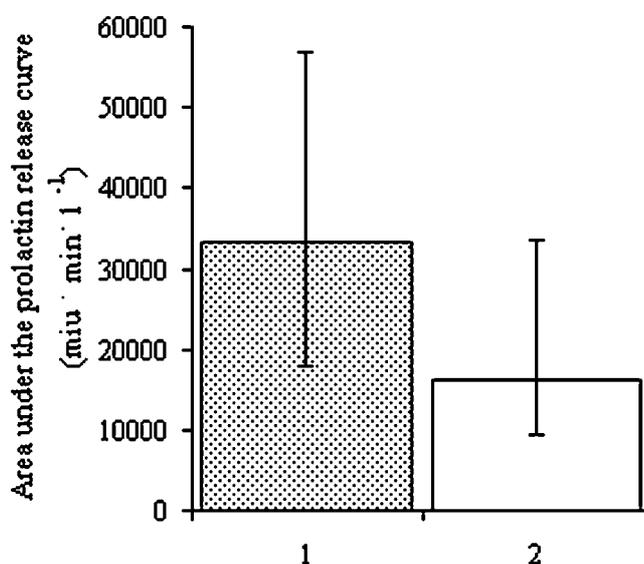
Figure 4.4 The components of the prolactin response to buspirone. The total response (pindolol-sensitive and pindolol-insensitive, shaded bar) and non 5-HT$_{1A}$ (pindolol-insensitive, open bar) components of the buspirone response. Data for the pindolol-insensitive components were obtained by subtracting the baseline corrected area under the curve of treatment II from that of treatment I. Values shown are medians and interquartile ranges.

Subtracting the prolactin response to buspirone in the presence of pindolol from the response to buspirone alone, as shown in Figure 4.2, gives the component of the buspirone-mediated prolactin response that was not attributable to its 5-HT$_{1A}$ agonist action (i.e. the pindolol-insensitive component). The two components (pindolol- sensitive and -insensitive) are shown in Figure 4.4. The pindolol-insensitive component of the prolactin response was later in onset with, on average, a peak at 105 min (IQ Range 75-130min, $p<0.05$) compared to the derived pindolol-sensitive component, which peaked at 105 min (IQ Range 70-130min). The pindolol-insensitive component peaked significantly later than the pindolol-sensitive component ($p<0.05$), the time to peak varied considerably and in eight subjects the order of the peaks was reversed. There were no obvious relationships between the magnitude of the total prolactin response (buspirone alone) and the proportions of the two components or the timing of the

peaks. No adverse effects were reported of the pindolol treatment alone. Pindolol treatment resulted in a lower severity of side-effects reported in the majority of subjects (Figure 4.3) whilst the reporting of side-effects still mirrored the change in prolactin in the blood. No subject exercised their right to withdraw from the study.

4.4 DISCUSSION

The investigations described here demonstrate the dual control of prolactin secretion. While the tonic prolactin levels were found to be predominantly (~82%) under the control of non 5-HT_{1A} pathways, the response to a buspirone challenge was, on average, attributable in equal measure to the two pathways.

The prolactin response to buspirone administration ($0.5\text{mg}^{.}\text{kg}^{-1}$ body mass) was clear and robust in all subjects as has been reported in a number of other human studies (e.g. Meltzer *et al.*, 1983; Coccaro *et al.*, 1990; Dinan *et al.*, 1990; Anderson & Cowen, 1992). Although all subjects demonstrated a prolactin response to buspirone, there was considerable inter-subject variation in terms of both magnitude and time to peak. Reasons for this variability are not clear, but buspirone undergoes extensive first-pass metabolism (Gammans *et al.*, 1986) which could differ between subjects, alternatively the variable responses might reflect genuine differences in central serotonergic sensitivity.

The prolactin response to a buspirone challenge has been used as an index of central serotonergic activity (e.g. Bakheit *et al.*, 1992; Jakeman *et al.*, 1994; Sharpe *et al.*, 1996) but it is clear that this ignores the complex actions of the drug. In addition to its 5-HT_{1A} agonist properties, buspirone is known to influence dopamine pathways (McMillen *et al.*, 1983; Eison & Temple, 1986) and is metabolised to 1-(2-pryrimidinyl)-piperazine, an α_2-adrenoceptor antagonist (Gammans *et al.*, 1986; Bianchi & Garattini, 1988). Pindolol treatment is one way of dissecting out the 5-HT_{1A} actions of buspirone from those mediated by other pathways.

The interpretation of the results presented here rests first on the selectivity and potency of the 5-HT_{1A} antagonist action of pindolol and secondly on the lack of any complicating actions. Pindolol has been shown to have a high affinity for 5-HT_{1A} receptors (Hoyer, 1988) and does not appear to possess any activity at dopamine receptors (Hjorth & Carlsson, 1986). Additionally PET scanning has shown that a 20mg dose of Pindolol, without a priming dose, resulted in 46% post-synaptic 5-HT_{1A} receptor occupancy (Rabiner *et al.*, 2000). Whilst this is by no means a total blockade of post-synaptic receptors, the results also show that the occupancy is dose dependant (Rabiner *et al.*, 2000). It is therefore likely that with the priming dose given on the two days before the buspirone challenge and the relatively high dose given an hour before the buspirone ($37\pm5\text{mg}$, $0.5\text{mg}^{.}\text{kg}^{-1}$ body mass) in this study, that a higher 5-HT_{1A}

receptor occupancy had been achieved. Indeed Rabiner *et al.* (2000) suggest that a 30mg dose would be enough to fully block the functional responses to Buspirone.

Pindolol is probably best known for its β-adrenoceptor antagonist action (Aellig, 1976) and the β-adrenoceptor antagonist propanolol has been reported to increase the prolactin responses to pharmacological challenges (Laakmann *et al.*, 1986). It is possible that pindolol may have a similar action but since pindolol has been found to consistently reduce prolactin levels, any stimulatory action will have led to an underestimation of the size of the 5-HT_{1A} component rather than falsely attributing this component to serotonergic activity. Pindolol, however, does have some intrinsic sympathomimetic activity (Aellig, 1976) and it is conceivable that pindolol could act in part, and/or in some individuals, as a β-adrenoceptor agonist which could lead to a reduction in prolactin release via this mechanism. This partial β-adrenoceptor agonist action of pindolol can not be ruled out when offering an explanation of the effects of pindolol on basal prolactin release although the β-adrenoceptor antagonist actions of pindolol are more likely to dominate (Aellig, 1976).

The observed reduction in prolactin release in both tonic and stimulated conditions after pindolol pre-treatment is consistent with previous findings (Park & Cowen, 1995; Meltzer & Maes, 1996) and supports the involvement of 5-HT_{1A} receptors in the release of prolactin. However, the relatively small pindolol-induced reduction of the basal levels (18%, Figure 1) suggests that tonic release of prolactin has only a small serotonergic stimulated component. Anderson and Cowen (1992) reported no reduction in tonic prolactin release following pindolol pre-treatment but only 3 baseline samples were taken following intravenous cannulation and it is possible that the elevated and changing baseline as a consequence of cannulation may have masked a small reduction in tonic prolactin as a result of pindolol treatment.

Pindolol pre-treatment reduced the prolactin response to the buspirone challenge in all subjects confirming the involvement of post-synaptic hypothalamic 5-HT_{1A} receptors in this neuroendocrine challenge. The prolactin response to the buspirone challenge that was not sensitive to pindolol can, in part, be attributed to the antagonist actions of buspirone at pituitary D_2 receptors (Meltzer & Fleming, 1982; Anderson & Cowen, 1992; Meltzer *et al.*, 1992; Maskall *et al.*, 1995) although other 5-HT receptor subtypes, for which pindolol has little affinity, have also been implicated in the control of basal prolactin secretion (Falaschi *et al.*, 1991).

The reporting of side-effects in response to buspirone administration mirrored the rise and fall in blood prolactin concentration in treatments I and II. With prior pindolol administration the severity of side-effects was reduced roughly in proportion to the reduced prolactin. This reduction after pindolol administration has not, to our knowledge, been reported before but would be expected if serotonergic pathways are involved. It is though difficult to draw any conclusions at to the cause of the reduction.

The present results suggest that approximately half the total prolactin response to buspirone could be attributed to 5-HT_{1A} agonist actions and the other half non 5-HT_{1A} agonist actions including putative D_2 antagonist activity. Coccaro *et al.* (1990) found a significant blunting of the prolactin response to buspirone by pindolol pre-treatment (5-20mg) in three subjects but the study lacked placebo control and led Meltzer *et al.* (1992) to conduct a further study using pre-treatment with 30 mg pindolol and a challenge with 30mg buspirone. These authors found no significant difference between the prolactin response to buspirone with or without pindolol pre-treatment, although the data showed a delay in the onset and peak after pindolol pre-treatment. The difference between these results and the present work may be due, in part, to the relatively low dose of buspirone and problems with the raised baseline, as mentioned before. In our experience, and from the work of others, 30mg buspirone produces only a modest prolactin response in healthy male subjects.

There was a large inter-subject variation in the extent to which pindolol blocked the prolactin response to buspirone in the current study. The most obvious explanation is that the relative importance of serotonergic and dopaminergic pathways controlling prolactin release differs between individuals and may depend on factors such as receptor density and sensitivity. Another possible explanation is a pharmacokinetic interaction between pindolol and buspirone, such that pindolol may lower plasma buspirone levels. We were not able to measure plasma buspirone concentrations in the present study and therefore cannot exclude this possible mechanism.

It is notable that, with several exceptions, the peak of prolactin release due to the non 5-HT_{1A} agonist actions of buspirone occurred later than that due to the 5-HT_{1A} agonist action this confirms the findings of Anderson & Cowen (1992). This could be due to an action of pindolol slowing the absorption of buspirone, which in turn may lead to its increased metabolism. It can therefore not be ruled out that with pindolol pre-treatment there may be a greater prolactin

release caused by the Buspirone's principle active metabolite 1-(2-pyrimidinyl)-piperazine (1PP) which results in the later peak on the release curve.

In conclusion, it would appear that the results of a challenge with buspirone alone must be interpreted with considerable care since a substantial, and variable, part of the prolactin response is due to pathways other than those mediated by 5-HT$_{1A}$ receptors. However, two challenges, one with buspirone alone, the other in the presence of pindolol, provides information about the relative sensitivities of both 5-HT$_{1A}$ and non-5-HT$_{1A}$ components and thus adds another dimension to the neuroendocrine challenge. It is possible that, in addition to differences in the absolute activity of the hypothalamic pathways, there may be changes in the relative importance of various pathways in different disorders or as a result of training or adaptation to changing environments.

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Chapter 5

RESPONSES TO EXERCISE IN THE HEAT RELATED TO MEASURES OF NEUROENDOCRINE FUNCTION

5.1 INTRODUCTION

The ability to undertake strenuous physical work at high ambient temperatures is an important attribute in many occupations as well as in sport and leisure activities. Heat tolerance varies between individuals and there is considerable practical importance in being able to detect individuals who may be heat intolerant, either as a result of an abnormal tolerance of increased body temperature, or due to impaired heat loss mechanisms.

As core temperature rises there is an increasing reluctance of subjects to continue working and this is thought to be a reflex inhibition (Bruck & Olschewski, 1987), probably arising in the hypothalamus or brain stem, and may involve serotonergic (5-HT) pathways that project to higher centres. Increasing core temperature, either passively through exposure to high ambient temperature at rest, or actively through exercise, increases central 5-HT activity (Hori & Harada, 1976b, a; Bridge *et al.*, 1999). Hypothalamic 5-HT and dopaminergic activity have both been implicated in the control of thermoregulation (Cox *et al.*, 1980) and are thought to thermoregulatory responses such as vasodilatation (Cox *et al.*, 1980). Additionally, activity of these pathways results in an increased release of neuroendocrine hormones (Meltzer *et al.*, 1983) and possibly behavioural changes that result in a loss of motivation to continue exercise. Differences in heat tolerance between individuals could be due to intrinsic differences of function of these hypothalamic pathways, through differences in the sensitivity (receptor density) or activity (neurotransmitter release for a given stimulus), giving rise to different thermoregulatory and/or behavioural responses to a given thermal load.

Hypothalamic activity cannot be assessed directly in man but the release of prolactin, which is stimulated by 5-HT receptors and inhibited by dopaminergic D_2 receptors, is often taken as an indirect measure. Drugs that directly, or indirectly, produce activation of 5-HT receptors increase plasma concentrations of prolactin. Data from a range of human and animal studies suggests that the serotonergic neurones of the dorsal raphe nucleus project to hypothalamic sites to stimulate prolactin secretion through activation of 5-HT receptors (Van de Kar *et al.*, 1996). Changes in prolactin levels in the blood therefore provide a useful marker for changes in central 5-HT activity as 5-HT is a prominent excitatory neurotransmitter for prolactin release (Struder & Weicker, 2001)

Circulating plasma prolactin concentrations rise during fatiguing exercise, largely in response to increases in core and skin temperature (Brisson *et al.*, 1986; Brisson *et al.*, 1991; Bridge *et al.*, 1999). A common technique used to assess hypothalamic 5-HT sensitivity in healthy subjects, is to measure the blood prolactin response to a neuroendocrine challenge with buspirone (Meltzer *et al.*, 1983; Anderson & Cowen, 1992; Bakheit *et al.*, 1992; Jakeman *et al.*, 1994; Sharpe *et al.*, 1996; Bridge *et al.*, 2001). (Jakeman *et al.*, 1994) found a reduced prolactin response to buspirone in highly trained endurance athletes compared to healthy controls. It has been suggested that a higher aerobic fitness provides an advantage during exercise in the heat with an improved ability to tolerate a high core temperature at exhaustion (Selkirk & McLellan, 2001). It is interesting therefore to investigate if a reduced prolactin response to buspirone is associated with an improved exercise tolerance in the heat.

Buspirone is primarily used as a 5-HT$_{1A}$ receptor agonist but it also has D$_2$ antagonist activity and thus causes the release of prolactin through these two actions (Eison & Temple, 1986). We have recently shown that it is possible to separate these actions by comparing the response to buspirone in the presence and absence of pindolol (Bridge *et al.*, 2001). Pindolol blocks 5-HT$_{1A}$ receptors and thus the prolactin response to buspirone in the presence of pindolol gives a measure of non-5-HT$_{1A}$ activation which is mainly due to D$_2$ receptor antagonism (Eison & Temple, 1986).

There is considerable evidence from animal studies to support a role for dopamine in the control of body temperature and regulation of heat loss (see Lee *et al.*, 1985 for review) and there has been a recent report of increased dopamine in the preoptic area and anterior hypothalamus of rats in response to raised body temperature (Hasegawa *et al.*, 2000). If hypothalamic activity is important in determining heat loss and therefore work capacity in the heat, it might be possible to predict exercise tolerance in the heat from a test of hypothalamic neuroendocrine function. The main purpose of this study was to evaluate this possibility.

We have assessed exercise tolerance at a high ambient temperature compared to the response to a neuroendocrine challenge with buspirone with and without pindolol. Our hypothesis was that sensitivity of 5-HT and dopaminergic pathways in the hypothalamus,

as assessed by a neuroendocrine challenge, are predictors of exercise tolerance when working in the heat.

5.2   METHODS

General Design

Subjects performed an exercise test to volitional fatigue on a cycle ergometer at an ambient temperature of 35°C (relative humidity, 30%) during which blood samples were collected every 10 min and rectal and skin temperatures measured every 5 minutes. The exercise tests were followed (on separate occasions) by two neuroendocrine challenges with buspirone, one of which was given in the presence of pindolol to block $5-HT_{1A}$ activity.

Subjects

Twelve recreationally active and healthy subjects participated in the study. Their mean age, body weight and maximal oxygen consumption (VO_{2max}) were, 22.9 ± 3.6 years (Mean $\pm$ SD), 72.8 ± 5.8 kg, and 4.21 ± 0.55 $l min^{-1}$, respectively. For the neuroendocrine challenges subjects were screened with a clinical interview to exclude any psychiatric history and to ensure they had been free of any medication for at least three weeks prior to the study. The study was approved by the Local Research Ethics Committee and subjects gave their informed consent in writing.

Experimental Design

Visit 1

Subjects completed an incremental exercise test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine maximal aerobic power output (W_{max}) and VO_{2max}. Workload was increased by 35 Watts (W) every 3 min until volitional fatigue. Expired gases were analysed and averaged over a 10 second period, using a computerised on-line system (Oxycon Alpha, Jaeger, Bunnik, The Netherlands). W_{max} was estimated using the following equation from (Kuipers *et al.*, 1985):

$$W_{max} = W_{final} + (t \times W) / T$$

Where W_{final} is the power (W) of the last completed stage, t is the exercise time (s) during the final uncompleted stage, T is the duration (s) of each stage and W is the workload (W) increment for each stage.

Visit 2

Subjects arrived at the laboratory at either 08:00 hours, having fasted from midnight, or at 13:00 hours having fasted for the previous 4 hours. A cannula was inserted into an antecubital vein to obtain blood samples. To ensure that subjects began each trial euhydrated they were given a bolus of water (8ml$\cdot$kg body weight (bw)$^{-1}$) to consume during the 45 min rest period between cannulation and the start of exercise. A resting blood sample was taken and subjects then began to exercise on the cycle ergometer. Exercise continued at a constant work rate of 65% of W_{max} (216±11W) and an ambient temperature of 35°C, and 30% relative humidity, until volitional fatigue. Subjects were asked to drink a minimum of 3ml$\cdot$kgbw^{-1} water every 15 minutes to maintain hydration during the exercise. Heart rate was continuously recorded (Polar Vantage NV, Polar OY, Finland). Venous blood samples (5ml) were taken every 10 minutes during exercise for determination of haematocrit, haemoglobin, lactate and glucose. Whole body ratings of perceived exertion (RPE, Borg, 1975) were obtained every 10 minutes during exercise.

Body temperature measurements

Rectal and mean skin temperature, the latter calculated using the four-site formula of (Ramanathan, 1964), were recorded every 5 minutes (Squirrel Meter Logger, Grant Instruments, Cambridge, UK).

Sweat rate

Subjects were weighed nude immediately before the start of exercise and at the point of fatigue after having first towelled down. Allowances were made for fluid ingested, respiratory water loss (Snellen, 1966), metabolic water loss (Mitchell *et al.*, 1972) and quantity of blood drawn to arrive at an overall sweat loss which was divided by time to give an average sweat rate over the entire period of exercise.

Neuroendocrine challenges visits 3 & 4

Subjects each made two visits to the laboratory and received placebo or pindolol as the pre-treatment followed by buspirone as the drug treatment (Challenge I and II, respectively). All drugs were administered orally, encased in identical gelatine capsules. Challenge I was preceded by 2 days during which subjects took a placebo and for the 2 days before Challenge II they took pindolol (10mg twice daily). Challenges I and II were randomised and balanced for order and were single-blind to the subjects in respect to pindolol.

Subjects arrived at the laboratory at 08:00 hours following an overnight fast although water was allowed *ad libitum* during the fast and throughout the challenge. After a 15 min rest a 21G cannula was inserted into a superficial forearm vein and kept patent with saline (Baxter 0.9%). After a further 45 min rest, a baseline blood sample (3ml) was taken and the subject ingested either placebo for Challenge I, or pindolol (0.5 mg·kgbw^{-1}, mean dose 37±3mg; Sandoz) for Challenge II. Serial blood samples were then taken at 15 min intervals for one hour. Subjects then took buspirone (0.5 mg·kgbw^{-1}, mean dose 37±3mg; Bristol-Myers Squibb) and further blood samples were withdrawn every 15 min for the next 150 min. Subjects rested but were not allowed to sleep in a room at an ambient temperature of about 22°C. Venous blood samples were collected in EDTA tubes and plasma was separated by centrifugation and stored at -70°C. All samples were analysed for plasma prolactin concentrations (PRL) within 3 months.

Blood analysis

Haematocrit was measured in triplicate by centrifugation. Blood glucose and lactate concentrations were measured using enzyme-linked assays (Sigma Diagnostics, Poole UK); haemoglobin concentration was measured using the cyanomethaemoglobin method (Sigma Diagnostics, Poole UK). PRL was measured by a radioimmunoassay (Skybio Ltd, UK). Average inter- and intra-assay coefficients of variation of the assay were 5.9% and 2.7% respectively. All plasma samples from a single subject were assayed in the same batch.

Statistical Analysis

Total hormone release in response to buspirone was measured from the area under the curve of hormone concentration with time (AUC) calculated using the trapezoid method from the time of buspirone administration and was corrected for the average resting concentration, from the preceding 60 minutes. When there was no increase, or a reduction, in prolactin concentration after buspirone administration the area under the curve was calculated as zero. Plasma volume changes were calculated from haematocrit and haemoglobin values using the equations of Dill & Costill, (1974). Data were tested for approximation to a normal distribution. Exercise data were analysed up to 40 minutes to include the maximum number of subjects and were tested using repeated measures ANOVAs (SPSS 10). P values were corrected for sphericity using the Huynh-Feldt method, and significant differences between time points were identified using Tukey's post-hoc test. Correlations were calculated using the Pearson's correlation. Data are reported as mean ± SEM unless otherwise stated.

5.3 RESULTS

Exercise Trials

Exercise time and perception of exertion

Mean VO_2 was 3.06 ± 0.13 l·min^{-1} during exercise which was $73 \pm 5\% VO_{2max}$. Exercise times ranged from 20 to 98 min (51.3 ± 6.6 min, Table 5.1). RPE increased during the exercise and was significantly higher than the initial value after 30 minutes (Figure 5.1) and values at fatigue were 20.

	VO$_2$max	Exercise time	Rectal Temperature at Fatigue	Sweat Rate	Area under curve for Buspirone Challenge	Non- 5-HT component	Plasma Lactate at 10 min	Plasma Lactate at Fatigue
	(l·min^{-1})	(min)	(°C)	(l·h^{-1})	(miU·min·l^{-1})	%	(mmol·l^{-1})	(mmol·l^{-1})
1	4.01	92.5	39.1	2.08	25943	100	2.74	5.90
2	4.35	98.7	39.1	2.32	41273	100	3.11	8.23
3	4.92	48.5	39.8	1.63	0	94*	8.34	11.64
4	4.32	53.9	39.1	1.94	25579	78	3.18	5.72
5	3.41	60.8	38.6	1.17	20145	52	3.04	4.54
6	3.77	35.8	38.4	1.11	67481	46	5.64	3.67
7	5.30	43.5	38.9	2.08	31183	44	4.30	6.66
8	3.46	43.0	38.2	1.48	42379	15	3.44	3.61
9	4.27	40.4	38.0	1.69	7838	0	1.93	2.65
10	3.91	43.4	39.1	1.02	2970	0	6.28	8.23
11	4.56	20.7	38.3	1.79	65138	0	4.35	3.95
12	4.21	34.3	38.7	1.75	23438	100	3.38	4.26
Mean	**4.21**	**51.3**	**38.8**	**1.67**	**29447**	**49**	**4.14**	**5.40**
SD	**0.55**	**23.0**	**0.5**	**0.41**	**21775**	**41**	**1.80**	**2.50**

Table 5.1 Individual subject data for maximal oxygen uptake, exercise times, prolactin response to buspirone and the non-5-HT component of the buspirone response. *This subject had no prolactin response above baseline to challenges with buspirone alone or to pindolol + buspirone and therefore a non-5-HT component was not calculated, as a result he is excluded from all correlations.

Body temperature and sweat rate

Rectal temperature rose steadily throughout the exercise (Figure 5.2) and was 38.8 ± 0.1°C at the time of volitional fatigue. Mean skin temperature rose significantly in the first 10 minutes of exercise ($p < 0.05$, Figure 5.2) and thereafter remained constant with a fatigue value of 34.9 ± 0.3°C. Sweat rate ranged from 1.02 to 2.32 l·h^{-1} with a mean value of 1.67 ± 0.12 l·h^{-1} (Table 5.1).
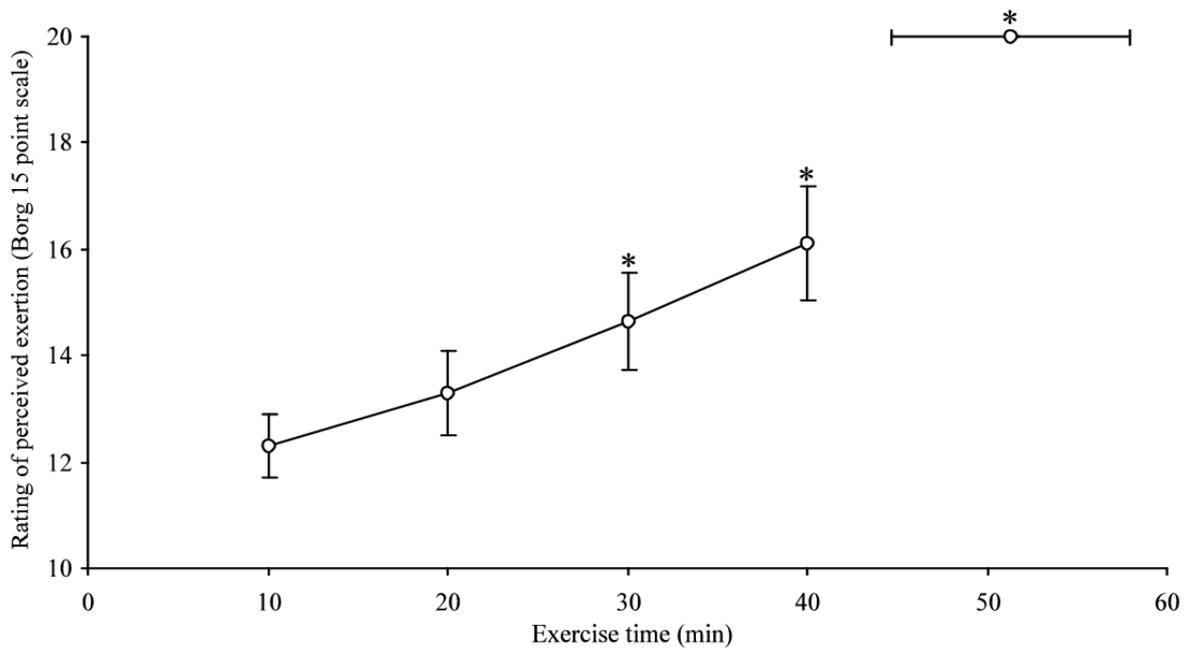
Figure 5.1 Rating of perceived exertion (Borg scale) during the course of the exercise. * Indicates significant difference from the 10 minute time point, $p < 0.05$; Data are Mean±SEM.
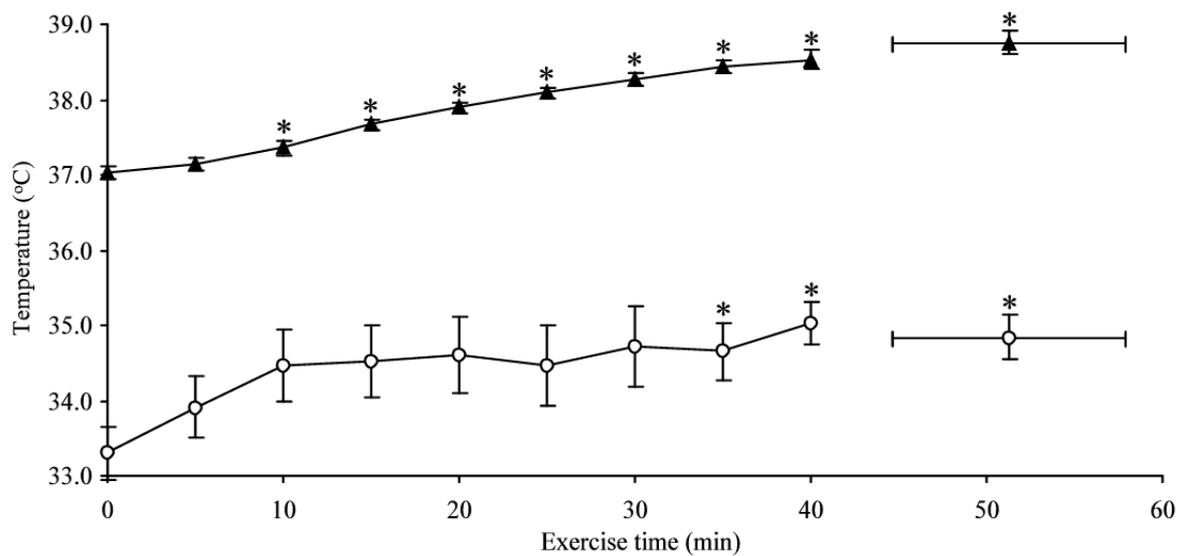
Figure 5.2 Rectal (triangles) and mean skin temperatures (squares) during exercise. * Indicates significant difference from time point 0, $p < 0.05$; Data are Mean ± SEM.

Blood and Metabolic parameters

There were no changes in haematocrit or haemoglobin concentration during exercise, at fatigue the mean calculated change in plasma volume was 3.9±1.0%. Blood lactate concentration increased during the first 10 minutes of exercise (Figure 5.3) but remained at this level to the time of volitional fatigue. Plasma lactate concentration at fatigue was 5.40 ± 0.72mM. Plasma glucose concentration did not deviate during exercise, and at fatigue

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was 5.86 ± 0.43mM (Figure 5.3). No change was seen in the respiratory exchange ratio during the course of exercise indicating a consistent source of fuel throughout the exercise.
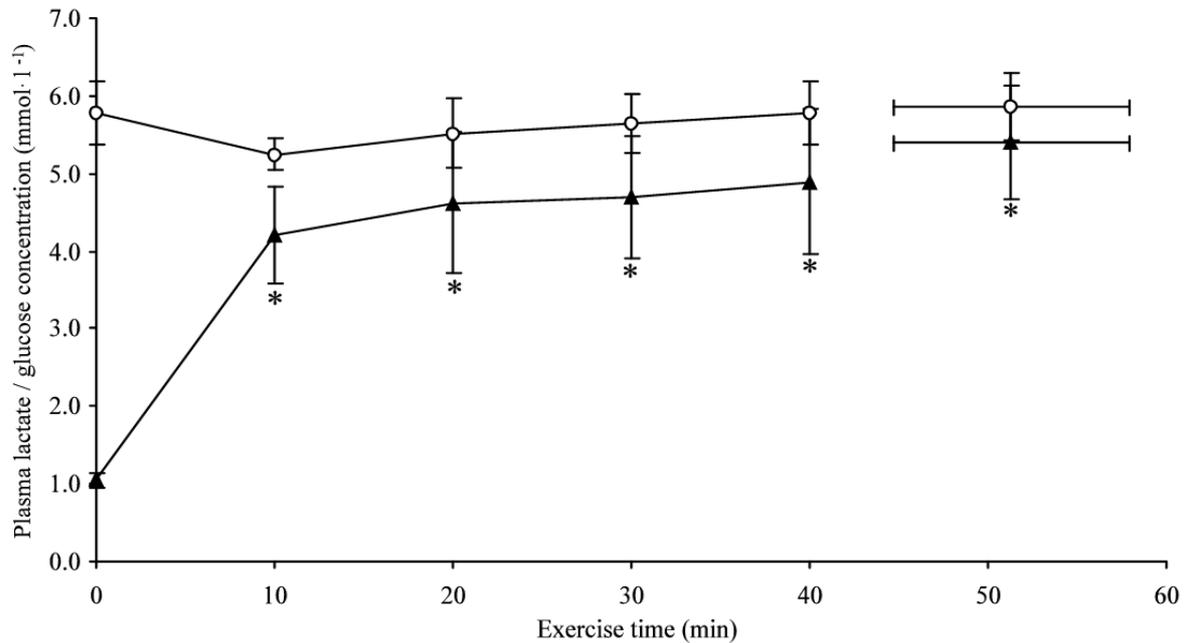

Figure 5.3. Plasma lactate (triangles) and glucose (circles) concentrations during exercise. Lactate concentrations rose significantly in the first 10 minutes but remained constant thereafter while glucose showed no significant fluctuations. * Indicates higher than time point 0 for lactate, $p < 0.05$. Data are Mean $\pm$ SEM.

Cardiovascular and respiratory parameters

Heart rate increased during exercise from 153 ± 4 beats·min^{-1} at 5 min to 168 ± 5 beats·min^{-1} after 40 min of exercise (i.e. from approximately 77% to 85% of maximum heart rate measured during the VO_{2max} test). Ventilation increased during exercise (76.8 ± 5.4 l·min^{-1} at 15min compare to 82.0 ± 6.8l·min^{-1} at 30min, $p < 0.05$).

Neuroendocrine Challenges

The oral administration of buspirone resulted in a robust prolactin response in all but one subject (Figure 5.4). In the combined challenges, pindolol was given 60 min before the buspirone and during this time the resting prolactin fell, on average, by 34%. Compared with buspirone alone, the prolactin response to buspirone in the presence of pindolol was reduced in all but two subjects, the peak response being reduced by about a third and the time of the peak response delayed by approximately 30 min (81 ± 10min buspirone compared to 113 ± 9min pindolol + buspirone, $p = 0.039$ Figure 5.4).
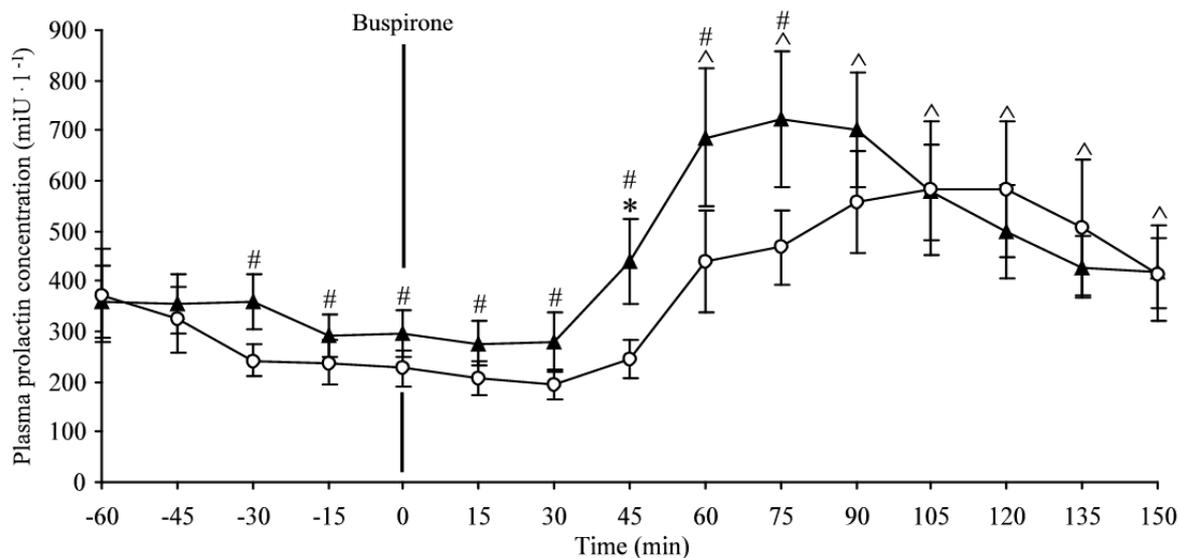
Figure 5.4. Prolactin response to challenges with buspirone (triangles) and pindolol + buspirone (Circles). Pindolol was administered at the -60 minute time point and buspirone at time point 0. * Indicates significant difference from time point 0 in buspirone trial, $p < 0.05$; ^ indicates significant difference from time point 0 in both trials, $p < 0.05$. # Indicates significant difference between trials at that time point, $p < 0.05$. Data are Mean ± SEM.

Subtracting the area under the curve of the pindolol + buspirone response from the area under the curve of the response to buspirone alone for each subject allowed the proportions of their serotonergic and non-serotonergic components to be calculated. The non-serotonergic component of the buspirone response ranged from 0 to 100% with a mean value of 49±12% (Table 5.1).

Correlations

Correlations were sought between values derived from the neuroendocrine challenges and indices of performance during exercise. Statistical data are presented in Table 5.2.

Variable		Area under curve for Buspirone Challenge	Non 5-HT component	5-HT component	% Non-5-HT
Time to fatigue	r	-0.155	0.661	-0.576	0.657
	p	0.630	0.019*	0.050	0.028*
	n	11	11	11	11
Rate of rectal temperature rise	r	-0.158	-0.616	0.234	-0.669
	p	0.625	0.033*	0.464	0.024*
	n	11	11	11	11

Table 5.2. Pearson correlation coefficients (r), significance values (p) and number of samples (n). * Indicates a significant linear correlation at the indicated p value.

5.4 DISCUSSION

The results support the view that with exercise at about 70% VO_{2max} the major limitation to performance in the heat is of central origin rather than arising in the peripheral musculature, the cardiovascular or respiratory systems. Results of the neuroendocrine challenges suggest that activity of hypothalamic pathways, probably those involving dopamine, play an important role in determining such performance.

The existence of central fatigue during submaximal exercise is difficult to prove since, to date, it can only be surmised from the absence of signs of peripheral muscle failure. Recently, (Nybo & Nielsen, 2001a) have shown that subjects made hyperthermic by exercise in the heat were unable to fully activate their quadriceps and that this effect was accompanied by changes in their electroencephalogram. These observations add weight to the suggestion that raised core temperature itself is the signal inhibiting exercise rather than some consequence of the elevated temperature (Nielsen *et al.*, 1997; Nybo *et al.*, 2001; Nybo & Nielsen, 2001b, a, c), such as altered blood flow to the working muscles.

In the work presented here there is evidence that fatigue was not the result of peripheral changes. Blood lactate levels rose initially during the exercise but then remained constant, indicating that the working muscles had achieved equilibrium, with energy supply from oxidative metabolism matching the energy demands. Subjects remained euglycaemic, and RER values remained stable, indicating that there was no change in the proportion of fat and carbohydrate oxidisation. Heart rate and ventilation tended to increase throughout exercise but at no stage did they approach values that would be considered to be limiting for exercise.

RPE increased throughout exercise in parallel with the increase in rectal temperature. The end point of exercise for our subjects occurred at somewhat lower values of core temperature than found by Nielsen *et al.*, (1997) and Gonzalez-Alonso *et al.*, (1999). This may, in some part, reflect a difference between rectal and oesophageal sites of measurement or differences in the type of subjects used. In their studies, Nielsen *et al.*, (1997) and Gonzalez-Alonso *et al.*, (1999) used subjects who were endurance trained cyclists, whilst our subjects, although generally fit and familiar with cycling exercise, were a more heterogeneous group. Additionally, our results do not show such a tight relationship

between fatigue and final core temperature, as suggested by Nielsen *et al.*, (1997) and Gonzalez-Alonso *et al.*, (1999), since some of our subjects reached volitional fatigue at nearly 40°C while others were only at about 38°C (Table 5.1). It has been suggested that in moderately fit subjects, as used in this experiment, the cause of voluntary exhaustion during exercise in the heat is more a combination of core and skin temperatures, rather than the attainment of a high core temperature (Cheung & McLellan, 1998).

The neural structures and pathways involved with central fatigue are poorly understood, but there has been interest in the possibility that serotonergic pathways are involved since the studies of Newsholme and colleagues (e.g. Newsholme *et al.*, 1987). Serotonergic pathways in the hypothalamus are involved in the control of prolactin secretion, an increase of which is associated with the development of fatigue (Marvin *et al.*, 1997). Central fatigue is most evident when working in the heat (Nybo & Nielsen, 2001a) and the hypothalamus is also the site of much of the body's thermoregulatory control (Boulant, 1981), suggesting that variations in activity or sensitivity of pathways in this region may account for some of the variations in endurance capability. The sensitivity of the hypothalamic pathways can be assessed by a neuroendocrine challenge with buspirone and patients with chronic fatigue syndrome have a very high prolactin response to this challenge (Bakheit *et al.*, 1992; Sharpe *et al.*, 1996). Conversely a low prolactin response has been associated with high endurance capacity in fit, young, male subjects (Jakeman *et al.*, 1994). In the latter study, associations were found between athletic ability, based on VO$_{2max}$ data, and response to buspirone, but no direct comparison was made with actual endurance performance under laboratory conditions.

Buspirone has a complex action in stimulating the release of prolactin since it is both a 5-HT$_{1A}$ agonist and a D$_2$ antagonist and both these actions result in the release of prolactin. We have recently shown (Bridge *et al.*, 2001) that the two actions of buspirone can be separated by the use of pindolol and, on average, approximately half the total response can be ascribed to a 5-HT stimulatory mechanism (i.e. blocked by pindolol) and the remainder to another mechanism which, although not identified, is most likely the removal of dopamine inhibition.

The work presented here is the first to make a direct comparison between exercise performance in the heat and hypothalamic sensitivity assessed by a neuroendocrine

challenge. It is also the first to dissect the buspirone challenge into its component parts so that we have been able to directly compare endurance with the different components of the response to buspirone. From the data presented in Table 5.1 it is evident that the total prolactin response to a buspirone challenge bears no relationship to performance in the heat. Comparison of two subjects illustrates the point. Subjects 2 and 8 had similar total prolactin responses (42000 and 41000 $miU^.min^.l^{-1}$) but the endurance time for subject 2 was over twice that for subject 8. Neither was there any relationship between prolactin response and VO_{2max} as was implied by Jakeman *et al.*, (1994).

Separating the buspirone response in the present study into serotonergic and non-serotonergic components (Figure 5.4) shows that, on average, the two release mechanisms were in very similar proportions (approximately 50%) to those reported previously (Bridge *et al.*, 2001). It is notable, however, that Table 5.1 shows a very wide variation in the proportions of 5-HT and non 5-HT components ranging from 100% of one, to 100% of the other. This is a feature that has been commented upon previously (Bridge *et al.*, 2001) and it prompts the question of whether the wide variation in neuroendocrine response is related to the wide variation in endurance performance seen in the heat (Table 5.1). We therefore sought evidence of relationships between the separate components of the buspirone response and endurance exercise performance. Table 5.2 presents the statistical data and while it is evident that there was no relationship between the total buspirone response and exercise performance, there was a high positive correlation between the non-5-HT component of the response and time to fatigue. A similar high correlation was found between time to fatigue and the proportion of the total prolactin response to buspirone attributable to the non-5-HT component. In these circumstances it is not clear whether it is a large non-5-HT component or a small 5-HT component that is the most appropriate predictor of performance.

Tolerance of high core temperature may be one factor determining endurance performance, the rate of temperature rise is clearly another key factor and it notable that while the best performers had the highest core temperatures at fatigue, the rate at which their temperatures rose was lower than for other subjects. There were strong negative correlations between the absolute size and the proportion of the buspirone response attributed to dopaminergic activity and the rate of rise of rectal temperature (non-5-HT component, Table 5.2).

In summary, our results show that, statistically, a substantial portion of the variation endurance capacity of normal subjects exercising in hot conditions may be explained by their differing responses neuroendocrine challenges with buspirone, coupled with pindolol used to block the 5-HT$_{1A}$ component. It appears that a high dopaminergic component is associated with better exercise performance and this may be related to the absolute magnitude of the response or to the high ratio of dopaminergic to serotonergic activities. Subjects with high sensitivity of these postulated dopaminergic pathways might benefit by a slower rate of rise of core temperature.

The neuroendocrine challenges we have described quantify the activity of hypothalamic pathways that appear to be involved in both thermoregulation and the perception of exertion and thereby the desire, or ability, to continue exercise. Such tests may throw light on the fundamental mechanisms of central fatigue while they could also prove to be a way of identifying individuals who are well adapted to exercise in the heat. Conversely those who are not well adapted may be at risk of developing heat illness since high heat tolerance but poor thermoregulation would be a dangerous combination.

Acknowledgement

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Chapter 6

PROLACTIN RESPONSE TO PASSIVE HEAT STRESS WITH AND WITHOUT 5-HT$_{1A}$ RECEPTOR BLOCKADE

6.1   INTRODUCTION

Increasing core-temperature either passively, through exposure to high ambient temperatures at rest, or actively, through exercise, results in a number of physiological and behavioural responses. These include vasodilatation, increased sweating, and a desire to move away from the heat. Additionally, as body temperature rises there are increased feelings of fatigue and lethargy. These feelings probably arise from the limbic area, hypothalamus or brain stem and involve serotonergic neurones that radiate from the raphe nuclei to higher centres concerned with motivation and motor drive.

Increased deep body temperature has been shown to increase central brain serotonin (5-HT) concentration in animals (Mohamed & Rahman, 1982; Sharma & Dey, 1987; Sharma *et al.*, 1992; Dey *et al.*, 1993) and is also likely to do so in man. Central serotonergic activity cannot be assessed directly in human subjects but many studies have used the release of prolactin (PRL) as an indirect measure. PRL is released from the anterior pituitary gland and its release is stimulated primarily by 5-HT receptor activation and inhibited by dopamine (DA) (Eison & Temple, 1986). During passive heat exposure, circulating PRL has been found to rise in a linear fashion with core and skin temperature (Christensen *et al.*, 1985; Brisson *et al.*, 1991). By measuring the PRL response for a given rise in temperature it may be possible to obtain an index of hypothalamic sensitivity. Additionally, there is also evidence to suggest that 5-HT may also have an important role in the regulation of body temperature (Yamawaki *et al.*, 1983).

There is considerable evidence from animals to support a role for DA in the control of body temperature, largely regulating heat loss (see Lee *et al.*, 1985 for review) and there has been a report of increased dopamine in the preoptic area and anterior hypothalamus in response to raised body temperature in rats (Hasegawa *et al.*, 2000). To interpret the results of studies where PRL is released as core temperature is raised the relative contributions of both 5-HT and DA mechanisms to this release need to be ascertained.

By administration of the 5-HT$_{1A}$ receptor antagonist pindolol, we have previously shown that approximately 82% of the basal PRL release is attributable to non-5-HT$_{1A}$

mechanisms, most likely of a dopaminergic nature (Bridge *et al.*, 2001), and that ~50% of the PRL release in response to buspirone can be attributed to each of the mechanisms. However no such information is available about the mechanisms responsible for the increase in PRL with rising body temperature.

Brain hypothalamic DA concentration is known to increase as a result of heat exposure in rats (Kao *et al.*, 1994; Hasegawa *et al.*, 2000), and this would be expected to lead to a reduced PRL secretion through a greater DA inhibition. However, in man increased PRL release is seen with rising body temperature, raising the possibility that PRL release in response to heat is entirely driven by 5-HT stimulation. This has important implications for exercise since it has been argued earlier (Chapters 2 & 3) that PRL release during exercise is primarily in response to raised core and skin temperature rather than any changes in circulating metabolites.

To ascertain the relative contribution of $5-HT_{1A}$ and non-$5-HT_{1A}$ (DA) mechanisms to PRL release in response to heat stress, we have measured plasma PRL concentration with and without pindolol pre-treatment.

6.2   METHODS

General design

Subjects underwent two passive heat exposures in a sauna maintained at 80°C to raise core temperature by 2.0°C. Before one of the heat exposures subjects took pindolol (10mg) twice daily for two days and 30mg 45 minutes before entering the sauna. Blood samples were taken during the heat exposures and analysed for PRL.

Subjects

Ten recreationally active non-acclimatised subjects participated in the study, which was of a randomised crossover design. Their mean age, and body weight were 23.3±3.9 y (Mean ± SD) and 74.1±5.6 kg, respectively. Subjects were screened with a clinical interview to ensure that there was no previous psychiatric history and they had been free of any medication for at least three weeks prior to the study. The study was approved by the Local Research Ethics Committee and subjects gave their informed consent in writing.

Experimental design

Prior to the heat exposures subjects either took pindolol (10mg) or a placebo twice daily for two days and 45 minutes (pindolol 30mg or placebo) before entering the sauna. Visits were balanced for order. Subjects arrived at the laboratory after an overnight fast and rested for 15 minutes before a cannula was inserted into a forearm vein. Subjects then drank 500ml of water to ensure adequate hydration. After a further 45 minutes rest, subjects entered a sauna at an ambient temperature of 80°C (RH 85-90%). Subject temperature was monitored continuously by a rectal probe (Grant Instruments Ltd, Cambridge, UK) and they remained in the sauna until their core temperature had increased by 2°C. Blood samples (5ml) were taken after every 0.5°C rise of core temperature. Water was allowed *ad libitum* and subjects were encouraged to drink freely.

Blood analysis

Haematocrit was measured in triplicate by centrifugation and haemoglobin was measured using the cyanomethaemoglobin method (Sigma Diagnostics, Poole UK). PRL was measured by radioimmunoassay (Skybio Ltd., UK).

Statistical Analysis

Total PRL release was measured from the area under the curve of the plasma PRL concentration with time (AUC) calculated using the trapezoid method and corrected for baseline concentrations. Plasma volume changes were calculated using the equations of [Dill, 1974 #1545]. Data were tested for approximation to a normal distribution. Data were analysed using repeated measures ANOVAs. P values were corrected for sphericity using the Huynh-Feldt method, and significant differences between time points were identified using Tukey's post-hoc test. Data are reported as mean ± sem.

6.3 RESULTS

All subjects completed the two heat exposure sessions attaining a core temperature increase of 2.0°C in a similar time in both sessions (36.9±2.5min normal v 32.6±1.9min pindolol, p=0.117). There were also no differences between the times taken to reach each 0.5°C temperature rise in each session (Figure 6.1).
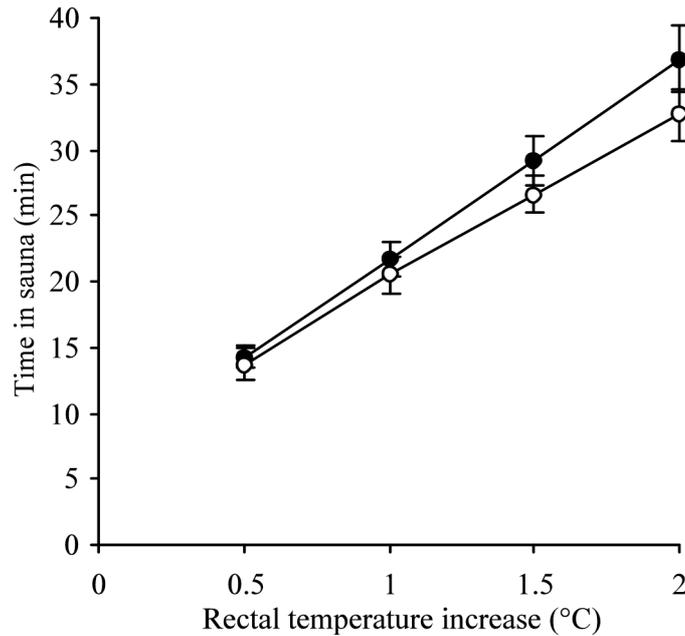

Figure 6.1. Time to increase rectal temperature by 0.5°C increments. Solid symbols show data from the placebo trial open symbols show data from pindolol trial. Data are mean±sem.

Hormonal response

In both heat exposures plasma PRL concentration rose and was significantly higher than basal levels after a 1.5°C rise in rectal temperature (394±54 baseline v 1381±227miU·l^{-1} placebo and 340±39 baseline v 1128±184miU·l^{-1} pindolol, p<0.05, Figure 6.2). No differences were found in plasma PRL concentrations between exposures at any increase in rectal temperature (Figure 6.2) nor was there a difference in plasma PRL concentration at a given rectal temperature (Figure 6.3). Total prolactin release (AUC) did not differ either between sessions (97,232±14,411 normal v 80,986±9980miU·min·l^{-1} pindolol, p=0.311).
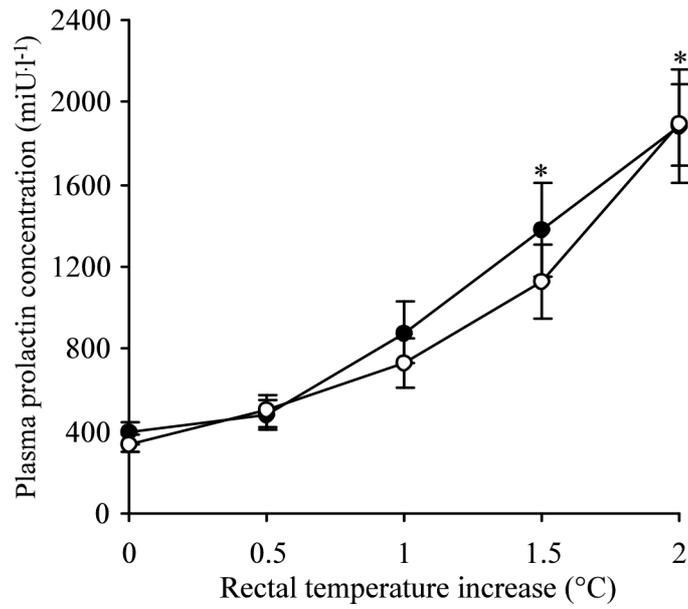
Figure 6.2. Plasma prolactin concentration at each 0.5°C rise in rectal temperature. Solid symbols show data from the placebo trial open symbols show data from pindolol trial. * indicates a significant difference from 0 time point for both trials. Data are mean±sem
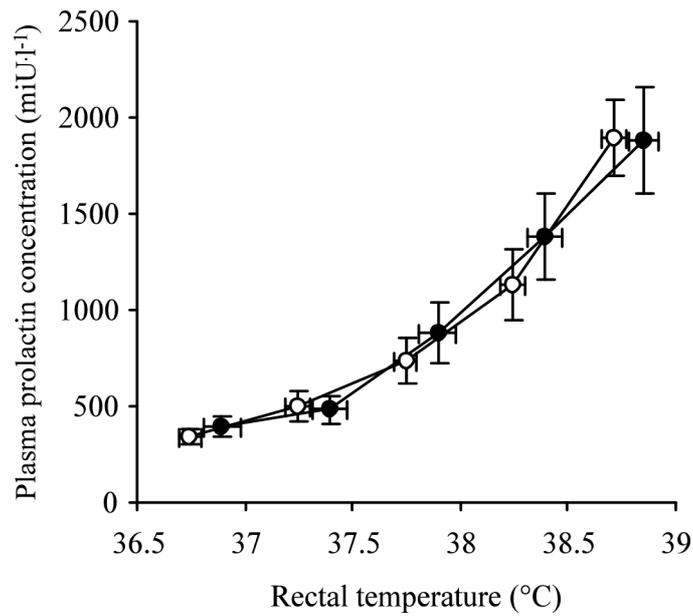

Figure 6.3. Plasma prolactin response to rectal temperature. Solid symbols show data from the placebo trial open symbols show data from pindolol trial. Data are mean±sem.

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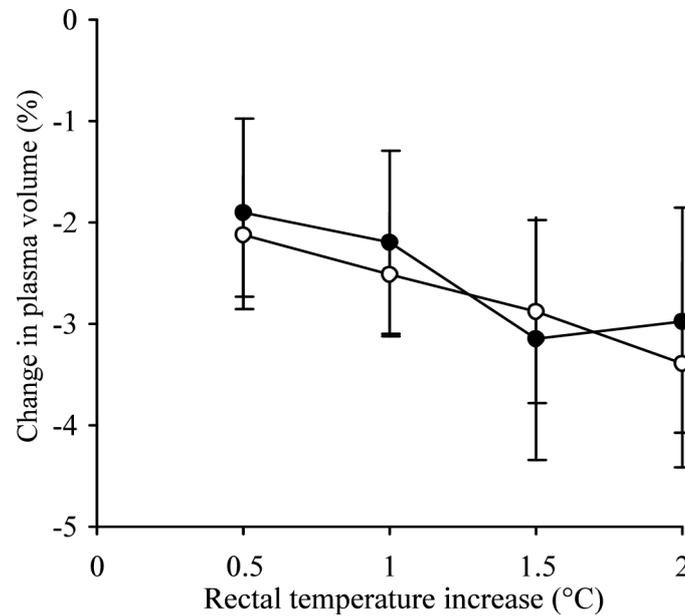
Figure 6.4. Changes in calculated plasma volume [Dill, 1974 #1545] during passive heat exposure. Solid symbols show data from the placebo trial open symbols show data from pindolol trial. Data are mean±sem.

Blood parameters

Whilst haemoglobin and plasma volume (Figure 6.4) remained fairly constant there was a significant increase in haematocrit (45.1±0.9 v 46.5±0.8%, $p<0.05$) after a 1.0°C rectal temperature rise in the normal session and after a 1.5°C rise (45.4±0.7 v 46.8±0.5%, $p<0.05$) in the pindolol session. No differences were found between sessions, at any core temperature increase, in haematocrit, haemoglobin or change in plasma volume.

6.4   DISCUSSION

The results presented here are interesting, but also very puzzling. It is important to know how PRL is released during fatiguing exercise since the release of PRL is closely associated with the development of central fatigue and there may well be common neural pathways involved. PRL release during exercise appears to be largely stimulated by increases in core and skin temperature (Chapter 2 & 3) and thus passive heating may be a good model for this aspect of exercise. The initial conclusion, therefore, might be that during exercise the release of PRL is largely due to a withdrawal of DA inhibition rather than an increase in serotonergic stimulation. The result is also puzzling, partly because there is a considerable amount of work, albeit sometimes controversial, going back to that of Newsholme et $al.$, (1987) suggesting that it is an increased serotonergic activity which is the stimulus for central fatigue. Secondly, the high temperature of the sauna stimulated a massive thermoregulatory response and this is normally associated with an increase in dopaminergic activity in the hypothalamus rather than a decrease implied by the present findings.

The interpretation of the results presented here rests first on the selectivity and efficacy of the $5\text{-}HT_{1A}$ antagonist action of pindolol and, secondly, on the lack of any complicating actions. Pindolol has been shown to have a high affinity for $5\text{-}HT_{1A}$ receptors (Andree et $al.$, 1999; Martinez et $al.$, 2000; Rabiner et $al.$, 2000a; Rabiner et $al.$, 2000b; Martinez et $al.$, 2001; Passchier et $al.$, 2001) and does not appear to possess any activity at DA receptors (Hjorth & Carlsson, 1986). Additionally PET scanning has shown that a 20mg dose of pindolol, without a priming dose, resulted in a 46% post-synaptic $5\text{-}HT_{1A}$ receptor occupancy (Rabiner et $al.$, 2000b). Whilst this is by no means a total blockade of post-synaptic receptors, the results also show that the occupancy is dose dependant (Rabiner et $al.$, 2000b). It is therefore likely that with the priming dose given for two days before the heat exposure and the relatively high dose (30mg) given an hour a higher $5\text{-}HT_{1A}$ receptor occupancy was achieved. Indeed Rabiner et al (Rabiner et $al.$, 2000b) suggest that a 30mg dose would be enough to fully block the functional responses to the $5\text{-}HT_{1A}$ agonist buspirone.

Brain 5-HT concentrations have been shown to increase in animals as a result of heat exposure (Mohamed & Rahman, 1982; Sharma & Dey, 1987; Sharma et $al.$, 1992;

Dey *et al.*, 1993) and it is likely that this also occurs in man but it would appear that this does not stimulate PRL release via 5-HT$_{1A}$ receptors. However, activation of other 5-HT receptor subtypes (e.g. 5-HT$_{2C}$) has also been shown to cause PRL release in man although not to the same extent as 5-HT$_{1A}$ receptors (Barnes & Sharp, 1999). The possibility exists that some, probably small, part of the PRL release might have been due to stimulation of other 5-HT receptor subtypes.

The stimulus for changes in central 5-HT and DA activity and therefore PRL release during heat exposure is not entirely certain but increasing core temperature and a higher skin temperature are both likely to be important factors (Chapters 2 & 3). The main thermoregulatory control centre of the body is located in the rostral hypothalamus and in particular the preoptic and anterior hypothalamus (Boulant, 1981, 2000). This area is known to contain the central brain thermoreceptors and is populated by temperature sensitive neurones which change their firing rates in response to changes in local or core temperature (Boulant, 1981, 2000). The same neurones also receive afferent information from skin and spinal thermoreceptors and integrate this information into the appropriate thermoregulatory response to a given heat load or cold stress (Boulant, 1981, 2000). There is good evidence that some of these temperature sensitive neurones are dopaminergic (Cox *et al.*, 1980) and that serotonergic neurones, which have previously been shown also to be temperature sensitive (Dickenson, 1977), project into the preoptic area from the dorsal raphe nuclei (Cox *et al.*, 1980). It is possible therefore that activation of these temperature sensitive neurones will result in an increase in activity of one and or both 5-HT and DA pathways.

It would be expected that hypothalamic DA concentrations would have increased in our subjects as a result of heat exposure. Evidence for this comes from animal studies that have shown hypothalamic DA concentrations to increase with heat exposure (Kao *et al.*, 1994; Hasegawa *et al.*, 2000) and that DA has an important role in heat dissipation (Cox *et al.*, 1980; Lin *et al.*, 1982; Lee *et al.*, 1985; Kendrick *et al.*, 1989; Canini & Bourdon, 1998). It is difficult to reconcile this with the present finding which suggests that PRL release is due to the withdrawal of DA inhibition. One explanation for this is that the pathways controlling PRL release and those affecting heat loss mechanisms are separate and distinct from each other, and that an increase in

the activity of one of these pathways may be accompanied by a decrease in the other, the different DA receptor subtypes may have some part to play in this behaviour. DA control of PRL secretion from the pituitary gland is largely governed by the D_2 type receptor (Chang *et al.*, 1997) whilst the both D_1 & D_2 receptor types control the hypothalamic heat loss mechanisms (Salmi *et al.*, 1993; Verma & Kulkarni, 1993), although they are unequal in effect (Verma & Kulkarni, 1993; Salmi, 1998) working through separate pathways (Salmi, 1998).

Pindolol may not provide complete blockade of all $5\text{-}HT_{1A}$ receptors and some component of PRL release may be stimulated by 5-HT receptors other than $5\text{-}HT_{1A}$. Nevertheless, we have previously shown pindolol to block the $5\text{-}HT_{1A}$ agonist activity of buspirone (Bridge *et al.*, 2001) and it is clearly an effective drug acting on serotonergic pathways in the hypothalamus. Consequently if there was a component of PRL release which was $5\text{-}HT_{1A}$ dependent, some reduction in PRL release with pindolol would have been seen. This was not the case and the final conclusion must be that there is no evidence of $5\text{-}HT_{1A}$ stimulation being involved in the heat-induced release of prolactin during passive heating. This raises doubts as to whether the release of prolactin during hyperthermia in exercise involves 5-HT pathways and in turn the role of 5-HT in central fatigue in the heat.

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Chapter 7

EFFECT OF CAFFEINE CONSUMPTION AND THE ROLE OF HYPOTHALAMIC PATHWAYS ON ENDURANCE PERFORMANCE AT DIFFERENT AMBIENT TEMPERATURES

7.1 INTRODUCTION

Caffeine, 1,3,7-trimethylxanthine, is one of the most widely taken drugs in the world and is believed to improve exercise capacity (Pasman *et al.*, 1995; Spriet, 1995; Graham & Spriet, 1996). There are reports of caffeine reducing perceived exertion during exercise (Rodrigues *et al.*, 1990; Alves *et al.*, 1995; Cole *et al.*, 1996) and it also has many positive subjective effects including increased feelings of well-being, energy, alertness, concentration and motivation (Griffiths *et al.*, 1990).

Despite this range of apparently beneficial effects, there is little information about the mechanism of action of caffeine. One possible peripheral mechanism might be to increase circulating free fatty acids which could stimulate fatty acid oxidation, sparing carbohydrate stores, and thus prolong exercise (Costill *et al.*, 1978; Erickson *et al.*, 1987; Graham & Spriet, 1991) and caffeine has been reported to reduce the rate of glycolysis in the first 15 minutes of exercise (Spriet *et al.*, 1992). However, more recent studies have failed to demonstrate a glycogen sparing effect either in the first few minutes of exercise (Graham *et al.*, 2000) or over the entire exercise period (Chesley *et al.*, 1995; Jackman *et al.*, 1996; Graham *et al.*, 2000; Laurent *et al.*, 2000). Additionally, caffeine has been found to raise blood lactate levels during exercise, indicating an increased muscle glcyogenolysis (Graham & Spriet, 1995; Jackman *et al.*, 1996; Laurent *et al.*, 2000) rather than glycogen sparing. Other investigators have found an elevation of circulating free fatty acids but without any indication of an increase in fat metabolism (Tarnopolsky, 1994; Graham *et al.*, 1998; Graham *et al.*, 2000). Jacobson *et al.* (2001) examined the action of caffeine taken together with fat or carbohydrate supplements on endurance exercise and found no effect on metabolic parameters while ratings of perceived exertion were consistently lowered.

Since there is no good evidence for a peripheral action of caffeine, attention has turned to possible central neural mechanisms during prolonged endurance exercise where the inability to continue exercising may be due to a loss of central drive or motivation (Newsholme *et al.*, 1987). Increasing feelings of effort and, eventually, fatigue are associated with a rise in blood levels of prolactin (PRL) (Marvin *et al.*, 1997) which is secreted from the anterior pituitary under the control of serotonergic and dopaminergic pathways in the hypothalamus (Struder & Weicker, 2001). It is

therefore possible that these pathways are also involved with the development of central fatigue. Endurance is reduced and perceived exertion increased when exercise is undertaken in hot environments, largely through an effect of raised body and skin temperature reducing central drive (Nybo & Nielsen, 2001a, b). Since the hypothalamus is intimately involved in both temperature regulation and neuroendocrine responses it has been suggested that the hypothalamus plays a role in integrating temperature regulation and the neuroendocrine response with the behavioural response (a disinclination to continue working, i.e. volitional fatigue) to prolonged exercise (Chapters 2 & 3). Studies with rodents have shown that caffeine can alter brain serotonin (5-HT) and dopamine (DA) concentrations, synthesis and turnover (Hadfield & Milio, 1989; Kirch *et al.*, 1990; Nehlig *et al.*, 1992), and it has been suggested that serotonin plays an important role in the mechanism of action of caffeine on the central nervous system (Hirsh, 1984). If, therefore caffeine acts through a central mechanism it could well do so by affecting hypothalamic 5-HT and DA pathways and it would be expected to be most effective in conditions where central fatigue is most evident, namely during exercise at high ambient temperatures.

Data from a range of studies suggests that serotonergic neurones of the dorsal raphe nuclei project to hypothalamic sites to stimulate PRL secretion through activation of central 5-HT receptors (Van de Kar *et al.*, 1996). Changes in PRL levels in the blood therefore provide a useful marker for changes in central 5-HT activity as 5-HT is a prominent excitatory neurotransmitter for PRL release (Struder & Weicker, 2001). However 5-HT does not have exclusive control of PRL secretion. Prolactin is also under a tonic inhibitory control by the hypothalamus, through DA secreted by the tuberohypophyseal pathway (Ben-Jonathan & Hnasko, 2001).

Although circulating PRL has often been measured as an indicator of central changes with fatiguing endurance exercise there are no reports of the action of caffeine on PRL release. Neither is there a substantial body of literature concerning the action of caffeine on exercise at high ambient temperatures. Cohen *et al.* (1996) found no effect of two doses of caffeine during competitive road races in hot and humid conditions, which runs counter to the prediction that caffeine would be more effective in hot conditions. However, exercise in a cool environment was not included in this

investigation and, to date, no direct comparison has been made of the effects of caffeine at high and low ambient temperatures.

The results presented here are from two separate experiments investigating the ergogenic action of caffeine, one carried out at a high ambient temperature and the other in a cooler environment. The first hypothesis tested was that the central action of caffeine is mediated by an action on hypothalamic 5-HT and DA pathways and this would be evident as a decrease in the PRL response to fatiguing exercise. This hypothesis was addressed in each of the two studies. The second hypothesis was that caffeine acts on pathways sensitive to body temperature and would thus be most effective during exercise at high ambient temperatures. This hypothesis was addressed by comparing the results of the two experiments one in the hot and the other in the cool conditions.

7.2 METHODS

General Design

Subjects performed two exercise tests to volitional fatigue on a cycle ergometer after ingestion of caffeine ($5mg^.kgbw^{-1}$) or placebo capsules. Tests were balanced for order and carried out double blind. The work reported here consists of two such experiments, the first at an ambient temperature of $18\pm0.3^{o}C$ (relative humidity 57%) and the second at $35\pm0.3^{o}C$ (relative humidity 29%).

Subjects

Seven trained male subjects participated in the first study; their mean age, body weight and VO_{2max} were 24 ± 4 yrs (Mean$\pm$SD), 75.5 ± 4.8 kg, and 4.52 ± 0.44 $l^.min^{-1}$, respectively. Four of these subjects also participated in the second experiment which, in all, involved eight male subjects of 23 ± 1 yrs, 70.5 ± 1.8 kg, and 4.45 ± 0.15 $l^.min^{-1}$. All subjects were familiar with the experimental protocols and were non heat acclimatised. Daily caffeine consumption of the subjects ranged from none to high (0-$450mg^.day^{-1}$) with similar ranges in both studies. The study was approved by the Local Research Ethics Committee and subjects gave their written informed consent.

Experimental Design

Visit 1

On their first visit to the laboratory subjects undertook an incremental exercise test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine maximal power output and VO_{2max}. Workload was increased by 35 Watts every 3 minutes until volitional exhaustion.

Visits 2 & 3

Subjects abstained from caffeine for three days before each visit. Subjects recorded their diet the day before visit 2 and were asked to adhere to the same diet on the day before visit 3. On the morning of a test subjects arrived at the laboratory at 8am, having fasted from midnight, or at 1pm, having fasted for the previous 4 hours. Each subject carried out their two tests at the same time of day. Rectal temperature was

measured in both trials and in the trial at 35°C mean skin temperature was determined from measurements at four sites (Ramanathan, 1964). A cannula was inserted into an antecubital vein and subjects then rested for 30 minutes before a blood sample (5ml) was taken and subjects ingested either caffeine (5mg·kgbw^{-1}, Roche) or placebo capsules. Subjects were given a bolus of water (8ml·kgbw^{-1}) with their tablets to ensure that they began each trial fully hydrated. After a further 60 minutes rest at room temperature (approx 18°C), subjects voided any urine and their body weight was recorded to the nearest 10g. They then began to exercise on an electrically braked cycle ergometer until volitional fatigue, or for 90 minutes (whichever was the shorter), at a constant work rate calculated to elicited 70% VO$_2$max from visit 1. To maintain hydration during the exercise subjects were asked to drink a minimum of 2ml·kgbw^{-1} at 18°C or 3ml·kgbw^{-1} at 35°C water every 15 minutes, although they were free to drink more.

Ratings of perceived exertion (RPE; Borg, 1971) were obtained every 10 min during exercise. Heart rate was recorded continuously (Polar Vantage NV, Polar OY, Finland) during the exercise period. Rectal (and when measured mean skin) temperature was recorded every 5 min (Squirrel Meter Logger, Grant Instruments, UK) and V$_E$, VO$_2$, and VCO$_2$ were measured every 15 min. Venous blood samples (5ml) were obtained before, every 10 minutes during exercise and at the point of fatigue for determination of haematocrit, lactate, glucose, free fatty acids and PRL. Body weight was recorded immediately at the end of exercise after sweat was towelled off the skin. Sweat loss was calculated as the difference in body mass between the start and end of the trials after correction for respiratory and metabolic water loss, fluid intake and the blood samples taken. Data are expressed as sweat rate by dividing sweat loss by the exercise time to give an average value in litres per hour.

Blood Analysis

Haematocrit was measured in triplicate by centrifugation. Blood glucose, lactate (Sigma Diagnostics, Poole UK) and free fatty acids (Wako Chemicals GmbH, Germany) were measured using enzyme-linked assays and haemoglobin using the cyanomethaemoglobin method (Sigma Diagnostics, Poole UK). Plasma PRL concentration was measured by a radioimmunoassay (Skybio Ltd, UK). Average inter- and intra-assay coefficients of variation of the assay were 5.9% and 2.7%

respectively. All plasma samples from a single subject were assayed in the same batch. Caffeine was measured in plasma samples using an ELISA method with an Olympus 600 analyser.

Statistical Analysis

All data was tested for approximation to a normal distribution. Significant differences within trials were determined using repeated measures ANOVA with Bonferroni corrections for multiple comparisons where data were normally distributed or, otherwise, a Friedman test was used. Between trials, where data were normally distributed, significance was determined at matched time points using Student's paired t-test. Significance was determined using Wilcoxon non-parametric tests if the data were found not to be normally distributed. Data are presented as mean±sem unless otherwise stated.

7.3   RESULTS

Experiment 1: ambient temperature 18°C

Exercise capacity and perceived exertion

Exercise intensity was the same in both trials (73±4% VO_{2max} for the placebo trial v 76±2% with caffeine, p=0.3). Two subjects were stopped after 90 minutes of exercise in the placebo trial and three in the caffeine trail. One of these subjects was stopped after 90 minutes in both trials and one subject cycled for longer on the placebo (90 minutes) compared to caffeine. However the remainder all exercised for longer in the caffeine trial (Figure 7.1).
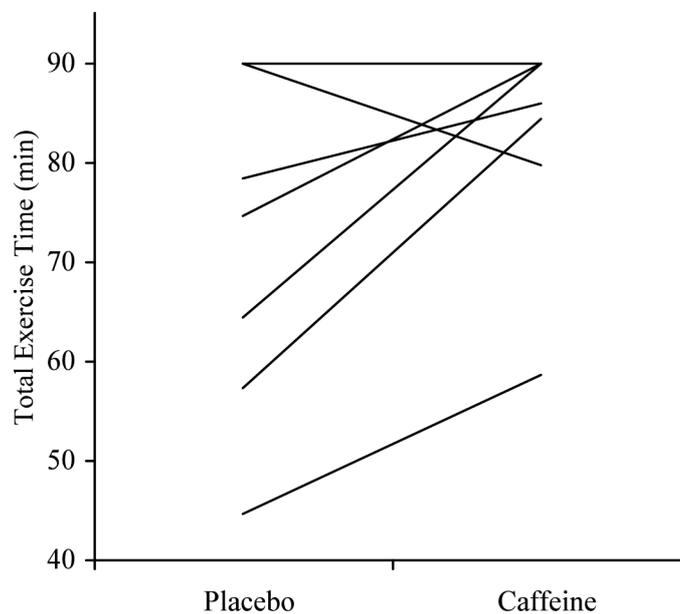

Figure 7.1. Exercise times for individual subjects during the placebo and caffeine trials at 18°C.

Excluding the subject who was stopped at 90 minutes on both occasions, the exercise time was 68.3±6.6 min for the placebo trial and 81.4±4.8 min for the caffeine trial, an average improvement of 23%.   The subject who exercised for longer on placebo had the highest habitual caffeine intake but, otherwise, there was no relationship between habitual caffeine intake and performance in the caffeine trial.   Irrespective of the effects on performance time, all subjects reported lower RPE scores during the caffeine trial, these being significantly lower from 20 minutes onwards (Figure 7.2, p<0.05).
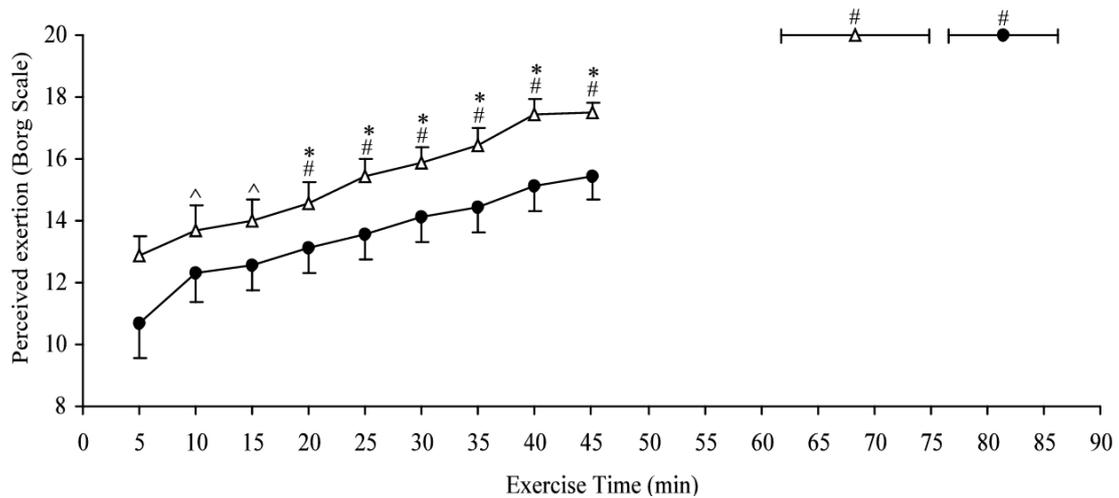
Figure 7.2. Perceived exertion during exercise at 18°C. Data are mean ± sem placebo - triangles. caffeine –circles. * indicates significant difference between trials ^ indicates significant increase from resting value in placebo trial # indicates significant increase from resting value in both trials (P<0.05).
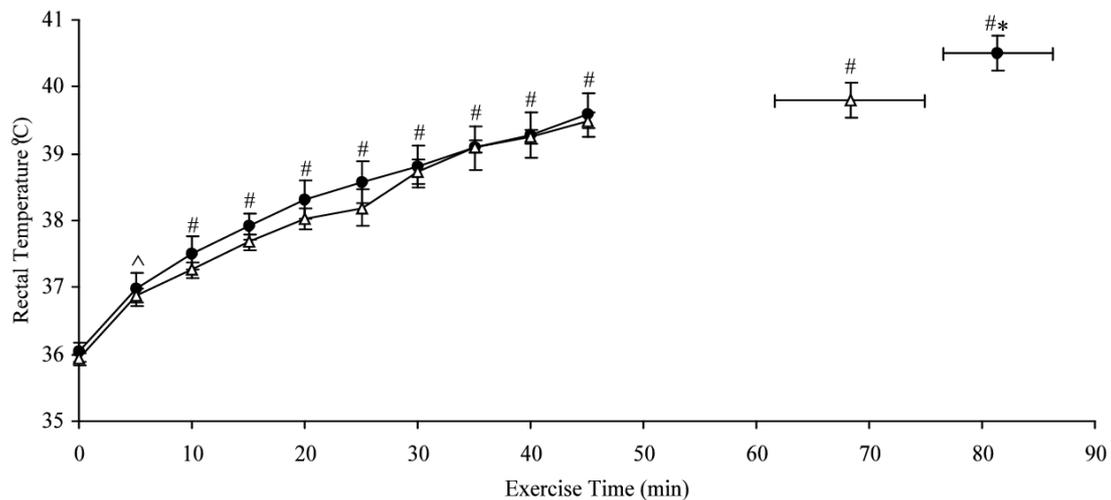
Figure 7.3. Rectal temperature during exercise at 18°C. Data are mean ± sem E – end of exercise. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference between trials ^ indicates significant increase from resting value in placebo trial # indicates significant increase from resting value in both trials (P<0.05).

Body temperature and sweating responses

Body temperature before exercise was the same with and without caffeine and there were no differences in rectal temperature between trials at identical time points up to 45 minutes (Figure 7.3). Rectal temperature at volitional fatigue was significantly higher in the caffeine trial (40.5±0.3°C) than with the placebo (39.8±0.3°C) (Figure 7.3, p<0.05). There was a tendency for sweat rate to be lower in the caffeine trial (0.88±0.14 $l·hr^{-1}$, compared to 1.05±0.07 $l·hr^{-1}$) although there was no statistically significant difference between trials.

Respiratory and Cardiovascular responses

There were no differences in any of the respiratory variables between trials (VO_2, VCO_2, V_E and RER, Table 7.1) which demonstrates that the energy consumption, gross efficiency and relative rates of carbohydrate and fat oxidation were not altered by taking caffeine. . Whilst heart rate increased during the exercise, values were similar for each trial with no differences found between trials in maximum (175 ± 4 beats.min^{-1} placebo v 175 ± 3 beats.min^{-1} caffeine) and average heart rate(157 ± 4 beats.min^{-1} placebo v 156 ± 3 beats.min^{-1} caffeine) before or at any time during exercise.

	18°C		35°C	
	Placebo	**Caffeine**	**Placebo**	**Caffeine**
VO_2 (l.min^{-1})	3.56±0.15	3.43±0.23	3.18±0.10	3.21±0.13
VCO_2 (l.min^{-1})	3.19±0.17	3.12±0.21	3.09±0.13	3.16±0.13
RER	0.89±0.05	0.91±0.01	0.97±0.01	0.99±0.01

Table 7.1. Mean respiratory parameters for each trial at both temperatures. Data are mean±sem.

Blood metabolites

Blood caffeine levels were 8.2 ± 0.7mg.l^{-1} 60 minutes after caffeine ingestion at the start of exercise and were undetectable in the placebo trial. Blood glucose concentrations remained stable throughout exercise with no change from rest to fatigue (6.1 ± 0.7 v 6.4 ± 0.7mmol.l^{-1} for the placebo trial and 6.0 ± 0.6 v 5.6 ± 0.4mmol.l^{-1} with caffeine). Blood lactate concentration increased in both trials in the first 10 minutes of exercise and then remained stable throughout exercise with no difference between levels at 10 minutes and fatigue (6.1 ± 0.6 v 6.4 ± 0.7mmol.l^{-1} for the placebo trial and 5.9 ± 0.6 v 5.5 ± 0.8mmol.l^{-1} with caffeine). Serum FFA concentrations were significantly higher in the trial with caffeine after 10 minutes of exercise and remained elevated above the concentrations in the placebo trial, however there were no significant differences between trials at the time of fatigue (Figure 7.4). There were no differences in haematocrit or haemoglobin between or within trials at any time point.
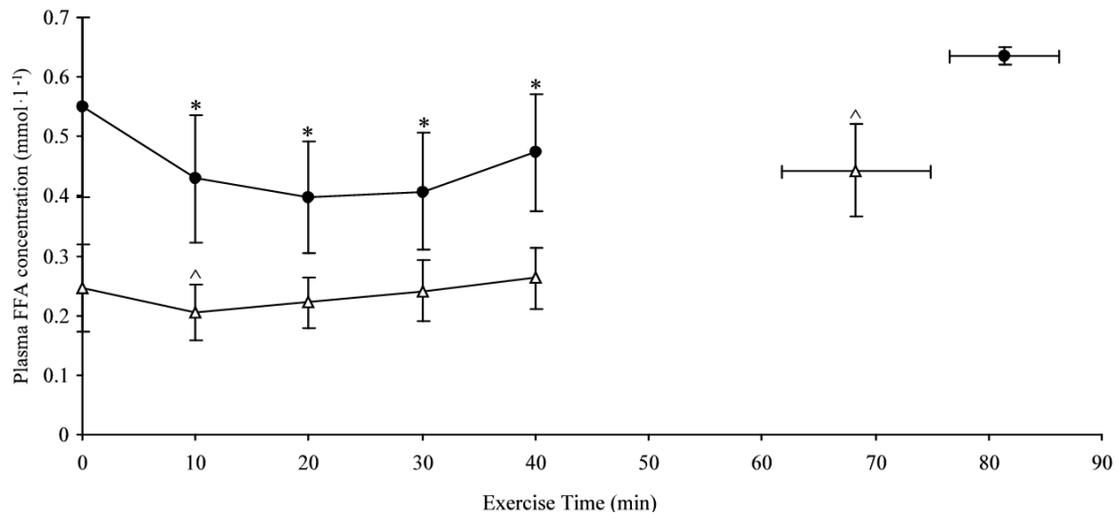
Figure 7.4 Plasma free fatty acid concentration (FFA) during exercise at 18°C. Data are mean ± sem. E – end of exercise. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference between trials ^ significant difference between time points (P<0.05).

Hormonal Responses

Plasma PRL levels rose although not significantly during the first 40 minutes of exercise in both trials and reached maximum values, which were higher than at rest (159 (147-177) v 443 (315-715) miU.l[-1] placebo trial and 163 (119-194) v 405 (345-498) miU.l[-1] caffeine trial, median (IQ range) p<0.05) at the time of fatigue. No differences were found between caffeine and placebo trials in terms of the values at given time points (Figure 7.5).
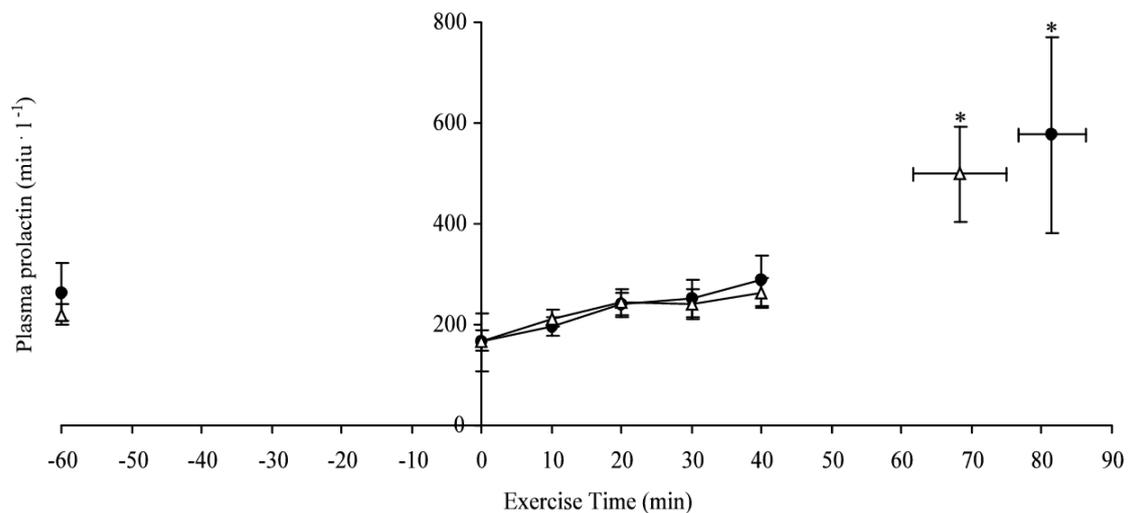

Figure 7.5   Plasma prolactin in response to exercise at 18°C.   Data are mean ± sem. E – end of exercise. Placebo trial – *triangles, open bar*. Caffeine trial –*circles, solid bar.* * indicates significantly difference from 0 minute time point, p<0.05.

Experiment 2: Ambient temperature 35°C

Exercise duration and perceived exertion

Endurance was approximately halved at the higher ambient temperature compared to exercise at 18°C, although the comparison is not strictly valid since only half the subjects participated in both trials. Exercise intensity was identical between trials (72±2%VO$_2$max in the placebo trial v 72±3% VO$_2$max with caffeine, p=0.7) and caffeine improved performance in all but one subject who rode for the same time in both trials. One subject was stopped at 90 minutes when on the caffeine trial. Ignoring the one subject who was stopped at 90 minutes in both trials, the times to volitional fatigue were 40:25±7:09 min for the placebo trial and 47:22±7:58 min with caffeine, the improvement in endurance on the caffeine trial being 18%, (Figure 7.6). Ratings of perceived exertion were significantly lower in the caffeine trial at all times during the exercise (Figure 7.7).
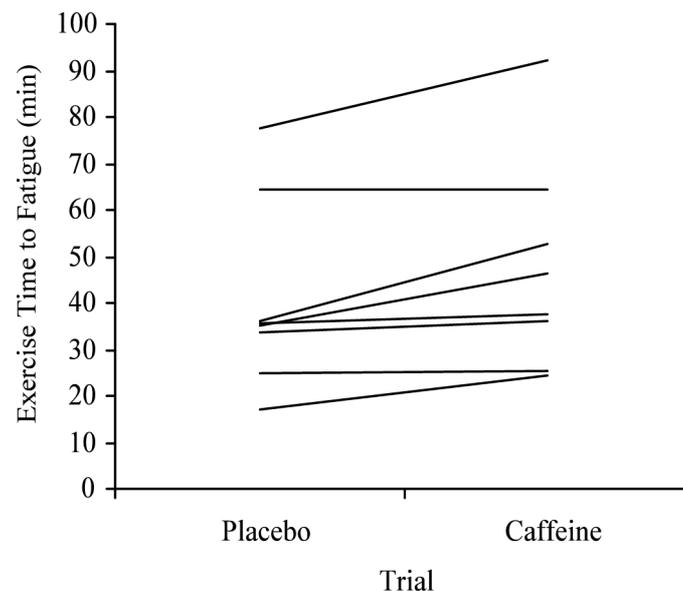

Figure 7.6. Exercise times for individual subjects during the placebo and caffeine trials at 35°C.
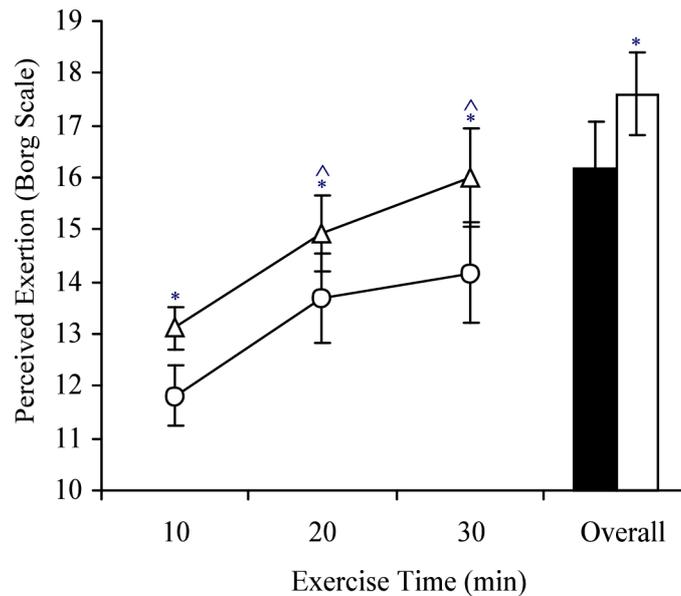
Figure 7.7 Perceived exertion during exercise at 35°C. Data are mean ± sem. Overall indicates subject rating of whole exercise period. Placebo trial – *triangles, open bar*. Caffeine trial –*circles, solid bar.* * indicates significant difference between trials ^ indicates significant increase from 10 minute value in both trials (P<0.05).

Body temperature and sweating responses

Rectal temperature increased throughout exercise, rising significantly above initial values after 25 minutes in the placebo trial and after 20 minutes in the caffeine trial. Rectal temperature was higher in the caffeine trial compared to placebo after 15 minutes of exercise and remained higher throughout the remainder of the exercise. At volitional fatigue rectal temperatures were 38.5±0.3°C for the placebo trial and 38.9±0.3°C for the caffeine trial (p<0.05; Figure 7.8). Sweat rates were not different between trials, 1.01±0.72 $l^.h^{-1}$ for the placebo trial and 1.23±0.82 $l^.h^{-1}$ for the caffeine trial. Mean skin temperature was the same in both trials, being 33.4±0.1°C during the placebo trial and 33.3±0.2°C for the caffeine trial and in neither case was there any change during exercise.
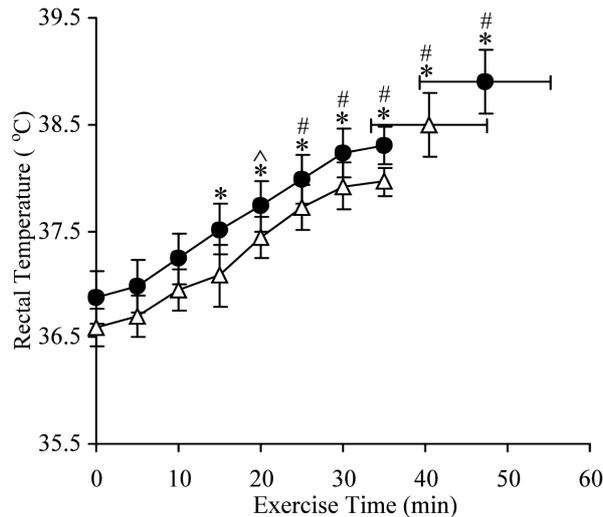
Figure 7.8 Rectal temperature during exercise at 35°C. Data are mean ± sem. F – indicates fatigue time point. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference between trials ^ indicates significant increase from resting value in caffeine trial # indicates significant increase from resting value in both trials (P<0.05).

Respiratory and cardiovascular responses

There were no differences in any of the respiratory variables (VO_2, VCO_2, V_E and RER, Table 7.1) between trials which demonstrates that the energy consumption, gross efficiency and relative rates of carbohydrate and fat oxidation were not altered by taking caffeine. Whilst heart rate increased during the exercise, values were similar for each trial with no differences found between trials in maximum (179±4 beats min^{-1} placebo v 181±5 beats min^{-1} caffeine) and average heart rate(163±4 beats min^{-1} placebo v 165±4 beats min^{-1} caffeine), before or at any time during exercise. No differences were found between trials in RER (0.99±0.0 caffeine v 0.97±0.0 placebo).

Blood metabolites

Blood caffeine levels were 7.9±0.8mg l^{-1} 60 minutes after caffeine ingestion at the start of exercise and were undetectable in the placebo trial. Blood lactate rose in the first 10 minutes of each trial to a level significantly higher than at the start of exercise (Figure 7.9). Although there was a tendency for lactate to continue to rise there were no statistically significant differences between values after 10 minutes of exercise and those at fatigue. Blood lactate was significantly higher at all time points during exercise with caffeine compared to the placebo trial (Figure 7.9, p<0.05). There were no changes in blood glucose concentrations during the first 20 minutes of exercise in either trial.   Thereafter concentrations tended to rise so that at fatigue the

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concentrations were higher than after 10 minutes exercise in the placebo trial (5.0±0.3 v 6.3±0.2mmol·l⁻¹, p<0.05), or after 20 minutes in the caffeine trial (5.9±0.4 v 7.1±0.6mmol·l⁻¹, p<0.05).
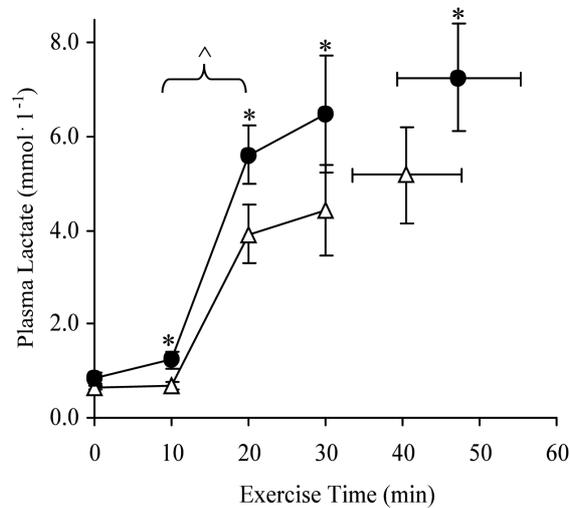
Figure 7.9 Plasma lactate during exercise at 35°C. Data are mean ± sem. F – indicates fatigue time point. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference between trials (P<0.05); ^ indicates significant increase between time points in both trials P<0.05.
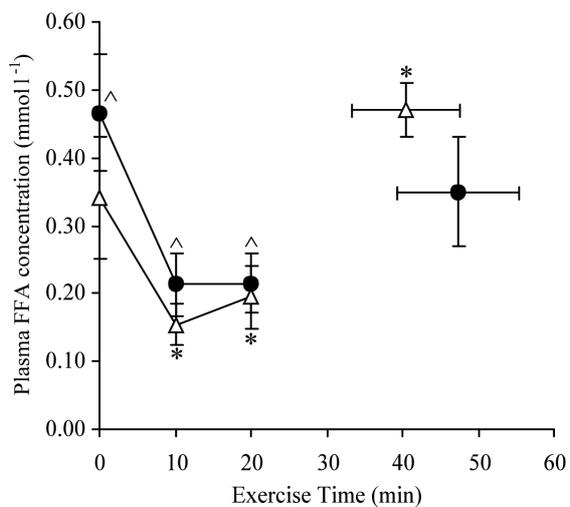
Figure 7.10 Plasma free fatty acid (FFA) during exercise at 35°C. Data are mean ± sem. F – indicates fatigue time point. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference between time points in placebo trial ^ indicates significant difference between time points in caffeine trial (P<0.05).

In the placebo trial FFA levels were higher at fatigue than at 10 and 20 minutes (Figure 7.10) in the caffeine trial FFA levels decreased in the first 10 minutes of exercise but were not significantly different from resting levels at fatigue (Figure 7.10). No statistically significant differences were seen between trials at any time point during the exercise. Haematocrit and haemoglobin did not change during

exercise and no differences were found between trials at any times. This was reflected in changes in plasma volume which were not different between trails, or at fatigue (-4.5±3.8% in the placebo trial v -4.2±2.0% with caffeine).

Hormonal Responses

The PRL response to exercise was more pronounced in the heat (Figure 7.11) than when exercising at $18^{\circ}C$ although the pattern of response, with little change in the early stages and then increasing rapidly to the point of fatigue, was similar in the two conditions (Compare Figures 7.5 and 7.11).
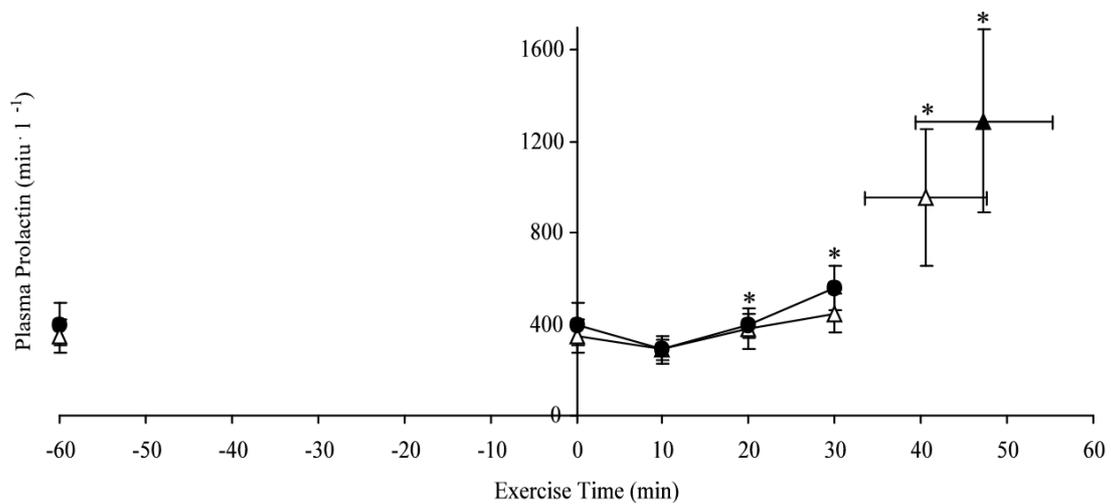


Figure 7.11 Plasma prolactin response to exercise at 35°C. Data are mean ± sem. F – indicates fatigue time point. Placebo trial – *triangles*. Caffeine trial –*circles*. * indicates significant difference from 0 time point within both trials (P<0.05).

During exercise at $35^{\circ}C$, PRL increased in both placebo and caffeine trials and was significantly higher than resting values after 10 minutes of exercise (p<0.05, Friedman). No differences were found at individual time points between the caffeine and placebo trials (Figure 7.11).

7.4 DISCUSSION

The results reported here confirm that caffeine reduces the subjective sensations of exertion and prolongs endurance when exercising at about 70% VO_{2max} and strongly support previous suggestions of a possible central nervous system mechanism rather than a peripheral action on substrate metabolism or the metabolic cost of exercise. The results do not, however, support our initial hypothesis that the effects are mediated through hypothalamic pathways nor does caffeine appear to influence pathways that are sensitive to body temperature. We postulate, therefore, that there are pathways leading to central fatigue in addition to those which are sensitive to core and skin temperature.

Despite its widespread use and the general belief in the efficacy of caffeine as an ergogenic aid, there is relatively little quantitative information about the benefits to be expected, let alone its mechanism of action. Our observation of a ~20% improvement in exercise duration is similar to previous reports when exercising at a similar intensity (Costill *et al.*, 1978; Graham & Spriet, 1991, 1995; Pasman *et al.*, 1995). It is likely that maximal benefits of caffeine will be seen with exercise of around 70-80% maximal aerobic capacity. Depletion of muscle glycogen is likely to be the limiting factor with exercise below this intensity while at higher intensities cardiovascular limitations and peripheral muscle fatigue will predominate. While improvements with caffeine administration have been reported in these latter conditions, they are not always significant (Spriet, 1995).

There was no evidence in the present study that caffeine altered substrate metabolism in any way that would lead to greater endurance. During exercise at 18°C, and in the early stages of exercise at 35°C, there were significant increases in circulating free fatty acids which might be expected to be oxidised in working muscles and thus spare glycogen. However, there were no changes in oxygen uptake or RER indicating that the mobilised fatty acids were not oxidised. Previous investigations (Tarnopolsky, 1994; Graham *et al.*, 1998) have made similar observations which raise interesting questions about the control of fat metabolism in working muscles, although these are outside the scope of the present study. With exercise in the heat there were

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consistently raised blood lactate levels in the caffeine, as compared to the placebo trial (Figure 7.8) as has been found by others (Graham & Spriet, 1995; Jackman *et al.*, 1996; Laurent *et al.*, 2000) indicating, if anything, an increased muscle glcyogenolysis rather than glycogen sparing.

The end point of exercise for our subjects in the second experiment at 35°C occurred at somewhat lower values of core temperature than found by Nielsen *et al.* (1997) and Gonzalez-Alonso *et al.* (1999b). This may, in some part, reflect a difference between rectal and oesophageal sites of measurement or differences in the type of subjects used. In their studies, Nielsen *et al.* (1997) and Gonzalez-Alonso *et al.* (1999b) used subjects who were endurance trained cyclists, whilst our subjects, although generally fit and familiar with cycling exercise, were a more heterogeneous group.

The higher core temperatures at fatigue during the caffeine trials (Figures 7.3 & 7.7) were partly a function of the longer exercise duration but there is also a suggestion that core temperature was slightly higher throughout the exercise while taking caffeine. This raises the possibility that caffeine may affect thermoregulation. Caffeine consumption is known to raise plasma catecholamines (Graham & Spriet, 1995; Jackman *et al.*, 1996; Van Soeren & Graham, 1998; Laurent *et al.*, 2000) and adrenaline infusion during exercise has been shown to cause vasoconstriction in the skin and increase oesophageal temperature (Mora-Rodriguez *et al.*, 1996). Limiting circulation to the skin might confer some benefits in that the central blood pool would be preserved and the working muscles might be better perfused with oxygenated blood. However it is unlikely that this explains the action of caffeine in our experiments since caffeine was associated with higher blood lactates which are more a feature of anaerobic metabolism.

Exercise at 35°C in the second experiment may have lead to dehydration resulting in a reduction in muscle blood flow (Gonzalez-Alonso *et al.*, 1998) and consequently fatigue. This is unlikely since lactate concentrations remained stable during exercise and did not increase at fatigue indicating that the working muscles were not in metabolic crisis. Additionally it has been shown that hyperthermia, rather than any

reduction of muscle blood flow, is the most important factor determining fatigue at high ambient temperatures (Gonzalez-Alonso *et al.*, 1999a)

The increase of circulating PRL during exercise is associated with increasing perceptions of exertion and it reaches its highest levels at the point of fatigue (Pitsiladis *et al.*, 1998). PRL secretion from the anterior pituitary gland is controlled by serotonergic and dopaminergic pathways in the hypothalamus and increases in blood levels are an indication of changes in hypothalamic activity (Van de Kar *et al.*, 1996). Activation of hypothalamic pathways with a 5-HT agonist or reuptake inhibitor increases the perception of exertion and causes premature fatigue (Wilson & Maughan, 1992; Marvin *et al.*, 1997). In the former case, where PRL was measured, the levels were raised in parallel with the reduced exercise tolerance. One physiological stimulus for PRL release appears to be an increase in core temperature and thus, presumably, the temperature of blood perfusing the brain, and this is also a powerful signal giving rise to central fatigue (Nybo & Nielsen, 2001a, b,; Chapters 2 & 3). However the effects of raised core temperature both on central fatigue and PRL secretion are modulated by skin temperature so that, for the same core temperature, not only does the exercise feel easier but circulating PRL is reduced when the skin temperature is lower (Chapters 2 & 3). The possibility was therefore considered that caffeine might have similar effects on PRL release and performance to those resulting from skin cooling.

Our first hypothesis was that the beneficial effect of caffeine are mediated by an action on 5-HT and DA in the hypothalamus and that this would be evident as a decrease in the secretion of PRL, possibly changing the relationship between PRL and core temperature, as is seen with exercise in cool ambient conditions (Chapters 2 & 3). The data (Figures 7.5 & 7.11) are quite clear, however, that although caffeine improved exercise performance it did not reduce the release of PRL in response to exercise in either the cool or hot conditions. If anything, PRL release tended to be higher with caffeine. The implication of this finding is that caffeine does not act by affecting the function of pathways in the hypothalamus which regulate the release of PRL from the anterior pituitary.

Our second hypothesis arose from the observations that body temperature is a major factor causing central fatigue. We argued that the action of caffeine would be most apparent during exercise in the heat where central fatigue is most pronounced. The action of caffeine might be to modify the pathways which are involved in sensing body temperature. However, again, the data clearly contradict our hypothesis. The improvement with caffeine was 23% with exercise in the cool environment and 18% when working in the heat. Time to fatigue is not an ideal measure and there were several instances where subjects were stopped at 90 minutes before they reached the point of volitional fatigue. It is possible that, had these subjects been allowed to continue to exhaustion, slightly different values would have been obtained for the effects of caffeine. However, it is most unlikely that it would alter the general conclusion that the effects of caffeine on exercise performance are no greater at high as compared with low ambient temperatures.

The negative findings in this study lead to an important conclusion. If caffeine does not act to improve performance by an action on hypothalamic pathways which respond to core and skin temperature, then there must be at least one other mechanism that is activated during exercise and leads to a decrease in the desire or ability to continue exercising. Clues as to the nature of this, or these, pathways come from the pharmacological action of caffeine in the central nervous system. Caffeine is a purinergic antagonist competing with adenosine at A_{2A} receptors in the brain (Garrett & Griffiths, 1997). Adenosine inhibits the release of dopamine and caffeine therefore enhances its release and, in this respect, has a similar effects to those of amphetamine and cocaine, although these latter agents act as re-uptake inhibitors. Amphetamine and cocaine are widely thought to improve work capacity, although there is very little published evidence to substantiate this belief, and their principle mode of action is to increase dopamine release in the nucleus accumbens thereby providing a reward stimulus. In addition to the nucleus accumbens, A_{2A} receptors are also present in the striatum (Ferre *et al.*, 1997) and it is possible therefore that caffeine may have an action on the basal ganglia. Caffeine has been shown to have a major protective effect against the development of Parkinson's symptoms in human populations and in experimental animal models (Ross & Petrovitch, 2001). A_{2A} receptors are particularly abundant in the nucleus accumbens and striatum and it is possible that caffeine may act by providing a reward stimulus at the nucleus accumbens or by

facilitating activity of the basal ganglia. These considerations point to a new direction for investigations of central fatigue.

In summary, the results presented show that caffeine has a significant effect both on performance and the perception of effort during exercise and this appears not to be the result of a change in substrate utilisation. The lack of effect on PRL release suggests that caffeine is not acting via hypothalamic pathways sensitive to core or skin temperature. The refutation of our two hypotheses raises the possibility of a mechanism of central fatigue which is in addition to those involving hypothalamic pathways and it is suggested that pathways involving the nucleus accumbens or basal ganglia warrant further investigation.

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Chapter 8

GENERAL DISCUSSION

The experiments presented within the body of this thesis confirm the findings of Nielsen *et al*. (Nielsen *et al.*, 1999; Nielsen *et al.*, 2001; Nybo & Nielsen, 2001) and Pitsiladis *et al*. (Pitsiladis *et al.*, 2002) that fatigue in the heat is largely a central process. The new aspects of the work have been to demonstrate that the perception of exertion is related to the release of prolactin, implicating the hypothalamus in the central mechanism and this is most clearly seen in the experiments at different ambient temperatures. While Brisson *et al*. (Brisson *et al.*, 1987; Brisson *et al.*, 1989) were the first to show the influence of skin temperature on prolactin secretion, the work in this thesis has shown that a cool environment has similar effects on perception and endurance.

The results discussed in Chapter 2 show that the increased perception of exertion during exercise in the heat is not only related to the increased heart rate, which is a consequence of thermoregulatory adjustments, but also to increases in skin and core temperature. These temperature signals are most likely integrated at the level of the brain stem and feed in to hypothalamic control of thermoregulatory responses. The release of the pituitary hormone PRL into the blood seems also to be under the influence of an integration of temperature signals, possibly in the hypothalamus and under the control of 5-HT and DA pathways. It is interesting to find that growth hormone and cortisol, other hormones under the control of the hypothalamus, are not subject to the same integrative effect of skin and core temperature.

There is little doubt that 5-HT and DA are involved in thermoregulation and the control of prolactin release and it is most likely that these neurotransmitters are also involved with pathways concerned with the perception of exertion and exercise endurance. Further work needs to be carried out in this area, possibly using PET or f-MRI scanning, to clarify the role of 5-HT in thermoregulation and to look at the changes in brain 5-HT and DA concentrations in response to heat exposure in humans. A large part of the work described here has been to determine the relative contributions of serotonergic and dopaminergic pathways in the control of prolactin release.

Studies in the past have looked at the control of prolactin release by use of neuroendocrine challenges with pharmacological agents such as buspirone and

measuring the PRL response in the blood. However, the work described in Chapter 4 shows that the response to buspirone is confused by the PRL releasing action of buspirone being on average only ~50% 5-HT$_{1A}$ with the remainder attributable to non-5-HT$_{1A}$ mechanisms, probably of a dopaminergic nature. The inter-individual variation in the proportion of the prolactin response attributed to serotonergic stimulation is considerable. Use of the 5-HT$_{1A}$ antagonist, pindolol, may be of use in obtaining a clearer picture of individual differences in 5-HT and DA sensitivity. The prolactin release in response to passive heat exposure seems to be more the result of a withdrawal of DA inhibition than a stimulation of 5-HT activity (Chapter 6).

Further work looking at the roles of 5-HT and DA mechanisms controlling prolactin release is needed to confirm the findings of Chapters 4 and 6, although we are currently limited by the selectivity of pharmacological tools and an inability to easily measure central changes in brain 5-HT and DA concentrations directly.

Chapter 5, in which the combination of buspirone and pindolol was used to assess the separate sensitivity of the 5-HT and DA pathways and to relate this to exercise capacity in the heat provides an insight into the importance of these neurotransmitters in fatigue during exercise in the heat. There was wide inter-subject variation in the prolactin response to buspirone and in the size of the separate 5-HT and DA components. The DA component strongly correlated both with exercise duration and rate of rise of core temperature suggesting that high activity of the dopaminergic pathways in the hypothalamus is a good predictor of exercise tolerance in the heat, possibly as the result of more effective heat loss. Further support for this notion comes from work in animals that has shown increases in brain DA to be involved in thermoregulatory heat loss mechanisms. This has implications for both athletes and individuals in hot environments as the ability to tolerate a high core temperature coupled with poor thermoregulation may result in individuals being at risk of exertional heat illness.

In terms of 5-HT and DA mechanisms and pathways concerned with fatigue in the heat, exercise may be an overly complicated and inefficient way of raising body temperature and all the other factors associated with prolonged exercise (e.g. pain, boredom etc) that affect performance are likely to increase the variance of the results

and reduce the power of the experiment. In this respect the use of passive heating may provide a clearer method to investigate the pathways involved. Chapter 6 produced unexpected results in that it appears the PRL release associated with heat stress has virtually no 5-HT component and may be the result of a withdrawal of DA inhibition at a time when brain DA would be expected to increase. This may to some extent be explained by differential pre and post-synaptic actions of pindolol.

The dosing regimen used for pindolol in this thesis results in a roughly ~44% occupancy at post-synaptic 5-HT$_{1A}$ receptors (Rabiner *et al.*, 2000). This is by no means a total blockade of post-synaptic receptors and a higher pre-synaptic receptor autoreceptor occupation (64%) at this dose has recently been reported (Martinez *et al.*, 2001). The ~44% occupancy at the post-synaptic receptors that mediate PRL release suggests that a 5-HT$_{1A}$ PRL releasing mechanism may still be functioning. A significant pre-synaptic 5-HT$_{1A}$ autoreceptor occupancy would result in an enhanced 5-HT neurotransmission (Rabiner *et al.*, 2000). The result of this enhanced synaptic release of 5-HT and the relatively small post-synaptic antagonist action of pindolol (accounting for only a ~44% receptor occupancy) may account for the lack of change seen in the PRL response to heat exposure. Nevertheless, we have shown pindolol to be effective in blocking the 5-HT$_{1A}$ agonist action of buspirone and it would be a remarkable situation if the pre and post-synaptic actions during passive heating were to cancel each other out exactly.

Caffeine has a significant action potentiating the release of DA in the brain through removal of an inhibitory action of purinergic 2A receptors on DA release. Results in Chapter 7 show that caffeine improves exercise capacity in both normal and hot environments without any peripheral changes that would indicate the existence of a peripheral ergogenic mechanism. However, the measurement of blood PRL showed no indication of changes in central 5-HT or DA activity in response to caffeine. It is possible, therefore, that caffeine acts in a totally different area of the brain to the hypothalamus. It may act in a similar way to cocaine and amphetamine by providing a reward stimulus at the nucleus accumbens or by facilitating motor output through actions on the nigrostriatal pathway of the basal ganglia. Certainly both of these suggestions would be in line with the observed reduction in perception of exertion after caffeine ingestion.

The increases in perceived exertion with prolonged exercise may be the result of reductions in voluntary muscle activation as the result of a central inhibition of motor output. Increases in motor output from the cortex required to overcome this inhibition probably result in the increases in perceived exertion seen during exercise, especially during exercise in the heat. The basal ganglia 'gates' motor output from the brain for tasks requiring coordination of different muscle groups and may therefore be involved in these feelings of increased exertion and fatigue.

The basal ganglia is thought to influence not only motor control, but also several other cognitive and limbic functions (Middleton & Strick, 2000). Indeed Nauta, (1986) suggests that the basal ganglia provide 'part of the neural substrate by which interoceptive or motivational influences can be channelled in to the motor system'. The same author proposed that specific pathways exist linking the limbic system and motor interaction in the basal ganglia in which dopaminergic and ascending serotonergic pathways were of prime importance.

The limbic system of the brain is associated with feelings of motivation and fatigue (Jacobs & Azmitha, 1992) and it is possible that through pathways linking it with the basal ganglia, motor output is modulated by motivation (Nauta, 1986). Evidence of this is seen in patients who have bilateral lesions of the basal ganglia separating it from the limbic system. In one case report, a patient when asked why he stayed in bed for half an hour with an unlit cigarette in his mouth, replied 'I am waiting for a light' (Ali-Cherif *et al.*, 1984), for him there was no motivation to perform the movement to light his cigarette. This limbic-to-motor link within the basal ganglia involving a neurotransmitter (e.g. dopamine and/or serotonin) is further supported by clinical observations in Parkinsonian patients and depressives (Chaudhuri & Behan, 2000). In these conditions a disturbance to dopamine or serotonin in the brain is usually manifested as a disinclination to move (Chaudhuri & Behan, 2000).

It is generally accepted that the limbic system functions to control and regulate emotional states, motivation, learning and memory. Behaviours such as feeding, drinking, sexual activity, sleep are all coded in the limbic system and another important behaviour is that of keeping warm and avoiding extreme temperatures.

Alongside this behavioural coding are those for experiences of pleasure and punishment both of which are associated with motivation. An example is obtaining warmth in a cold environment which is pleasurable as is being cooled in a hot environment. We tend to perform behaviours because we are rewarded by them i.e. they make us feel better and as a result we are motivated to do them. It is possible that the eventual reason why we cease exercising is the reward that is gained through stopping (i.e. heat production drops, we can move to a cooler environment).

5-HT and DA are only two of a number of neurotransmitters in the brain that are involved in controlling movement, thermoregulation and other autonomic responses. A major inhibitory neurotransmitter in the brain is γ–aminobutyric acid (GABA) and the importance of this neurotransmitter within the mammalian brain is unquestionable (Malcangio & Bowery, 1996). Studies that have assessed central fatigue using double-pulse TMS found reduced intracortical facilitation after fatiguing exercise (Tergau *et al.*, 2000). Extensive pharmacological studies have suggested that intracortical inhibition and facilitation are mainly controlled by GABAergic mechanisms (Ziemann *et al.*, 1996) and it is likely that the change in intracortical facilitation seen at fatigue is the result of changes in these GABAergic mechanisms (Tergau *et al.*, 2000). Other glutamaminergic agents as well as dopaminergic and antidopaminergic drugs are also through to affect motor cortex neurones through GABAergic mechanisms (Ziemann *et al.*, 1997; Ziemann *et al.*, 1998). It is interesting to note that dopamine agonists were found to increase intracortical inhibition (Ziemann *et al.*, 1997). Motor cortical excitability is also affected by the 5-$HT_{1B/1D}$ receptor agonist zolmitriptan which has been found to reduce excitability as assessed by TMS (Werhahn *et al.*, 1998). GABA is one of a number of other neurotransmitters that may form an integrated fatigue mechanism involving 5-HT and DA. As easier and quicker techniques are developed for investigating *in vivo* changes within the human brain during exercise and heat exposure a clearer picture of central fatigue mechanisms will undoubtedly evolve probably involving a combination of neurotransmitters and neuronal pathways.

8.1   Future work

At the end of any series of experiments there are always further questions to be answered or new avenues to explore, outlined below are areas for further work and new research.

- The results of Chapter 5 could be expanded upon by the use of DA antagonists and agonists to further explore the relationships between function of the DA pathways and exercise tolerance in the heat.

- Whilst several training studies have looked at relatively short-term training effects on the function of the 5-HT system, no studies have yet looked at any possibly changes that may occur in the DA system in either short or long-term training.

- It is possibly that the adaptations seen with heat acclimation may be related to changes in 5-HT and DA function within the hypothalamus and this is an important area to be looked at using the buspirone challenge in conjunction with pindolol.

- The precise roles of DA and 5-HT in prolactin release during exercise and in passive heating experiments need further investigation. Whether DA agonists block the prolactin response to heat is of great interest as is any subsequent effect on thermoregulation and perception.

- Does passive heating really mimic exercise in terms of changes in perception of exertion, EEG and ability to voluntarily activate muscle during fatiguing contractions?

- The finding that caffeine does not alter hypothalamic 5-HT or DA function opens up another possible range of mechanisms leading to fatigue. Caffeine may act upon the basal ganglia to facilitate motor function or provide a reward

stimulus at the nucleus accumbens. In this respect a comparison with the effects of amphetamine and cocaine would be interesting and informative.

- If there is another mechanism of central fatigue involving the basal ganglia or nucleus accumbens, what is the stimulus that activates this fatigue pathway? Potential candidates include pain and feed-forward signals for motor programs. Basal ganglia function may be evaluated by tests involving rapid and coordinated movements at rest and at fatigue. It is possible that any action of the nucleus accumbens may be blocked by administration of the GABA agonist Baclofen.

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