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Chemoreceptors and cardiovascular control in acute and chronic systemic hypoxia

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Abstract

This review describes the ways in which the primary bradycardia and peripheral vasoconstriction evoked by selective stimulation of peripheral chemoreceptors can be modified by the secondary effects of a chemoreceptor-induced increase in ventilation. The evidence that strong stimulation of peripheral chemoreceptors can evoke the behavioural and cardiovascular components of the alerting or defence response which is characteristically evoked by novel or noxious stimuli is considered. The functional significance of all these influences in systemic hypoxia is then discussed with emphasis on the fact that these reflex changes can be overcome by the local effects of hypoxia: central neural hypoxia depresses ventilation, hypoxia acting on the heart causes bradycardia and local hypoxia of skeletal muscle and brain induces vasodilatation. Further, it is proposed that these local influences can become interdependent, so generating a positive feedback loop that may explain sudden infant death syndrome (SIDS). It is also argued that a major contributor to these local influences is adenosine. The role of adenosine in determining the distribution of O_2 in skeletal muscle microcirculation in hypoxia is discussed, together with its possible cellular mechanisms of action. Finally, evidence is presented that in chronic systemic hypoxia, the reflex vasoconstrictor influences of the sympathetic nervous system are reduced and/or the local dilator influences of hypoxia are enhanced. *In vitro* and *in vivo* findings suggest this is partly explained by upregulation of nitric oxide (NO) synthesis by the vascular endothelium which facilitates vasodilatation induced by adenosine and other NO-dependent dilators and attenuates noradrenaline-evoked vasoconstriction.

Key words
- Hypoxia
- Adenosine
- Chemoreceptors
- Vasodilatation
- Noradrenaline

Introduction

The peripheral chemoreceptors have been known about at least since the beginning of this century. In the 1920's and 1930's it was recognised that stimulation of the carotid and aortic chemoreceptors could produce reflex effects both on the respiratory and cardiovascular system. Since then, a great deal has been discovered about the respiratory, cardiac and vascular responses evoked when the chemoreceptors are selectively stimulated and about the ways in which the responses interact with one another. However, still relatively little is known about the functional importance of peripheral chemore-

ceptors in cardiovascular control under natural, physiological conditions, or in pathological situations. Over the last 10-15 years we have been investigating their role in regulating the cardiovascular system in acute and chronic systemic hypoxia and in particular, we have been trying to establish how the reflex responses interact with the local influences of hypoxia. This is the major subject matter of this review. However, in order to put this work into context, it is first necessary to briefly review what is known of the effects of selective stimulation of the peripheral chemoreceptors.

Primary and secondary responses

Between the 1930's and 1950's, several studies were published on the cardiovascular changes that could be evoked by selective stimulation of the carotid bodies, either by local injection of substances such as sodium cyanide or saline equilibrated with CO_2, or by perfusion with hypoxia and hypercapnic blood. The results obtained were somewhat confusing, some studies showing a rise in arterial pressure, others a fall, some showing bradycardia, and others tachycardia (e.g. 1,2). However, already it was beginning to be recognised that the respiratory consequences of chemoreceptor stimulation could have a large impact on the magnitude and direction of the cardiovascular changes (3). The nature of these respiratory and cardiovascular interactions was largely established by the carefully controlled work of M. de Burgh Daly and colleagues (see Figure 1).

Vascular responses

In a series of studies on dogs anaesthetised with chloralose or barbiturates, Daly and co-workers perfused the vascularly isolated carotid regions with blood from a donor dog in such a manner that the perfusion pressure and therefore the stimulus to the carotid baroreceptors was maintained constant, whilst the carotid bodies were exposed either to normoxic, normocapnic blood or to hypoxic, hypercapnic blood. In early studies, the systemic circulation was performed by a pump at a constant volume per minute so that any change in perfusion pressure reflected changes in systemic vascular resistance. In this way they were able to show that when ventilation was spontaneous, selective stimulation of the carotid chemoreceptors induced a fall in systemic vascular resistance indicating peripheral vasodilatation. However, when ventilation was maintained constant during

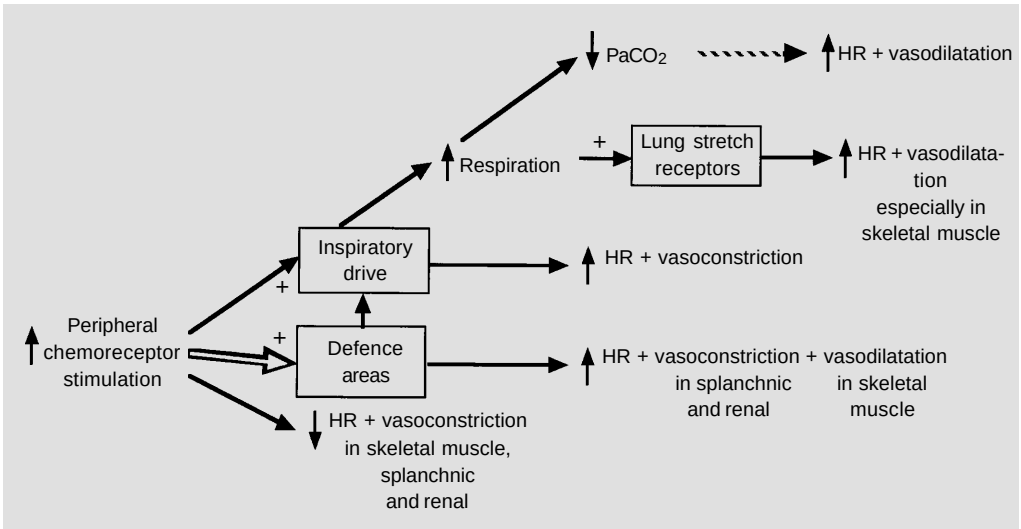

Figure 1 - Schematic diagram showing the primary and secondary effects of selective stimulation of peripheral chemoreceptors. + indicates an excitatory effect. Broken arrow indicates direct or local rather than reflex effects. Open arrow to defence areas indicates a higher threshold effect. For further details see text. HR, Heart rate.

artificial ventilation, the same stimulus evoked an increase in systemic vascular resistance reflecting peripheral vasoconstriction (4,5). This suggested that the primary reflex response to carotid chemoreceptor stimulation was vasoconstriction and that the vasodilatation was secondary to the chemoreceptor-induced hyperventilation. Other experiments showed that the peripheral vasodilatation induced by chemoreceptor stimulation was also greatly reduced or reversed to vasoconstriction if the dog was allowed to hyperventilate, but with the vagus nerves cut, or if the partial pressure of CO_2 in the arterial blood ($PaCO_2$) was maintained constant (4,6). Thus, it was concluded that the two major factors that contributed to the secondary vasodilatation were a reflex triggered by vagal afferent fibres and the hypocapnia that arose from hyperventilation. Further experiments demonstrated that vasodilatation secondary to hyperventilation occurred in skeletal muscle and skin of the limbs, and in renal and splanchnic vasculature, the response being most pronounced in muscle (7,8). As far as the vagal afferents were concerned, the important receptors turned out to be the slowly adapting pulmonary stretch receptors (6,9), while the efferent arm of the reflex was shown to be an inhibition of sympathetic noradrenergic tone (7). The mechanisms underlying the vasodilator effects of hypocapnia have received far less attention: they seem to be mediated mainly by the unloading of the central chemoreceptors for CO_2 and the resulting fall in sympathetic noradrenergic activity (see 10-12).

It is worth noting that in the early studies of Daly and Ungar (5), selective stimulation of the aortic chemoreceptors by perfusion of the aortic arch with hypoxic hypercapnic blood generally evoked peripheral vasoconstriction whether or not ventilation was held constant. This was consistent with the evidence that the vasodilatation that followed carotid chemoreceptor stimulation was secondary to the hyperventilation, for stimulation of aortic chemoreceptors evoked only a small ventilatory response.

Cardiac responses

The heart rate response was also investigated in other early experiments on the dog. Selective stimulation of the carotid chemoreceptors was shown to be more likely to evoke tachycardia when the ventilatory response was large (~200% higher compared to control); when the ventilatory response was small, a bradycardia was often seen (6). Careful examination of these responses revealed that just as with the vascular responses, two aspects of the hyperventilatory response to chemoreceptor stimulation affected the cardiac response: both pulmonary stretch receptor stimulation and hypocapnia contributed to the tachycardia, while the underlying primary response was apparently bradycardia (6-8). However, even when ventilation was maintained constant, carotid chemoreceptor stimulation still sometimes evoked tachycardia in a way that seemed related to the stimulatory drive to ventilation and which was most evident when the cardiac vagal fibres were intact (6,8). This effect was reminiscent of the sinus arrhythmia that persists after lung denervation and which was at first ascribed to “irradiation” of the cardiac vagus with impulses from the respiratory centre (13). More recently, it has become clear that collaterals of central inspiratory neurones exert an inhibitory effect on cardiac vagal neurones (14,15). This helps to explain the increase in heart rate that normally occurs with every inspiration (sinus arrhythmia) and reflects a central neural mechanism by which heart rate tends to increase whenever inspiratory drive increases. There is an additional effect of central inspiratory drive that tends to increase the excitability of sympathetic neurones, thus facilitating tachycardia and vasoconstriction (16-18). However, this effect is much smaller than that exerted on the cardiac vagus.

As far as the aortic chemoreceptors are concerned, the nature of the primary cardiac response evoked by their stimulation is somewhat controversial (see 19). Angell-James and Daly (20) demonstrated in their experiments on the dog, in which the aortic bodies were perfused with hypoxic, hypercapnic blood, that the primary cardiac response was bradycardia. By contrast, Hainsworth and colleagues (21), who performed similar experiments, maintained that the primary response was tachycardia.

Species differences

From all the experiments that have been performed on a range of different species, it seems that during selective stimulation of peripheral chemoreceptors, vasodilatation and tachycardia secondary to the chemoreceptor-induced hyperventilation are more pronounced in the dog than in other species (see Ref. 19). This is probably because the magnitude of the hyperventilatory response is greater in the dog for the same secondary effects can be demonstrated in other species if the conditions are carefully managed to maximise their effects. Nevertheless, among the commonly used laboratory animals, both the tachycardia and peripheral vasodilatation secondary to the hyperventilation of selective chemoreceptor stimulation are weak or non-existent in the cat (22,23), a small tachycardia has been recorded in the rabbit (24,25), although whether or not a secondary vasodilatation influence occurs has not been studied. In the rat, a mild secondary tachycardia has been demonstrated, but the vasodilatation is apparently absent (26). The limited information available on selective chemoreceptor stimulation in primates, including man, suggests that tachycardia and peripheral vasodilatation secondary to hyperventilation can occur but are weak, the influence of central inspiratory drive on heart rate being more important than the influences of pulmonary stretch receptor stimulation or hypocapnia (27-29).

The alerting or defence response

In the middle of the 1960's a report appeared (30) which suggested that primary bradycardia and vasoconstriction, with the possibility of tachycardia and vasodilatation secondary to hyperventilation, may not be an adequate description of the cardiovascular effects of selective peripheral chemoreceptor stimulation. The Italian group led by Zanchetti (30), who were interested in stimuli that might activate or inhibit the areas of the central nervous system concerned with aggressive or defensive behaviour, performed a series of experiments on cats that had been decerebrated at a high level so as to remove the cerebral cortex, but leaving the hypothalamus and brain stem intact. Since the usual cortical inhibitory influence over the brain stem has been removed, such preparations show "spontaneous" episodes of sham rage that are characterised by the somatic and autonomic correlates of range or defensive behaviour in the cat, i.e., clawing and struggling movements, piloerection, retraction of the nictitating membrane, rapid ventilation and a rise in arterial pressure. Zanchetti and colleagues (30) showed that such sham rage episodes could be evoked not only by light mechanical stimulation of the skin, but by selective stimulation of the carotid bodies. This led them to suggest that peripheral chemoreceptors have an excitatory input into the brain regions that mediate the somatic and autonomic components of defensive and aggressive behaviour.

It must be emphasised that at this stage in the investigative process, such a proposal would have had to be treated with caution, for removal of the normal cortical inhibitory influences may have revealed a pathway that was of no functional significance. However, it was very difficult to test the hypothesis more fully because the common anaesthetics of the time, including chloralose, barbituates

and halothane were found to block afferent activation of the brain stem defence areas. In other words, noxious stimuli that were known to evoke defensive or aggressive behaviour with the characteristic autonomic changes in conscious animals could not evoke the autonomic components of the response in animals anaesthetised with these agents (31,32).

Nevertheless, the studies that were performed in the 1950's, 1960's and 1970's by Folkow and Neil (33) in Sweden and by Hilton (34) in England on anaesthetised cats and dogs told us a great deal about what became known as 'the defence response'. They concluded from experiments in which brain sites were stimulated electrically that the regions that integrate the defence response run from the amygdala via an efferent pathway to the ventral hypothalamus, that a contiguous or further region runs through the central grey matter of midbrain and medulla and that all of these regions feed through a single pathway that runs through the ventral medulla. More recently, this pathway was found to synapse onto the neurones of the rostral ventrolateral medulla (RVLM) that is now recognised as being of major importance in setting the resting level of vasomotor tone and in integrating the major cardiovascular reflexes (35-37). The pattern of response that was evoked in anaesthetised animals by electrical stimulation within the integrating regions for the defence response and which was, in fact, crucially important in allowing these regions to be identified, characteristically included a rise in arterial pressure, tachycardia, vasoconstriction in cutaneous renal and splanchnic regions, but vasodilatation in skeletal muscle. This muscle vasodilatation was not secondary to hyperventilation or muscle contraction, but was a true primary component of the 'defence response'. In cats and dogs it was largely mediated by sympathetic cholinergic fibres, but was attributable also to inhibition of sympathetic noradrenergic activity to muscle and to the dilator influence of circulating adrenaline. This cardiovascular pattern of response was accompanied by other autonomically mediated changes including pupillary dilatation, retraction of the nictitating membrane, piloerection and even urination and defaecation (33-37).

Other experiments performed on conscious animals by Folkow and Hilton and their research group (33,34,37) showed that the characteristic cardiovascular pattern of response and the other autonomic changes could be evoked by electrical stimulation in the identified regions, by painful stimuli and by a variety of environmental stimuli ranging from sudden sound to confrontation with an angry cat or dog. The behavioural changes that accompanied these responses ranged from arousal to mild alerting, or a display of defensive behaviour, only reaching overt rage, aggression or the full "defence response" when the stimulus was particularly strong. Indeed, given that i) the pattern of the cardiovascular response was consistent irrespective of the strength of the behavioural response, ii) defensive or aggressive behaviour was only seen *in extremis* and iii) the same cardiovascular pattern was seen in human subjects when they were exposed to relatively mild arousing stimuli, such as sound and mental tasks, the term "defence response" is a misnomer. By emphasising one end of the spectrum, it associates the characteristic pattern of cardiovascular response with extreme circumstances, when in fact this pattern of response probably occurs in every one of us many times during every day, whenever we experience a novel or unexpected situation, or become emotionally stressed by events around us. For these reasons, the term "alerting response" is generally far more useful and accurate (see 37). It is used in the remainder of this review.

A "new" anaesthetic and a "new" facet of the chemoreceptor response

Experiments performed in Birmingham,

UK, around 1980 demonstrated that the steroid anaesthetic alphaxalone-alphadalone (Althesin or Saffan) does not have the same depressant actions on transmission through the defence areas of the brain as the commonly used anaesthetics (see 38 and above). It can be given by intravenous infusion at a rate that produces anaesthesia and full analgesia and yet which does allow the characteristic cardiovascular and autonomic components of the alerting response to be evoked by stimulation of known afferent inputs to the defence areas, for example nociceptor afferent fibres in peripheral nerves. This finding was extremely important for it paved the way for detailed studies of the organisation of the areas of the brain that are responsible for the alerting response, from the afferent inputs, and including the presumed integrating areas of the amygdala and hypothalamus and the efferent pathway of the ventral medulla. In particular, it allowed a more complete study of the responses evoked by peripheral chemoreceptor stimulation. In fact, all of the research we have performed on anaesthetised animals, which is discussed below, was performed under Althesin or Saffan anaesthesia and much of it would not have been possible without it. Since Althesin was the agent originally introduced for use in human patients, but Saffan, which is chemically identical, replaced it as the veterinary product, both agents are referred to below reflecting the year in which the experiments were performed.

In cats anaesthetised with Althesin and in rats anaesthetised with Saffan, we were able to show that selective stimulation of the carotid chemoreceptors evokes the full cardiovascular pattern of the alerting response as described above, including tachycardia and muscle vasodilatation that are not secondary to the hyperventilation (39,40). This response is accompanied by the other autonomic features of the alerting response. In individual animals the pattern of response evoked by chemoreceptor stimulation was virtually identical with the pattern evoked by stimulation in the amygdala or hypothalamic defence areas, or by stimulation of nociceptor afferent fibres in the radial nerve (39). In the cat, the muscle vasodilatation evoked by chemoreceptor stimulation was mediated by sympathetic cholinergic fibres, inhibition of sympathetic noradrenergic fibres and circulating adrenaline (39), while in the rat it was mediated by the last two influences only, as is characteristic of the muscle dilatation of the alerting response in these species (40).

In view of such evidence, one might question whether the primary and secondary responses to chemoreceptor stimulation studied under the more commonly used anaesthetics (see above) have any functional significance. The answer to this is undoubtedly, yes they do! Experiments on the cat showed that when the depth of Althesin anaesthesia was increased by increasing the infusion rate of the agent, then the tachycardia and muscle vasodilatation of the response to carotid chemoreceptor stimulation disappeared and were commonly replaced by bradycardia and muscle vasoconstriction (39). Furthermore, at a light level of anaesthesia, the muscle vasodilatation and tachycardia that were evoked by carotid chemoreceptor stimulation were converted to vasoconstriction and bradycardia simply by reducing the concentration of the solution used to stimulate the carotid chemoreceptors. In other words, under deep Althesin anaesthesia or with mild chemoreceptor stimulation, the response that remained was a mild hyperventilation, generalised vasoconstriction in muscle and other tissues (41) with bradycardia, just as would be expected on selective stimulation of carotid chemoreceptors under chloralose or barbiturates, given that the secondary effects of hyperventilation upon the cardiovascular system are weak in the cat (see above). Essentially similar results were obtained in the rat under Saffan except that the tachycardia was more persistent, consistent with the more pronounced tachycardic response to

hyperventilation in this species (see above and 40).

On this basis, it seemed reasonable to propose that in the absence of anaesthesia, mild chemoreceptor stimulation would evoke the primary bradycardia and peripheral vasoconstriction, complicated by the tachycardia and/or vasodilator effects of hyperventilation in those species in which these secondary influences are evident, but that strong chemoreceptor stimulation would evoke the full cardiovascular pattern of the alerting response (see Figure 1). Correspondingly, behavioural arousal or alerting would be expected to accompany mild chemoreceptor stimulation, while obvious aggressive or defensive behaviour might be expected on strong chemoreceptor stimulation (39).

Unfortunately, these proposals have not yet been fully tested: it is difficult to selectively stimulate carotid chemoreceptors in a conscious animal and it is technically difficult to record a full range of cardiovascular variables. It is also difficult to establish in conscious animals whether any muscle vasodilatation and tachycardia are integral components of the alerting response rather than secondary effects of hyperventilation, since this requires the use of paralysing agents. Nevertheless, the fragmentary evidence that is available is promising (for a more complete discussion, see Ref. 19). In particular, Franchini and Kreiger (42) showed in experiments on conscious rats that systemic administration of potassium cyanide which stimulates peripheral chemoreceptors evoked a dose-dependent behavioural response that ranged from arousal to escape behaviour. The accompanying cardiovascular response was a graded rise in arterial pressure, with tachycardia when the dose of potassium cyanide was low and bradycardia when it was higher: regional flows were not recorded. The increasing dominance of the bradycardia is surprising in view of the results discussed above but does not necessarily argue against the general hypothesis. Rather, it may reflect the fact that potassium cyanide given into the systemic blood stream does not provide a simple, selective stimulus to the carotid chemoreceptors. For example, in the rat, carotid chemoreceptor stimulation commonly evokes an augmented breath or respiratory gasp, probably because chemoreceptor stimulation facilitates the input of rapidly adapting irritant receptors: an augmented breath may be accompanied by a transient, but pronounced bradycardia (40,43).

The evidence available from studies on systemic hypoxia in conscious animals and human subjects lends good support to the general idea that the defence areas can be activated by carotid chemoreceptor stimulation (see below).

Functional implications of chemoreceptor-induced activation of the defence areas

If it is accepted that experimental stimulation of the carotid chemoreceptors can activate the brain defence areas, then it is reasonable to assume that in everyday life stimuli that activate these receptors, such as hypoxia, hypercapnia and acidaemia, are some of many inputs, together with visual, auditory, tactile, noxious and emotional inputs, that have the potential to produce the various facets of behavioural arousal and the characteristic cardiovascular and autonomic components of the alerting response. Since peripheral chemoreceptors are tonically active even at normal values of arterial PO_2, PCO_2 and pH, it can be argued that their input contributes to the general level of arousal in the awake state (19,40,41). Further, since efferent activity from the defence areas contributes to the tonic level of arterial pressure through its influence on the RVLM, it can be argued that peripheral chemoreceptors help to set the resting level of arterial pressure in the awake state by their influence on defence areas, as well as by their more direct influence on the RVLM or other descending path-

ways to sympathetic preganglionic neurones (40,41). On the other hand, since during sleep, hypoxic stimulation of the peripheral chemoreceptors is well known as a stimulus that can serve to wake the individual, it is now reasonable to argue that this reflects specific activation of the defence areas by the chemoreceptors, and that the increased activity in the defence areas contributes to the accompanying rise in arterial pressure (see 19). It is then a simple step to propose that longer term activation of peripheral chemoreceptors, as occurs for example in chronic obstructive sleep apnoea, and the hypertrophy and increased sensitivity of the carotid bodies that has been reported in essential hypertension might contribute to the hypertension of these conditions by activating the defence areas (44,45). This hypothesis gains weight when one appreciates the effects of defence area activation on the baroreceptor reflex and realises that chemoreceptor stimulation can mimic these effects, as is discussed in the next section.

Interactions of chemoreceptor stimulation with the baroreceptor reflex

Arterial baroreceptor stimulation, as occurs when pressure in the carotid sinus and aortic arch is raised, produces reflex bradycardia and vasodilatation which tends to return arterial pressure to the normal level. From experiments performed on animals anaesthetised with the commonly used anaesthetics such as chloralose and barbiturates, it has generally been reported that selective stimulation of the baroreceptors inhibits the peripheral vasoconstriction evoked by selective chemoreceptor stimulation (46-49). This may occur, at least in part, by an inhibitory influence of baroreceptor afferent input on chemoreceptor afferent input within the nucleus tractus solitarius (50). Since chemoreceptor stimulation itself raises arterial pressure by causing vasoconstriction, this suggests that even the concurrent stimulation of the baroreceptors this produces must limit the reflex effects that are produced by the chemoreceptors. Moreover, in the presence of any other stimulus to the individual which is simultaneously raising arterial pressure, one can infer that this would tend to further reduce the reflex vasoconstriction that the chemoreceptors can produce.

However, it is also known, from experiments in which the hypothalamic defence areas were electrically stimulated, that activation of the defence areas causes inhibition of both the bradycardia and peripheral vasodilatation evoked by stimulation of the baroreceptors (51). In other words, the pattern of the defence response predominates and the baroreceptor reflex is suppressed. This suppression is explained, at least in part, by a pathway from the hypothalamus which influences the baroreceptor reflex pathway within the nucleus tractus solitarius, close to the site of termination of the baroreceptor afferent fibres (52). In view of this evidence it was reasonable to question how the effects of chemoreceptor and baroreceptor stimulation would interact under conditions in which peripheral chemoreceptors could activate the defence areas.

In fact, experiments on cats anaesthetised with Althesin, in which the level of anaesthesia was light enough and the chemoreceptor stimulus was strong enough to evoke the full pattern of the alerting response, showed that during this response even a supramaximal stimulus to the baroreceptors, an increase in carotid sinus pressure to 250 mmHg, produced no detectable effect on the cardiovascular system. Furthermore, if a baroreceptor reflex was initiated first and then the carotid chemoreceptors were stimulated, the cardiovascular pattern of the alerting response completely overcame the cardiac and vascular components of the baroreceptor reflex. On the other hand, if the depth of Althesin anaesthesia was increased, or the stimulus to the chemoreceptors reduced, so

that carotid chemoreceptor stimulation evoked bradycardia and vasoconstriction even in muscle, then the reflex effects of baroreceptor stimulation interacted in an additive way and the bradycardia of chemoreceptor stimulation was accentuated, while the vasoconstrictor responses were reduced, or reversed to vasodilatation, as might have been predicted from the experiments on animals anaesthetised with the commonly used anaesthetics (53).

Thus, from these results it can be argued that a strong stimulus to the carotid chemoreceptors occurring acutely, as for example on sudden exposure to a hypoxic environment, would not only activate the defence areas to produce the cardiovascular pattern of the alerting response, but would suppress the baroreceptor reflex so that the rise in arterial pressure would be greater than otherwise expected and the perfusion pressure for the delivery of blood flow to the skeletal muscles would be consequently greater. This, as has been argued for other stimuli that activate the defence areas, may confer a split second advantage on the individual, in this case allowing him or her to run away from the hypoxic environment more readily (34,37). Further, more prolonged, but strong stimulation of the carotid chemoreceptors as might occur in sleep apnoea might, by activating the defence areas, tend to inhibit the normal ability of the baroreceptor reflex to buffer increases in blood pressure and so further contribute to the development of hypertension.

Acute systemic hypoxia

The information that is reviewed above about the effects of selective stimulation upon the cardiovascular system is the information that provided the background for our studies on the effects of acute systemic hypoxia. But it was also important for us to recognise that systemic hypoxia can have additional effects on the respiratory and cardiovascular systems that may interfere with the effects of selective stimulation of the carotid chemoreceptors. All of these effects are indicated schematically in Figure 2. Briefly, there is substantial evidence that hypoxia of the central nervous system can depress ventilation by influences on central respiratory neurones. Further, there is evidence that central neural hypoxia can increase sympathetic activity to the heart and circulation, probably in part by acting on neurones of the ventrolateral medulla (see

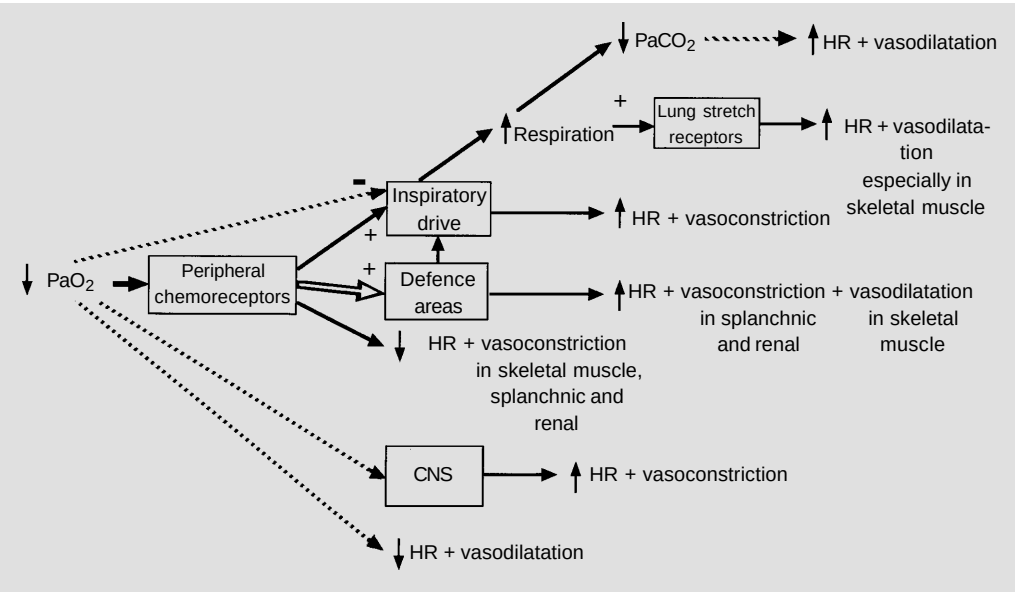

Figure 2 - Schematic diagram showing the possible effects of systemic hypoxia. + indicates an excitatory effect; - indicates an hibitory effect. Arrows as in Figure 1. CNS, Central nervous system; HR, heart rate.

19,54). On the other hand, it is well known that by direct actions on the heart, hypoxia causes bradycardia and myocardial depression, while by direct actions on the tissues of the systemic circulation hypoxia causes vasodilatation. Thus, we were aware that the final or observed response to systemic hypoxia must represent a balance of these individual effects. It was our intention to establish their relative importance.

Systemic hypoxia and the alerting response

In cats and rats anaesthetised with Saffan, it became clear that systemic hypoxia achieved by breathing 12, 8 or 6% O_2 could evoke the full cardiovascular pattern of the alerting response (55,56). In such experiments it is difficult to deduce the threshold for the response, but a response pattern that was recognisable as that of the alerting response was commonly seen when PaO_2 dropped to below 66-65 mmHg. This is not only consistent with the evidence that selective stimulation of carotid chemoreceptors can evoke the cardiovascular pattern of the alerting response under Althesin or Saffan anaesthesia, but is consistent with many reports in the literature that systemic hypoxia elicits behavioural arousal or even escape behaviour in the rat, rabbit, dog and in human subjects (see 19 for references) and with evidence that episodes of behavioural arousal during hypoxia were associated with tachycardia and a rise in arterial pressure (57). The fact that signs of behavioural arousal were reported when PaO_2 reached relatively low values of below 50 mmHg may indicate that the behavioural components of the alerting response require a more severe level of hypoxia than the cardiovascular components. However, the apparent discrepancy may simply reflect differences in the observer's ability to detect the cardiovascular pattern of the alerting response in an anaesthetised animal and a change in behaviour in a conscious individual.

In a recent study on conscious rats instrumented with an ultrasonic Doppler probe for recording hindquarters blood flow and an arterial cannula for recording arterial pressure and heart rate (58), we have attempted a more complete analysis of the behavioural and cardiovascular changes evoked by exposure to 8% O_2. Within the first 3 min of hypoxia, we have seen the full repertoire of behaviour that is typical of the rat in states ranging from mild arousal to overt fear, or aggression. Concomitantly, arterial pressure, heart rate and hindquarters vascular conductance tended to rise, but it would be difficult to associate these changes specifically with the alerting response, rather than with the exercise that is occurring as well. For the remainder of a 20-min period of hypoxia the signs of arousal were less marked and the cardiovascular variables returned towards or below baseline, consistent with the observations we have made under Saffan anaesthesia (see below).

Interestingly, when the same animal was exposed to the same level of hypoxia on the following day, then the signs of behavioural arousal were much less pronounced (58). This suggests that at least the behavioural components of the alerting response evoked by chemoreceptor stimulation habituate on repetition of the stimulus, as is known to occur with repetition of other alerting stimuli. The important question, that we cannot yet answer, is whether when hypoxic stimulation of the chemoreceptors is repeated, their ability to evoke the cardiovascular components of the alerting response and thereby to suppress the baroreceptor reflex also habituates. As far as other alerting stimuli are concerned, such as sudden sound, cold water or emotional stress, it is known that the cardiovascular components of the alerting responses and behavioural alerting do not necessarily habituate together. Moreover, there may be variation between individuals, so that some show habituation of one or more cardiovascular components of the alerting response on repetition of

the stimulus, while others show no change, or even sensitisation of one or more components (37). For systemic hypoxia, it is tempting to speculate that those individuals who show no habituation of the cardiovascular components of the alerting response to chemoreceptor stimulation would be most at risk of suffering the acute effects of a sudden rise in arterial pressure and tachycardia, such as aneurysm or myocardial infarction during repeated asthmatic attacks and more at risk of developing hypertension as a consequence of sleep apnoea.

The gradual effects of hypoxia

From our experiments on Saffan anaesthetised cats and rats, it is clear that the cardiovascular pattern of the alerting response is most likely to be evoked within the first 1-$1^1/_2$ min of hypoxia. This pattern seems to be superimposed upon more gradual changes that are graded with the level of hypoxia (55,56). In the cat, there is hyperventilation which is well maintained at least during a 3-min period of hypoxia, accompanied by a rather small tachycardia and rise in arterial pressure and no change, or slight vasodilatation in mesenteric, renal and skeletal muscle vasculature (56). In the rat, there is also hyperventilation, but with a more pronounced tachycardia and substantial vasodilatation in mesenteric, renal, cerebral and skeletal muscle vasculature. Moreover, the hyperventilation and tachycardia tend to return towards, or below the baseline, this becoming more obvious if the period of hypoxia is prolonged to 5 or 10 min (55,59,60; see Figure 3). The net effect of these changes is that arterial pressure falls in the rat to an

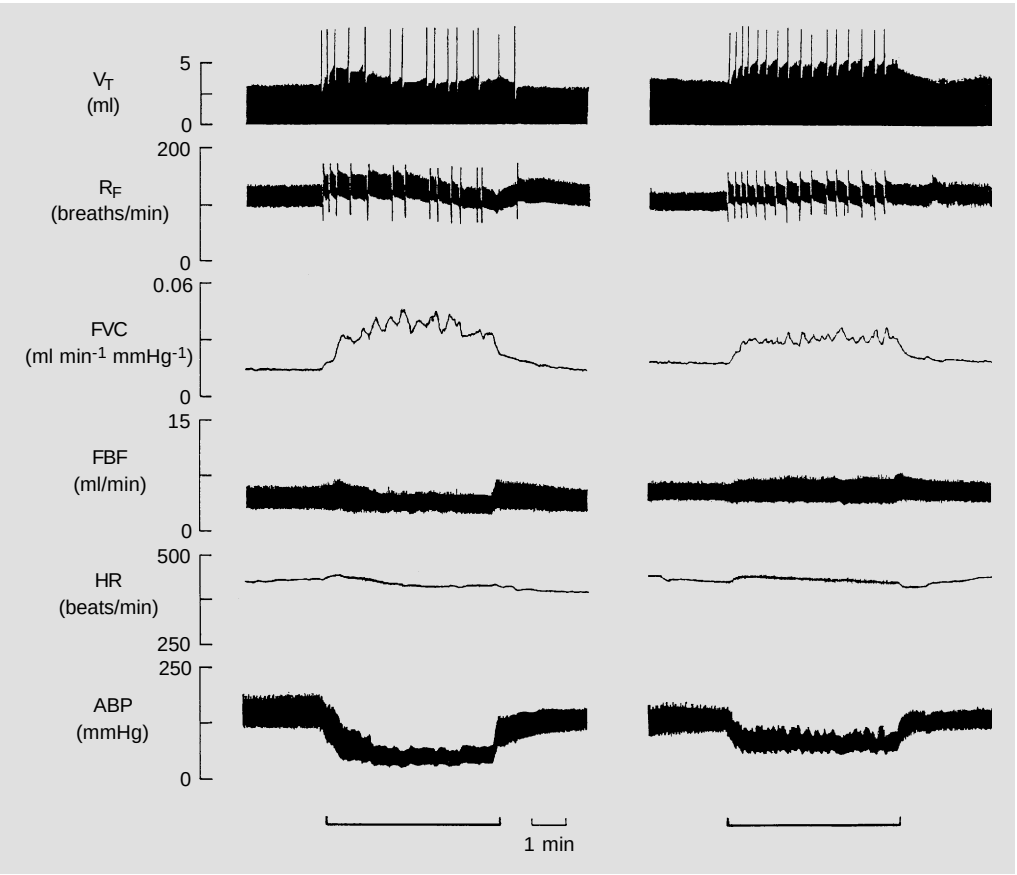

Figure 3 - Original traces showing the effect of the adenosine receptor antagonist 8-phenyltheophylline (8-PT) on the respiratory and cardiovascular responses evoked in the anaesthetised rat by a 5-min period of breathing 8% O_2. V_T, Tidal volume; R_F, respiratory frequency; FVC, femoral vascular conductance; FBF, femoral blood flow; HR, heart rate; ABP, arterial blood pressure. 8-PT was given between the left and right hand panels. Note that after 8-PT the V_T and HR were better maintained during hypoxia while the increase in FVC and fall in ABP were reduced.

extent that is graded with the level of hypoxia (55).

By performing experiments in which we have vagotomised, kept $PaCO_2$ constant, or paralysed and artificially ventilated the animals, we have been able to establish the roles of the secondary effects of hyperventilation in these gradual changes. They are relatively weak in both species in agreement with their effects on the responses evoked by selective stimulation of carotid chemoreceptors (see above). In the cat, the lung stretch receptors with vagal afferents apparently have no significant influence on the heart rate or vascular responses to systemic hypoxia, but if $PaCO_2$ is prevented from falling with the hyperventilation, then there is bradycardia rather than tachycardia, vasoconstriction in the renal and mesenteric circulation, and no change or slight dilatation in skeletal muscle (56). This strongly suggests that in the normal situation, the fall in $PaCO_2$, plays a major role in counteracting the bradycardia and vasoconstriction that would be expected as a primary response to hypoxic stimulation of carotid chemoreceptors. Since vasoconstriction did not occur in muscle, even when $PaCO_2$ was controlled, it seems likely that in this tissue, the neurally mediated vasoconstriction was opposed by the local dilator influences of hypoxia.

By contrast, in the rat, the fall in $PaCO_2$ makes only a minor contribution to the tachycardia and muscle vasodilatation in severe hypoxia (61). On the other hand, the lung stretch receptors with vagal afferents do contribute to the tachycardia, but not to the peripheral vasodilatation (62). Indeed, when ventilation was kept constant by artificial ventilation, then hypoxia induced bradycardia, but with substantial vasodilatation in skeletal muscle (60). By considering these results together with the effects of various antagonists of the sympathetic nervous system, we have concluded that the primary bradycardia of hypoxic stimulation of carotid chemoreceptors is opposed both by the secondary effects of hyperventilation on lung stretch receptors and by the ability of central nervous hypoxia to increase cardiac sympathetic activity (56,60,62). The late bradycardia that occurs when hypoxia is prolonged can be attributed not only to the primary bradycardia of carotid chemoreceptor stimulation, but also to the local effects of hypoxia on the heart (60,63), while the late decrease in ventilation towards or below the baseline reflects the central effect of hypoxia on respiratory neurones (63). Clearly, this later decrease in the ventilatory response means that lung stretch receptors make less contribution to the tachycardia as the period of hypoxia progresses (see 60). The peripheral vasodilatation in mesenteric and in muscle vasculature largely reflects the local dilator effects of tissue hypoxia. These influences are so strong that they apparently overcome the primary reflex vasoconstriction expected from carotid chemoreceptor stimulation and are largely responsible for the fall in systemic arterial pressure (55).

The rat is, therefore, far more dominated by the local effects of hypoxia on central respiratory neurones, heart and vasculature than the cat. This is of particular interest because there are reports in the literature indicating that the respiratory and cardiovascular responses evoked by systemic hypoxia in neonates are very similar to those we have seen in the rat: characteristically, ventilation increases and then falls and generally arterial pressure decreases (see 64,65). We have suggested this may arise because small mammals in general, whether the neonates of large mammalian species or the adults of small mammalian species, have a higher rate of O_2 consumption per gram body weight than larger mammals: they may therefore be more susceptible to the local metabolic effects of hypoxia when O_2 supply is reduced (55). Unfortunately, the pattern of response that is produced by the local effects of hypoxia is potentially life-threatening as is explained below.

A positive feedback loop?

The fact that respiration increases initially in neonates who become hypoxic, but then falls, has been put forward as an explanation for sudden infant death syndrome (SIDS) or cot death (66). We believe the concomitant cardiovascular changes are crucially important in this situation. In experiments on rats in which we have recorded cerebral blood flow, we have demonstrated that although there is cerebral vasodilatation in response to systemic hypoxia, cerebral blood flow begins to fall when ventilation and heart rate drop below their resting values and arterial pressure drops below the autoregulatory range of about 60 mmHg (60). We have proposed that the respiratory and cardiovascular changes may generate a positive feedback loop (Figure 4). Thus, when ventilation begins to fall, this exacerbates the fall in PaO_2 and the peripheral vasodilatation and bradycardia that are caused by the local effects of hypoxia, so potentiating the fall in arterial pressure. Once the arterial pressure is below the autoregulatory range, then cerebral blood flow falls, the O_2 supply to the brain is further reduced and the central neural hypoxia is exacerbated so that the central respiratory neurones are further depressed (60).

Our experiments on newborn piglets indicate that this positive feedback loop is particularly likely to develop in individuals in whom the stimulatory effect of hypoxia on respiration is weak (64). Furthermore, our studies on rats that have been kept chronically hypoxic from birth in a hypoxic chamber, have shown that the bradycardia fall in arterial pressure and fall in cerebral blood flow induced by acute hypoxia is greatly exaggerated in these animals relative to those seen in controls (67). Thus, it seems that chronic hypoxia from birth accentuates those very responses that contribute to the positive feedback loop. This is entirely consistent with studies indicating that babies who are most at risk of SIDS are those who have been hypoxic from birth (66).

The mediator of the local effects of hypoxia - adenosine

Although the section above has emphasised the importance of the local effects of hypoxia in the rat and other small mammals, it is important to recognise that the local effects do contribute to the overall response to hypoxia in larger mammals as well. It is simply that in larger mammals the balance is tipped more strongly towards the neurally mediated, reflex effects of hypoxia. Thus, a fall in ventilation when hypoxia is severe or prolonged that can be attributed in part to a depressive effect of hypoxia on the central nervous system (68) is a generally recognised phenomenon occurring, for example, in the cat and in human subjects (69,70). Similarly, it is known that heart rate and arterial pressure begin to fall when systemic hypoxia is severe or prolonged, for example, in both the cat and human subjects (56,71). It is also known that in human subjects systemic hypoxia induces vasodilatation in splanchnic circulation and forearm muscle (71,72) and that when the influence of the sympathetic nerve fibres on forearm muscle is blocked, systemic hypoxia still induces substantial muscle vasodilatation, as would be consistent with a local dilator influence of hypoxia

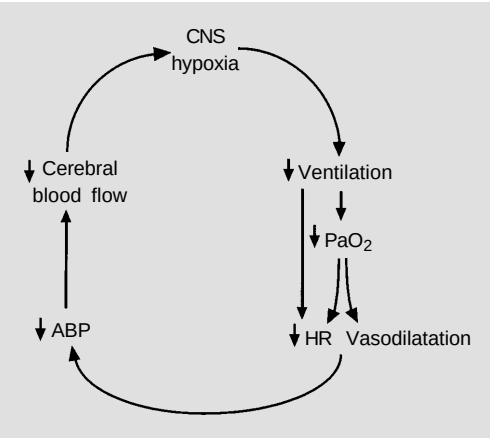

Figure 4 - The proposed positive feedback loop that may develop during systemic hypoxia, particularly in small adult mammals and neonates. CNS, Central nervous system; HR, heart rate; ABP, arterial blood pressure.

(72). The question of what produces these local effects is therefore important and may be of general relevance.

It is generally accepted that adenosine is produced in tissues when they become hypoxic. When we began our experiments adenosine concentrations had been shown to rise substantially in the brain soon after the onset of hypoxia (73). Moreover, adenosine had not only been implicated in hypoxia-induced cerebral vasodilatation (74), but it had been shown to depress ventilation by actions on respiratory neurones. In fact, substances such as aminophylline and theophylline whose effects include blockade of adenosine receptors have been shown to reduce respiratory depression associated with systemic hypoxia in neonates (75), as well as in adult cats and human subjects (69,70). Further, adenosine is well known to be released in the heart under hypoxic conditions and adenosine not only induces coronary vasodilatation (76), but can induce bradycardia by direct actions on the sinoatrial node and by interfering with sympathetic and vagal transmission (77). Adenosine had also been shown to be released by skeletal muscle during muscle contraction when there is a relative hypoxia (78) and by the use of various adenosine antagonists, adenosine had been implicated in the muscle vasodilatation that occurs during contraction (e.g. 79). We therefore hypothesised that adenosine plays a major role in the cerebral and respiratory and cardiovascular changes that occur in the rat during systemic hypoxia.

To test this hypothesis we have used 8-phenyltheophylline (8-PT) which is a selective adenosine receptor antagonist that does not inhibit phosphodiesterase activity like aminophylline and theophylline. In full accord with our hypothesis, 8-PT given systemically greatly reduced the late decrease in ventilation and bradycardia, the muscle vasodilatation and fall in arterial pressure induced by a 5- or 10-min period of breathing 12, 8 or 6% O_2 (59,63; see Figure 3). In addition, when 8-PT was applied topically to the cerebral cortex, the hypoxia-induced cerebral vasodilatation was attenuated (80). On the other hand, when 8-sulphophenyltheophylline, an adenosine receptor antagonist that does not cross the blood brain barrier, or adenosine deaminase, which breaks down adenosine, but does not cross the blood brain barrier, were given systemically, they did not affect the respiratory response to hypoxia, had less effect on the late fall in heart rate than 8-PT, but still caused a substantial reduction of the muscle vasodilatation (63). These results provide strong evidence that adenosine released locally in the brain, heart and skeletal muscle is responsible respectively for the respiratory depression and cerebral vasodilatation, the bradycardia and the muscle vasodilatation. They also reinforce our proposal that the respiratory and cardiovascular changes are interdependent in a potentially positive feedback manner (60,63; see Figure 4): if the antagonist used had no effect on the respiratory component of the response then its effects on the cardiac and vasodilator components were less pronounced than for an antagonist that had the potential to affect all components of the response. By using specific antagonists for the subtypes of adenosine receptor, we have recently shown that the adenosine that is released in systemic hypoxia probably acts via A_{2A} receptors to produce cerebral vasodilatation (81), but via A_1 receptors to produce muscle vasodilatation (82,83). Our own evidence, together with that of others, indicates the respiratory depression and bradycardia are probably mediated by A_1 receptors (82,84).

Since adenosine is generally released in hypoxic tissues and since its ability to depress respiration by a central action and to cause bradycardia and cerebral and muscle vasodilatation is common to a range of mammalian species, it seems reasonable to assume that adenosine plays a major role in the local effects of hypoxia on respiration and

the cardiovascular system in all mammalian species. Experiments already performed on hypoxia-induced respiratory depression and on hypoxia-induced bradycardia in isolated hearts in a number of species including adult man, cat, rabbit and guinea pig are consistent with that view (69,70,77).

Interactions between reflex and local effects of hypoxia in muscle microcirculation

The experiments of the type discussed above in which pharmacological antagonists are given systemically are useful in that they can indicate whether particular nerve-mediated, hormonally mediated or local influences contribute to the gross change in vascular conductance or resistance that occurs in skeletal muscle or other tissues during systemic hypoxia. However, they tell us little of how these factors interact at the level of tissue microcirculation to determine the O_2 supply to the capillaries and thereby to the tissue cells. Further they suffer from the disadvantage that many pharmacological antagonists when given systemically affect arterial pressure and therefore their effect on the change in regional vascular conductance induced by systemic hypoxia may be in part related to their effect on perfusion pressure. To address these issues we have performed many experiments on the microcirculation of the spinotrapezius muscle of the rat with the muscle prepared for intravital microscopy. Pharmacological agonists and antagonists can then be applied topically to the muscle in doses that achieve local effects, but which do not affect systemic variables, or the changes induced in them by systemic hypoxia.

Experiments of this type have shown that systemic hypoxia induces both increases and decreases in the diameter, dilatation and constriction, respectively, of individual arterioles of the spinotrapezius muscle (85). On balance, dilator responses are more common than constrictor responses and dilator responses are more common and larger in the distal, or terminal arterioles, than in the proximal arterioles (85). Thus, the fact that gross muscle vascular conductance increases during systemic hypoxia disguises the fact that within muscle some vessels constrict while a majority dilate.

When an α adrenoceptor antagonist, phentolamine, was applied to the spinotrapezius, mean increases in arteriolar diameter produced in particular sections of the arteriolar tree by systemic hypoxia were potentiated, while mean decreases in arteriolar diameter were reduced, or reversed to dilator responses. Moreover, the sections of the arteriolar tree that were most affected were the primary and secondary arterioles of 18-40 µm that are most strongly constricted by direct stimulation of the sympathetic nerves (81). More detailed analysis of each section of the arteriolar tree showed that only vessels that constricted in hypoxia were affected by phentolamine, while those that dilated were not (86). These observations are consistent with the occurrence of an increase in sympathetic nerve activity to skeletal muscle during systemic hypoxia, as would be expected from the primary response to carotid chemoreceptor stimulation (see above) and suggest that the sympathetic nerve activity preferentially constricts the primary and secondary arterioles. However, they also indicate that many arterioles from all sections of the arteriolar tree do not respond to sympathetic activation in hypoxia, even though we know they are innervated by sympathetic fibres.

On the other hand, when the adenosine receptor antagonist, 8-PT, was topically applied to the spinotrapezius muscle it reduced mean increases in diameter induced in particular sections of the arteriolar tree by systemic hypoxia, or converted them to mean decreases in diameter, the responses of the terminal arterioles being particularly affected (87). These observations are, of course, fully consistent with the evidence discussed above

that adenosine plays a major role in the hypoxia-induced muscle vasodilation and indicate that the terminal arterioles, the vessels that are traditionally thought to be most responsive to locally released vasodilator metabolites, are particularly affected by locally released adenosine. Nevertheless, only those arterioles that were dilated by systemic hypoxia were affected by 8-PT, even though we were able to show that all arterioles were responsive to exogenous adenosine (87).

Our observations on the effects of antagonists of the actions of circulating hormones are comparable with those just described in that a vasopressin receptor antagonist affected the arterioles that constricted in response to systemic hypoxia, but had no effect on those that dilated (88), whereas a ß adrenoreceptor antagonist affected the arterioles that dilated in response to systemic hypoxia, but had no effect on those that constricted (86). These observations were in turn consistent with evidence that vasopressin is released in response to selective stimulation of carotid chemoreceptor and that vasopressin receptor blockade reduces the increase in gross muscle vascular conductance induced by systemic hypoxia (89) and with evidence that adrenaline is released into the blood stream by hypoxic stimulation of carotid chemoreceptors and makes a contribution, albeit small, to the hypoxia-induced increase in gross muscle vascular conductance (90). However, we have to conclude from the microcirculatory studies that some arterioles "escape" the constrictor influence of vasopressin or "escape" the dilator influence of adrenaline during systemic hypoxia, even though one would expect the concentrations of the two hormones reached in individual arterioles to be very similar and even though we could show they were all capable of responding appropriately to exogenous vasopressin or adrenaline (88).

The most obvious explanation for the heterogeneity of the responses seen amongst the arterioles during systemic hypoxia is that the major determinant of the response in any given arteriole is in fact the local level of hypoxia (see 91 and Figure 5). It is known that there is considerable variation in the level of tissue PO_2 found in different regions of skeletal muscle during normoxia (92). This reflects several factors including i) regional differences in the oxygen consumption of the nearby muscle fibres, ii) distance along the arteriolar tree from the major supplying artery given that O_2 diffuses out of arterioles along their length, and iii) proximity of arterioles and venules with opposite directions of blood flow, given that O_2 may diffuse from an arteriole to a venule, thus short-circuiting O_2 supply to tissue downstream of the arteriole. It would be expected that adenosine would reach higher concentrations during systemic hypoxia in regions of the muscle where the local level of hypoxia is relatively severe, either because of a high level of O_2 consumption or a poor "anatomical" distribution of O_2. The arterioles in such regions would be preferentially dilated by adenosine and this may in turn increase the susceptibility to other dilator influences such as the ß adrenoreceptor mediated effect of adrenaline. On the other hand, the concentration of adenosine would be low in regions of the muscle where the local level of hypoxia is relatively mild. The arterioles in such regions may then be particularly vulnerable to constrictor influences such as those of sympathetic nerve activity and circulating vasopressin (91).

This heterogeneity in the responses of the muscle arterioles during systemic hypoxia may be functionally important in allowing a more homogenous distribution of O_2 in the various parts of the muscle at a time when the gross O_2 supply is reduced. In other words, the behaviour we have seen in individual arterioles of muscle during systemic hypoxia may explain the finding that the variation in the levels of tissue PO_2 within muscle is considerably reduced during systemic hypoxia concomitant with the fall in

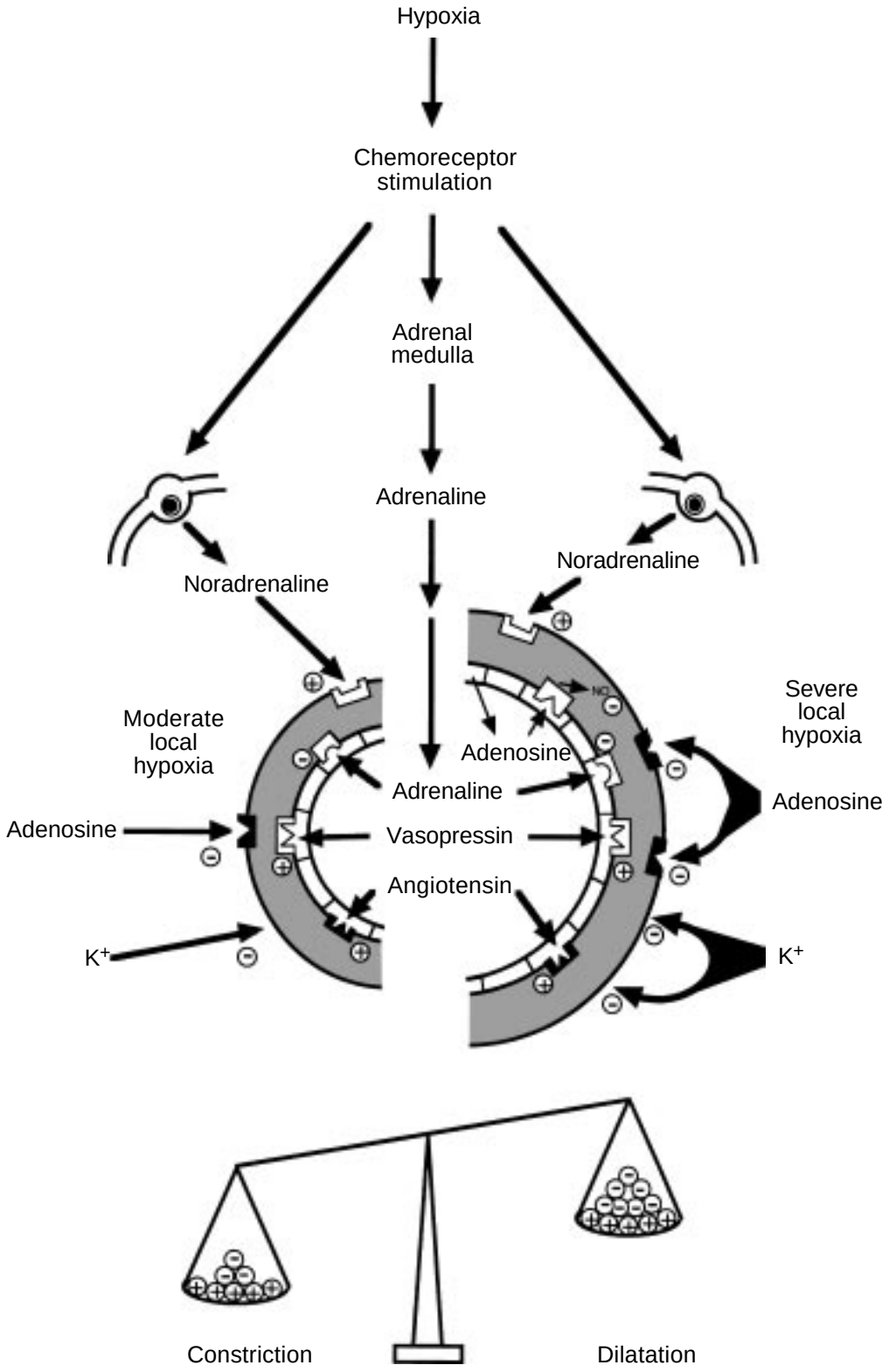

Figure 5 - Schematic diagram showing how the balance of the nerve and hormonally mediated constrictor influences and the locally and hormonally mediated dilator influences of systemic hypoxia on blood vessels within skeletal muscle may be different depending on the local level of hypoxia. In regions where the level of hypoxia is more severe the balance may be more readily tipped towards vasodilatation. For further discussion see text.

average tissue PO_2 (92). It may also demonstrate how the O_2 consumption of resting muscle can be maintained constant during systemic hypoxia, for a more homogeneous distribution of blood flow and therefore O_2 supply through the capillary network would help muscle fibres to maintain their O_2 consumption by increasing their O_2 extraction (93).

Mechanisms of action of adenosine

On the basis of the literature, it is theoretically possible that during systemic hypoxia adenosine is released from skeletal muscle fibres, vascular smooth muscle, or endothelium. Also ATP released from these sites or from sympathetic nerve varicosities or red blood cells may be hydrolysed extracellularly to adenosine by the action of 5'ectonucleotidase. Further, it is theoretically possible that adenosine induces vasodilatation by acting on the vascular smooth muscle or endothelium, or more indirectly, by acting on the skeletal muscle fibres, or at prejunctional sites on the sympathetic nerve varicosities (91). So far, we have only investigated a few of these possibilities. Firstly, we found that αß methylene ADP, which inhibits the action of 5'ectonucleotidase, had no significant influence on the dilatation induced in hind limb muscles of the rat by systemic hypoxia. This indicates that most of the adenosine that is vasoactive is released as such from the intracellular sites (94).

Since it was already known that systemic hypoxia leads to an increase in plasma levels of K^+ (95) and since we had shown that the action of circulating adrenaline upon ß adrenoreceptors of skeletal muscle was important in stimulating the re-uptake of K^+ during systemic hypoxia, probably by acting on $ß_2$ receptors coupled to Na^+-K^+ATPase (90; see Figure 6), we were particularly interested in whether adenosine might also regulate K^+ balance in skeletal muscle. At the time we began our experiments, electrophysiological experiments had shown that in cardiac myocytes and coronary artery myocytes, adenosine receptors are coupled to ATP-sensitive K^+ (K_{ATP}) channels in such a manner that adenosine opens the channels (96,97). It was also known that K_{ATP} channels are present on skeletal muscle fibres (98). As a further part of the background, it was known that K^+ can induce vasodilatation by stimulating Na-K^+ ATPase activity in vascular smooth muscle and by opening inwardly rectifying K^+ channels (99). Thus, we hypothesised that adenosine released in skeletal muscle during systemic hypoxia opens K_{ATP} channels on the skeletal muscle fibres leading to the release of K^+ which then contributes to the muscle vasodilatation (100).

In agreement with this hypothesis we demonstrated that glibenclamide, which inhibits K_{ATP} channels, abolished the increase in the K^+ concentration of venous blood draining skeletal muscle ($[K^+]_v$) that was induced by systemic hypoxia, without affecting the increase in the arterial concentration of K^+ ($[K^+]_a$); glibenclamide also reduced the accompanying muscle vasodilatation. Similarly, 8-PT not only reduced the muscle vasodilatation of systemic hypoxia (see above) but also abolished the increase in $[K^+]_v$. Further, glibenclamide reduced muscle vasodilatation evoked by adenosine, while 8-PT abolished both the muscle vasodilatation and the increase in $[K^+]_v$ evoked by adenosine (100).

Thus, it seemed reasonable to conclude that there are adenosine receptors on skeletal muscle fibres that are coupled to K_{ATP} channels: this has since been confirmed by electrophysiological recordings (101). Our results indicate they can be stimulated to open and to allow K^+ efflux by adenosine that is released in systemic hypoxia and they raise the possibility that this K^+ contributes to the muscle vasodilatation. However, it seems likely that the contribution K^+ makes is relatively small given that glibenclamide had a much smaller effect than 8-PT on the muscle

vasodilatation (100). Indeed, the fact that glibenclamide affected the early and not the late part of the muscle vasodilatation of hypoxia (100) suggests that the opening of K_{ATP} channels is particularly important in initiating the vasodilatation rather than maintaining it and raises the possibility that the K_{ATP} channels that are of major importance in the vasodilatation are on the vascular smooth muscle, or endothelium, rather than on the skeletal muscle fibres. On the other hand, the very fact that 8-PT had a much larger effect than glibenclamide on hypoxia-induced dilatation (100) strongly suggests that adenosine also acts in a manner that is independent of K_{ATP} channels.

Our experiments with nitro L-arginine methyl ester (L-NAME) which inhibits NO synthesis are consistent with this suggestion (94). For L-NAME greatly reduced both the muscle vasodilatation induced by systemic hypoxia and that induced by infusion of adenosine. Thus, it seems that the great majority of the component of the hypoxia-induced dilatation that is mediated by adenosine is also dependent on NO synthesis by the endothelium (94). It could be that the K_{ATP} channels that initiate the muscle vasodilatation are on the endothelium and coupled to adenosine receptors so that their activation triggers synthesis of NO by hyperpolarising the endothelial cells. In agreement with this proposal, our recent studies, which have shown that the hypoxia-induced muscle dilatation is mediated by A_1 adenosine receptors, have also indicated that muscle vasodilatation mediated by A_1 receptors is dependent both on the opening of K_{ATP}channels and on NO synthesis (102,103; Figure 6).

Thus, we can summarise our results to date by stating that the adenosine that makes the major contribution to the muscle vasodilatation that occurs in the rat during acute systemic hypoxia probably acts by stimulating adenosine A_1 receptors on the endothelium and increasing the synthesis of NO which then induces relaxation of the vascular

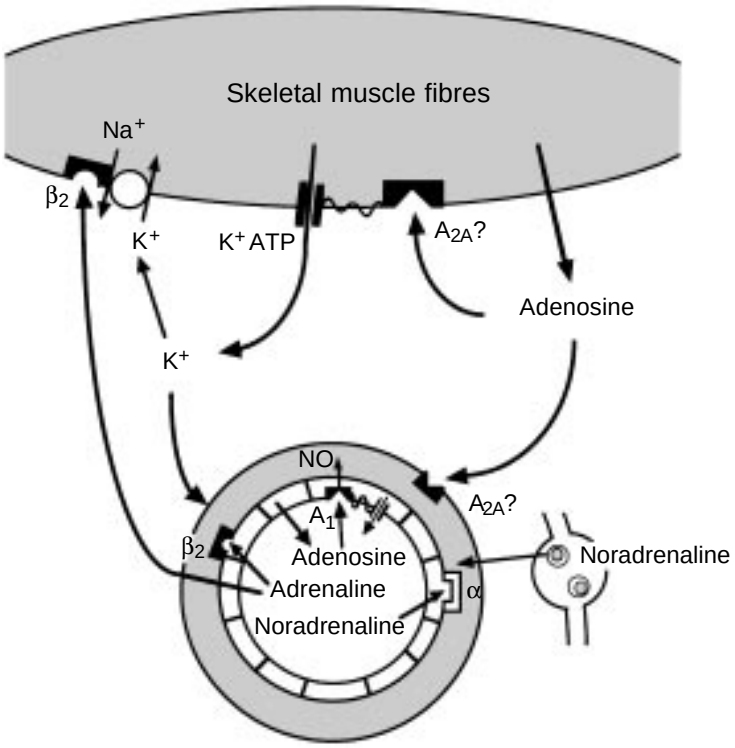

Figure 6 - Schematic diagram showing some of the factors that influence the arterioles of skeletal muscle during systemic hypoxia and the cellular mechanisms by which they may act. Noradrenaline released from sympathetic nerve fibres and circulating in the blood stream exerts a constrictor influence via α receptors. Circulating adrenaline exerts a dilator influence via ß$_2$ receptors on the vascular smooth muscle, but may also stimulate the Na^+-K^+ ATPase pump on skeletal muscle fibres via ß$_2$ receptors, thus increasing the uptake of K^+. Adenosine that is released from skeletal muscle fibres may cause the efflux of K^+ from those fibres by stimulating adenosine receptors that are coupled to K^+ ATP channels. The final concentration of K^+ in the interstitial fluid is therefore determined by the balance of the influences of adrenaline and adenosine: K^+ is a vasodilator. Adenosine may also act directly on the vascular smooth muscle. In addition, adenosine that is released from the endothelial cells acts on receptors on the endothelial cells, possible by opening K^+ ATP channels, to increase the synthesis of NO which exerts a major dilator influence on the vascular smooth muscle. Current evidence indicates that the adenosine receptors on the endothelium that are stimulated in hypoxia are of the A_1 subtype while those on the vascular smooth muscle and skeletal muscle fibres are of the A_{2A} subtype.

smooth muscle. Since the endothelium is a very effective metabolic barrier to the transport of adenosine (84), and since adenosine deaminase, which does not cross the vascular endothelium, was just as effective in reducing hypoxia-induced vasodilatation as 8-PT, an adenosine receptor antagonist (63), it seems most likely that the majority of the adenosine is released from the endothelium and acts on the endothelium in an autocrine fashion. Adenosine that is released from skeletal muscle fibres may contribute in a small way to the dilatation by acting on K_{ATP} channels on the skeletal muscle fibres to stimulate efflux K^+ which relaxes the vascular smooth muscle (Figure 6).

Chronic systemic hypoxia

Acute systemic hypoxia is of interest in its own right because it can occur in a number of clinical conditions, on immediate exposure to high altitude and on accidental or experimental exposure to hypoxic gas mixtures. However, chronic systemic hypoxia, and the adaptations that occur in this condition, is arguably more important because chronic hypoxia is so common in many respiratory and cardiovascular disorders and because it occurs in individuals who acclimatise to living at high altitude. The number of laboratory studies that have been performed on respiratory and cardiovascular adaptations to chronic systemic hypoxia is still rather small. The results of studies performed on chronically hypoxic patients are complicated by the pathological condition that underlies their hypoxic state, while those performed on healthy individuals who climb to high altitude are complicated by many factors including the effects of exercise and exposure to low temperature.

Our own experiments have been performed on rats housed in a hypoxic chamber at 12% O_2 with temperature, humidity and day/night cycle kept constant. A level of 10-12% O_2 has been shown to be sufficient to induce the right ventricular hypertrophy, increased pulmonary vascular resistance and pulmonary vascular remodelling that are characteristic of chronic systemic hypoxia (104). When adult rats that have been made chronically hypoxic for 3-4 weeks (CH rats) are studied under Saffan anaesthesia while breathing 12% O_2, they have increased ventilation relative to control, normoxic (N) rats breathing air, which might be attributed to a tonic drive to respiration exerted by the carotid chemoreceptors and probably to an increased sensitivity of the central chemoreceptors to CO_2 (105). However, the systemic arterial pressure, heart rate and gross vascular conductance of hind limb muscle are comparable in CH rats breathing 12% O_2 and N rats breathing air (105). This suggests that cardiovascular adaptations must have occurred: the muscle vasodilatation and reduction in arterial pressure that might be expected from the response to acute systemic hypoxia are apparently absent, as are the sympathetically mediated tachycardia that might be expected as a secondary consequence of hyperventilation and as a result of hypoxia of the central nervous system (see above). This new state may be partly explained by the fact that the increase in haematocrit in the CH rats allows their arterial O_2 content when they are breathing 12% O_2 to equal that of the N rats when they are breathing air (105). However, since the adenosine receptor antagonist, 8-PT, increased the control level of ventilation in the CH rats it seems there must still be sufficient hypoxia of the central nervous system to cause a tonic release of adenosine and depression of central respiratory neurones (105). On the other hand, since 8-PT had no influence on the control levels of arterial pressure, heart rate or muscle vascular conductance in the CH rats, this indicates there are no tonic influences of adenosine upon the systemic circulation. This suggests the heart and peripheral tissues are no longer hypoxic (105).

Interactions between local and reflex effects

When the CH rats were made acutely hypoxic by breathing 8% O_2 rather than 12% O_2, then they showed a similar pattern of response to that which occurs in acute hypoxia in N rats. There was an increase in ventilation and heart rate, a fall in arterial pressure and an increase in muscle vascular conductance with a later fall in ventilation and heart rate towards control levels. Moreover, the magnitude of the fall in arterial pressure and muscle vasodilatation induced in the CH rats was almost as great as those that occurred in N rats when they were subjected to the much larger change from air-breathing to 8% O_2 (105). This suggests that the vasodilator effects of acute hypoxia may be potentiated in CH rats, and/or that the reflex vasoconstrictor responses to carotid chemoreceptor stimulation are impaired.

Our experiments on the microcirculation of the spinotrapezius muscle were consistent with both of these possibilities. We found that the changes in arteriolar diameter induced when CH rats were switched from breathing 12% O_2 to 8% O_2 were just as great as those induced in N rats when they were switched from breathing air to 8% O_2 (106). Further, the effects of adenosine receptor blockade on these responses were fully comparable in the CH and N rats: in each section of the arteriolar tree, mean increases in diameter were reduced or reversed to mean decreases in diameter and quantitatively the sizes of these effects were similar in CH and N rats (106). The maximal dilatation produced by topical application of adenosine in each section of the arteriolar tree was also similar in the CH and N rats, so there was no reason to suppose that the arterioles of the CH rats were capable of greater maximal dilatation in response to adenosine or any other dilator influence than those of N rats (106). We have made very similar observations in the microcirculation of the intestinal mesentery in CH and N rats (107).

There are a number of possible explanations for these results. It may be that adenosine is more readily released by an acute hypoxic stimulus in the CH rats than in the N rats and/or the arterioles of CH rats are more sensitive to adenosine than those of N rats. Another explanation, which is not mutually exclusive, is that the arterioles of CH rats are less affected by the reflex vasoconstrictor influences of acute hypoxia (see above) than those of N rats and so they are more readily overcome by the dilator influences.

The last possibility seemed to be a particularly interesting one to us. Firstly, it was reported that chronically hypoxic patients with respiratory disease showed a reduced ability to maintain their arterial pressure when they were subjected to lower body negative pressure (108): this might be explained if they show impaired vasoconstrictor responses to the sympathetic neurotransmitter noradrenaline. Secondly, it had also been reported that the dorsal aorta of CH rats shows a reduced ability to constrict to phenylephrine, vasopressin and angiotensin as compared with N rats (109).

We therefore performed experiments on the spinotrapezius muscle and mesentery of CH and N rats, to obtain dose-response curves for the effects of noradrenaline on the arterioles. For both the mesentery and muscle, the most obvious difference between the CH and N rats was that the maximum vasoconstrictor responses evoked in the arterioles by noradrenaline were greatly reduced in the CH rats and the size of this effect was similar in the mesentery and muscle (110). Since arterioles of the intestinal mesentery have little or no tissue parenchyma around them, there was no reason to argue that the responses to noradrenaline were suppressed by some factor released by tissue cells. Rather, it seems the constrictor responses to noradrenaline must be reduced by some factor that is intrinsic to the blood vessel wall.

As a way of investigating this phenomenon, we chose first to study the iliac artery of

the rat *in vitro*, this being an artery that supplies the skeletal muscles of the hind limb. When we compared sections of iliac artery taken from CH and N rats we found, in complete agreement with the results obtained *in vivo*, that the maximum of the noradrenaline response-curve arteries from the CH rats was greatly reduced relative to that of the N rats, but there was no difference between CH and N rats in the concentration of noradrenaline that produced 50% of the maximum response. This indicates that there was no difference in the number of noradrenaline receptor sites, nor in the binding of noradrenaline to the receptors. However, the disparity between the iliac arteries of CH and N rats only existed when the endothelium was present: when the endothelium was removed, the maximum response to noradrenaline was similar in the arteries from the CH and N rats (111,112).

An obvious possibility was that NO might be involved. We therefore tested the effect of blocking NO synthesis with L-NAME. Whereas L-NAME had no effect on the basal tone of iliac arteries of N rats, it substantially increased that of CH rats. Furthermore, whereas L-NAME produced only a small increase in the maximum response to noradrenaline in the iliac arteries from the N rats, it substantially increased the maximum response of the arteries from the CH rats so that their maximum response became comparable to that of the N rats. By contrast, L-NAME had no significant effect on the noradrenaline-dose response curves of endothelium denuded iliac arteries from either CH or N rats. This provided strong evidence that the basal synthesis of NO by the endothelium is increased in the iliac arteries of CH rats and suggested that NO was responsible for the impaired vasoconstrictor responses to noradrenaline (112).

Having obtained this evidence *in vitro* it was then important to establish whether a similar effect could be produced *in vivo*. In fact, in experiments on mesenteric arterioles of CH rats, the maximum constrictor response evoked by noradrenaline was greatly enhanced by topical application of L-NAME to the mesentery, so that it equalled the maximum vasoconstrictor response recorded in N rats (Marshall JM, unpublished observations).

Thus, our current hypothesis is that chronic hypoxia causes an up-regulation of NO synthesis in the endothelium of the systemic circulation. This may be attributed at least in part to the effect of an increase in shear stress on the endothelium, caused by the hypoxia-induced increase in haematocrit for shear stress is known to stimulate NO synthesis (113). As a consequence of increased NO synthesis, we propose that the dilator influence of any substance that achieves its dilator effects in an NO-dependent manner, such as adenosine, may be enhanced in chronic hypoxia. This would be expected to lead to an increase in the vasodilator effect of acute hypoxia, just as our results have demonstrated. On the other hand, up-regulation of NO synthesis would also be expected to reduce the effects of the reflex vasoconstrictor influences of acute hypoxia, again, just as our results imply.

Concluding remarks

From a sound foundation of knowledge about the responses that can be evoked by selective stimulation of carotid chemoreceptors, we have been able to show that activation of the defence areas by the carotid chemoreceptors and that elicitation of the characteristic pattern of the alerting (defence) response is an integral part of the full response to acute systemic hypoxia. But we have also shown how this response, as well as the classical primary cardiovascular reflex responses to carotid chemoreceptor stimulation of bradycardia and generalised vasoconstriction and the cardiovascular changes that are secondary to chemoreceptor-induced hyperventilation can be modified, or even

overcome, by the local effects of hypoxia on the central nervous system, heart and peripheral tissues. It seems that these local effects of respiratory depression mediated by the influence of hypoxia on central respiratory neurones, bradycardia and peripheral vasodilatation generally become manifest in severe, or longer periods of acute hypoxia, but are more likely to predominate in small adult mammals and neonates: they have the potential to form a positive feedback loop that leads to death.

Our results suggest that adenosine plays a major role in producing these local effects of tissue hypoxia and have shown some of the cellular mechanisms by which adenosine achieves these effects. In particular, our observations on the microcirculation of skeletal muscle have demonstrated how adenosine, acting in part in a NO-dependent manner, can overcome the vasoconstrictor influences of chemoreceptor-induced activation of sympathetic noradrenergic fibres and of circulating hormones on individual arterioles and so can increase the homogeneity of the O_2 supply through the capillary network.

The sum total of experimental studies performed on chronic systemic hypoxia leaves many questions unanswered. To date, the results suggest that within 3-4 weeks of chronic hypoxia, adaptations have taken place such that ventilation is tonically raised, but arterial pressure and heart rate are normal. This alone suggests that the normal ability of hyperventilation to induce tachycardia is impaired. In addition, when a further acute hypoxic challenge is superimposed upon the chronically hypoxic state, the normal ability of chemoreceptor-induced stimulation to cause tachycardia secondary to hyperventilation and reflex vasoconstriction seems impaired (105). These results may be explained, at least in part, by down-regulation of cardiac ß adrenoreceptors, as has been reported in chronic hypoxia (114) and by up-regulation of NO synthesis by the endothelium of the systemic vasculature, such that functional responses to the sympathetic neurotransmitter, noradrenaline, are reduced. A question that is of particular interest is whether the impairments in the functional responses to peripheral chemoreceptor stimulation that occur in chronic hypoxia are accompanied by increases or decreases in the cardiac vagal and sympathetic nerve activity that produces them.

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