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THE ROLE OF ADENOSINE IN THE RESPIRATORY AND CARDIOVASCULAR RESPONSE TO SYSTEMIC HYPOXIA IN THE RAT

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SUMMARY

1. In rats anaesthetized with Saffan we have studied the effects of the adenosine receptor antagonists, theophylline and 8-phenyltheophylline, upon the respiratory and cardiovascular responses evoked by 5 min periods of systemic hypoxia.

2. In the group of animals that were to receive theophylline (15 mg kg^{-1} I.V.), arterial O_2 pressure (P_{a,O_2}) fell from 83 ± 2 mmHg during air breathing to 38 ± 3 or 34 ± 3 mmHg during the 5th minute of two different control periods of hypoxia, while in the group that were to receive 8-phenyltheophylline (10 mg kg^{-1} I.V.), P_{a,O_2} fell from 83 ± 1 to 53 ± 2 mmHg. Neither drug significantly altered the levels of P_{a,O_2} reached during hypoxia.

3. During the control periods of hypoxia respiration increased, but the increase evoked at the 5th minute was significantly less than that evoked at the 2nd minute of hypoxia. This secondary waning of the hyperventilation was abolished by both drugs.

4. Similarly, both drugs attenuated the tendency for the hypoxia-induced tachycardia to wane between the 2nd and 5th minute.

5. Further, both drugs substantially reduced both the hypoxia-induced fall in arterial pressure and the increases in vascular conductance in hindlimb muscle, carotid vasculature and kidney.

6. Thus, we propose that in the rat the release of adenosine by hypoxic tissues makes a major contribution to the secondary decrease in respiration and heart rate that occurs during systemic hypoxia and to the accompanying vasodilatation in muscle and fall in arterial pressure. The effects of the adenosine antagonists on the carotid and renal vasculature are more equivocal and may be partly explained as a smaller autoregulatory dilatation to a smaller fall in systemic arterial pressure.

7. These results and proposals are discussed in relation to the conditions that are known to cause release of adenosine and in relation to its known effects upon the respiratory and cardiovascular systems.

INTRODUCTION

We have recently reported that in both the anaesthetized, and unanaesthetized rat a 3 min period of systemic hypoxia produced hyperventilation, tachycardia and a pronounced fall in arterial pressure (Marshall & Metcalfe, 1988*b*; 1990). However,

during the 3rd minute of hypoxia, the hyperventilation and tachycardia tended to wane, so that ventilation and heart rate returned towards or even below the control levels. Our measurements of regional blood flows and vascular conductances showed that there was vasodilatation in the major tissues, the response being particularly pronounced in skeletal muscle and the brain. However, in the kidney the vasodilatation induced by administering 6% O_2 was significantly smaller than that induced by 8% O_2, suggesting that during severe hypoxia a vasoconstrictor influence on renal vasculature may compete with a dilator influence. Our analysis of the effects of vagotomy and sympathetic blockade led us to conclude that the secondary hyperventilation, bradycardia and the peripheral vasodilatation reflected the local effects of hypoxia, viz. the central depressant action of hypoxia upon neurones that control respiration and the myocardial depressant and vasodilator actions of locally released metabolites respectively (Marshall & Metcalfe, 1988*b*, 1990).

As a pattern of response, hyperventilation and tachycardia waning to hypoventilation and bradycardia respectively, accompanied by a fall in arterial pressure and widespread peripheral vasodilatation, is comparable to that induced in the neonates of both large and small mammalian species by systemic hypoxia (e.g. Gootman, Buckley & Gootman, 1979; Darnall, 1985; Gootman, Gootman, Buckley, Peterson, Steele, Sica & Gandi, 1990). Although the mechanisms underlying the pattern of response induced in neonates have not been fully analysed, we proposed that the similarity arises because small mammals in general, whether adult or neonate, have a higher rate of O_2 consumption/body weight which is readily compromised by hypoxia and renders them particularly susceptible to the local effects of hypoxia (see Marshall & Metcalfe, 1988*b*).

In the neonate there is evidence that the secondary hypoventilation can be inhibited by theophylline, which is an adenosine antagonist (e.g. Darnall, 1985). Indeed, aminophylline (theophylline with ethylenedianine added to increase solubility) is used clinically to ameliorate respiratory depression in the newborn (Aranda & Turmen, 1979). In accord with this, adenosine is known to be released by brain tissue within a few seconds of the onset of hypoxia (Winn, Rubio & Berne, 1981). Moreover, centrally administered adenosine has been shown to induce respiratory depression as well as cause cerebral vasodilatation (Eldridge, Millhorn, Waldrop & Kiley, 1983; Wessberg, Hedner, Persson & Jonassan, 1985; Morii, Ngai, Ko & Winn, 1987). However, adenosine is also known to be released by other tissues under hypoxic conditions, including heart, skeletal muscle and kidney (Osswald, Schmitz & Kemper, 1977; Berne, Winn, Knabb, Ely & Rubio, 1983). Further, adenosine can induce bradycardia by an action on adenosine A_1 receptors on the sino-atrial node, vasodilatation in skeletal muscle by acting on vascular A_1 receptors, but vasoconstriction in the kidney via a preferential action on A_1 receptors on renal afferent arterioles (see Collis, 1989; Kellet, Bowmer, Collis & Yates, 1989). This raises the possibility that adenosine plays a major role in the patter of cardiovascular and respiratory responses that we have observed in the rat during hypoxia.

To test this hypothesis we have examined the effect of theophylline upon the pattern of response induced by 5 min periods of systemic hypoxia. By using a 5 min period of hypoxia, rather than 3 min as in our previous studies, we hoped to potentiate the proposed local effects of hypoxia. The attraction of using theophylline

was that it is used clinically. However, theophylline is not only an adenosine antagonists but, at high concentrations, it can inhibit cyclic AMP phosphodiesterase activity (see Collis, 1989). Thus, we carried out a second smaller series of experiments using the more potent and specific adenosine antagonist 8-phenyltheophylline, which is reputed to have no action on phosphodiesterase activity (Smellie, Davis, Daly & Wells, 1979). Some of our findings have already been reported in brief (Marshall & Neylon, 1990).

METHODS

Experiments were performed on male and female rats. The animals used in the experiments involving theophylline and 8-phenyltheophylline were of the Sprague–Dawley and Wistar strains respectively; their body weights were 330 ± 5 g (mean $\pm$ S.E.M., $n = 26$) and 355 ± 6 g ($n = 11$) respectively.

In all experiments, anaesthesia was achieved in the manner fully described by Marshall & Metcalfe (1988*a*, *b*). Briefly, anaesthesia was induced with a halothane–oxygen mixture, followed by an injection of Saffan (Glaxovet 4 mg kg^{-1}) given via a cannula placed in the jugular vein. Anaesthesia was maintained by a continuous infusion of Saffan at 9·5–13·6 mg total steroids kg^{-1} h^{-1} during surgery and at 4·5–7 mg kg^{-1} h^{-1} during the experimental period.

The animal was prepared for recording respiratory and cardiovascular variables as described by Marshall & Metcalfe (1988*b*) using the equipment described in that paper. Briefly, a tracheal cannula was inserted to allow tidal volume and respiratory frequency to be recorded via a flow head and electrospirometer. Throughout the experiment, air, or one of the hypoxic gas mixtures (see below), was blown across the end of the flow head by an air pump. Arterial pressure was recorded from the right femoral artery, heart rate being derived electronically from the pressure recording. The right femoral vein was cannulated to allow injection of pharmacological agents, while the right brachial artery was cannulated to allow removal of blood for blood gas analysis (see below); all samples removed were replaced with equal volumes of saline.

Regional blood flows were recorded via electromagnetic cuff-type flow transducers placed on the left renal artery and left femoral artery, the paw being excluded by a stout ligature around the ankle. In addition, to give an indication of brain blood flow, carotid blood flow was recorded via an electromagnetic transducer placed on the common carotid artery, all branches of the common carotid except the internal artery having been ligated. Zero flow was regularly obtained during each experiment by temporary occlusion distal to the transducer. The flow transducers were calibrated *in vitro* by constant-flow perfusion through a freshly excised artery. In most experiments, flow was recorded from at least two regions. When two flows were recorded, they were recorded simultaneously throughout the experiment. When three flows were recorded in a given experiment, the response to each stimulus was tested twice, both before and after the adenosine antagonist (see below), and two flows were recorded on each occasion. In fact, femoral flow was usually recorded with either renal or carotid flow, the order of recording renal and carotid flow being randomized. Vascular conductance was computed on-line for each vascular bed by electronic division of arterial flow by arterial pressure. All variables were displayed on an eight-channel pen recorder. Gas mixtures (8 or 6% O_2 in N_2) were freshly prepared in PVC Douglas bags before each experiment. Gas concentrations were measured using a Nova Stat Profile analyser (Stat 3). The samples for blood gas analysis were taken anaerobically into 150 μl heparinized capillary tubes and analysed using the Nova Stat 3.

Experimental protocol

After completion of surgery, the animal was allowed to equilibrate for at least 30 min at the experimental level of anaesthesia. Then, continuous recordings were made during normoxia (air breathing) and during 5 min periods of breathing an hypoxic mixture. In the experiments in which theophylline was administered (see below), in which the two different hypoxic mixtures were used, at least two sets of recordings were made for each level of hypoxia; in the experiments in which 8-phenyltheophylline was used 8% O_2 was administered at least twice. Arterial blood samples were taken for measurements of blood gases and pH once during normoxia and once at each level of hypoxia, the sample being taken during the 5th minute of hypoxia.

Following this two separate doses of adenosine were given (0·3 ml 10^{-4} M I.V., 9-β-D-ribofuranosyladenine, Sigma), each being flushed in with saline. Several minutes were allowed between the doses for the recorded variables to stabilize. Then, in most experiments, the response evoked by the β-receptor agonist isoprenaline (1 μg kg^{-1} I.V.) was tested. Following this a competitive antagonist of adenosine was administered. In the first series of experiments, theophylline (1,3-dimethylxanthine anhydrous, Sigma) was given (15 mg kg^{-1} I.V.) dissolved in saline and flushed in with saline. In the second series 8-phenyltheophylline (Sigma) was used (10 mg kg^{-1} I.V.). This was prepared by dissolving 8-phenyltheophylline in polyethylene glycol 400: 0·1 M-NaOH (50:50 v/v) and then diluting it with saline. A solution containing 10 mg ml^{-1} was administered over 4 min and flushed in with twice the volume of saline (Wormald, Bowmer, Yates & Collis, 1989).

A 15 min period was allowed to elapse after administration of the antagonist and then the responses evoked by adenosine, isoprenaline and each level of hypoxia were re-tested, blood samples being taken as described above.

Analysis of results

The absolute values and percentage changes quoted represent the mean of the values and of the percentage changes recorded for each animal, ±standard error of the mean. Statistical analyses were carried out using Student's unpaired or paired t test, as appropriate, $P < 0.05$ being considered significant.

RESULTS

Control responses evoked by systemic hypoxia

The control levels of arterial pressure and heart rate in the Sprague–Dawley rats, which received theophylline ($n = 26$), were 115±4·2 mmHg and 428±9·6 beats min^{-1} respectively and in the Wistar rats, which received 8-phenyltheophylline ($n = 11$), they were 145±2·1 mmHg and 431±6·4 beats min^{-1} respectively. The control levels of tidal volume, respiratory frequency and respiratory minute volumes (the product of tidal volume and respiratory frequency) were 2·0±0·1 ml, 117±6·7 breaths min^{-1} and 218·8±0·9 ml min^{-1} respectively in the group that received theophylline and 2·1±0·1 ml, 114±4·0 breaths min^{-1} and 234·5±8·8 ml min^{-1} in the group that received 8-phenyltheophylline. The control values of arterial blood gases and pH for the two groups are shown in Table 1. There was no significant difference between the two groups for any of the above variables, nor for the blood flows and vascular conductances recorded in femoral, renal or carotid vasculature.

Examples of the control responses evoked by systemic hypoxia are shown in Fig. 1. The means of the percentage changes evoked in each of the cardiovascular and respiratory variables at each level of hypoxia are shown in Fig. 2 for the theophylline experiments and in Fig. 3 for the 8-phenyltheophylline experiments. The changes induced in arterial O_2 and CO_2 pressures (P_{a,O_2}, P_{a,CO_2}) and arterial pH are shown in Table 1.

The pattern of response evoked in both groups of animals was consistent with that described previously for rats exposed to 3 min periods of hypoxia (Marshall & Metcalfe, 1988*b*). Briefly, there was an increase in tidal volume, respiratory frequency and minute volume, the increase in tidal volume and minute volume tending to be smaller at the 5th than at the 2nd minute of hypoxia. Meanwhile, there was a pronounced fall in arterial pressure and an increase in heart rate, but the tachycardia tended to wane between the 2nd and 5th minute, this being more obvious in some animals than in others (see below). There were pronounced increases

in both femoral and renal vascular conductance, the vasodilatation in the kidney tending to keep renal blood flow constant despite the fall in arterial pressure. In addition, carotid vascular conductance also increased substantially, so that mean carotid blood flow increased by about 10%.

TABLE 1. Blood gas values recorded during air breathing and during hypoxia before and after theophylline or 8-phenyltheophylline

	Before			After		
	P_{a,O_2}	P_{a,CO_2}	pH_a	P_{a,O_2}	P_{a,CO_2}	pH_a
			Theophylline (15 mg kg^{-1})			
Air	83·3±2·1 (10)	35·9±1·5 (10)	7·263±0·017 (11)	83·4±1·3 (9)	39·2±1·1 (10)	7·211±0·007 (9)
Hypoxia (1)	38·4±2·7 (11)	28·6±1·5 (10)	7·325±0·023 (11)	40·9±4·0 (8)	27·3±2·6 (8)	7·354±0·011 (8)
Hypoxia (2)	35·4±3·0 (8)	27·6±1·6 (8)	7·318±0·018 (8)	39·4±4·3 (8)	28·1±1·8 (8)	7·353±0·012* (8)
			8-Phenyltheophylline (10 mg kg^{-1})			
Air	83·4±1·3 (9)	39·2±1·1 (10)	7·211±0·007 (9)	91·9±2·69** (10)	37·0±1·0 (10)	7·255±0·013** (10)
Hypoxia	52·8±2·1 (9)	34·3±1·3 (10)	7·226±0·018 (10)	54·9±3·1 (10)	33·3±0·9 (10)	7·297±0·016** (10)

Values of P_{a,O_2}, P_{a,CO_2} and arterial pH (pH_a) were recorded during the 5th minute of hypoxia before and after theophylline (above) or 8-phenyltheophylline (below). For further details of the hypoxic mixtures see text.

Asterisks indicate significant difference between value recorded before and after drug: $^{**}P < 0.01$, $^{*}P < 0.05$. Numbers in parentheses indicate total number of animals from which these values were recorded.

It may be noted that in the group of animals that received theophylline, the levels of P_{a,O_2} attained during administration of 8% O_2 were substantially lower, while the levels of P_{a,CO_2} were substantially higher than in the group that received 8-phenyltheophylline (Table 1). Further, if the respiratory and cardiovascular changes evoked in the two groups of animals are compared (compare Figs 2 and 3), it can be seen that they tended to be larger in the theophylline experiments. These differences can be explained, for in the theophylline experiments the air pump used to blow gas mixtures across the end of the tracheal cannula delivered at $> 1{\cdot}1$ l min^{-1}, which is the rate we have used previously (Marshall & Metcalfe, 1988*b*) and which we estimate exceeds the maximum inspiratory flow rate. However, for the 8-phenyltheophylline experiments which were performed 6 months later, our calibrations showed that the delivery rate of the pump had decreased substantially. Therefore, it is reasonable to assume that in these experiments the animals were not actually inspiring 8% O_2 but were taking in some atmospheric air as well, via the open end of the T-piece that was attached to the tracheal cannula. Judging from our previous data (Marshall & Metcalfe, 1988*b*, Fig. 2) we estimate that the actual percentage of O_2 inspired was $\sim$ 12%. For this reason we cannot make direct comparisons between the results obtained in the theophylline and 8-phenyltheophylline experiments. However, this

does not affect the aim of these experiments which was to test the effect of each antagonist on the response to hypoxia.

The effects of theophylline and 8-phenyltheophylline during normoxia

The effects of theophylline and 8-phenyltheophylline on the baseline levels of cardiovascular and respiratory variables during air breathing (normoxia) are shown in Table 2.

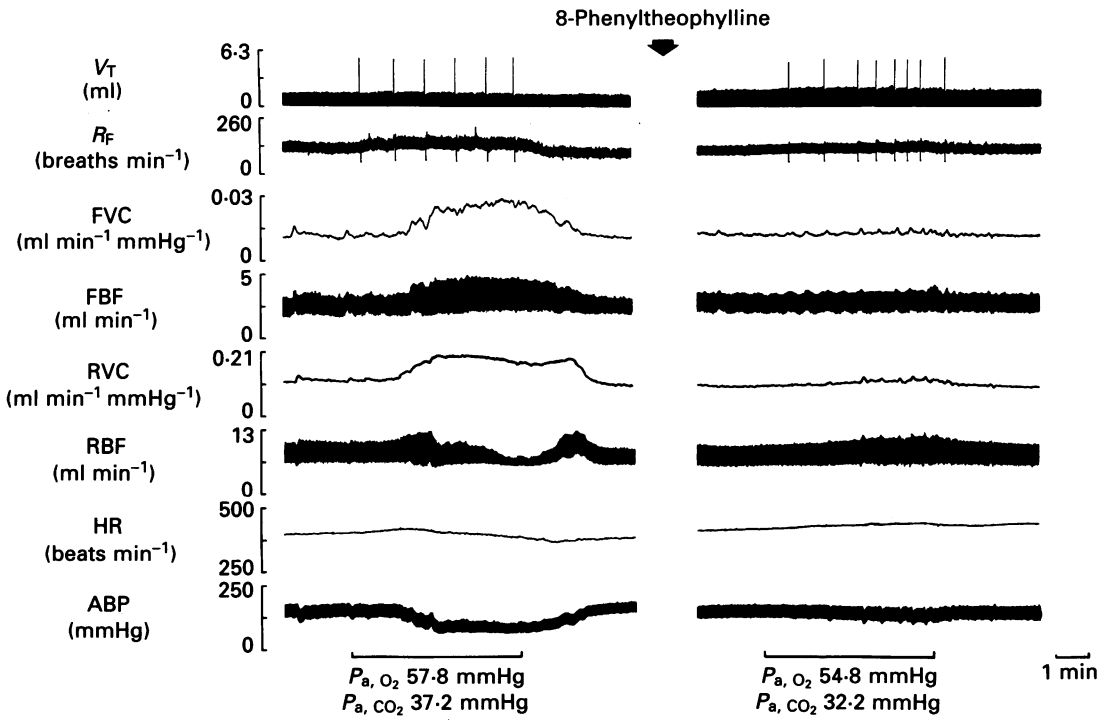

Fig. 1. The effect of 8-phenyltheophylline upon respiratory and cardiovascular evoked by moderate hypoxia. Traces from above down: tidal volume (V_T) in ml; respiratory frequency (R_F) in breaths min^{-1}; femoral vascular conductance (FVC) in ml min^{-1} mmHg^{-1}; femoral blood flow (FBF) in ml min^{-1}; renal vascular conductance (RVC) in ml min^{-1} mmHg^{-1}; renal blood flow (RBF) in ml min^{-1}; heart rate (HR) in beats min^{-1}; and arterial pressure (ABP) in mmHg. The bar beneath each set of traces indicates a 5 min period of hypoxia. The blood gas values are those measured at the end of the 5th minute of hypoxia.

After theophylline there was a significant increase in tidal volume which led to a significant increase in minute volume. By contrast, after 8-phenyltheophylline, the tendency for minute volume to increase was due to an increase in respiratory frequency, rather than tidal volume. Associated with these changes there was a significant increase in arterial pH and in P_{a,O_2} after 8-phenyltheophylline (Table 1). After theophylline there was a significant increase in heart rate; this did not occur after 8-phenyltheophylline. However, both drugs caused a significant, though small, fall in arterial pressure (Table 2). Moreoever, at least in some animals, both drugs caused a substantial increase in femoral vascular conductance: in thirteen out of

twenty animals after theophylline and in five out of eight animals after 8-phenyltheophylline. This effect considering the full groups was just below the level of statistical significance; $P < 0.055$ and $P < 0.07$ for theophylline and 8-phenyltheophylline respectively. After theophylline, there was an accompanying significant increase in femoral blood flow. Carotid vascular conductance did not change

TABLE 2. Effects of theophylline and 9-phenyltheophylline upon baseline levels of respiratory and cardiovascular variables during air breathing

	Theophylline (15 mg kg^{-1})	8-Phenyltheophylline (10 mg kg^{-1})
Tidal volume	7·1 ± 1·6***	−0·3 ± 1·8
	(25)	(10)
Respiratory frequency	0·5 ± 1·4	5·0 ± 2·4
	(25)	(10)
Minute volume	8·2 ± 2·7*	4·7 ± 2·7
	(24)	(10
Heart rate	12·4 ± 1·9***	−0·4 ± 1·4
	(25)	(11)
Mean arterial pressure	−1·5 ± 1·5*	−6·8 ± 2·6*
	(26)	(11)
Femoral blood flow	21·7 ± 7·2**	19·5 ± 7·4
	(20)	(8)
Femoral conductance	20·5 ± 10·8	21·0 ± 8·3
	(20)	(8)
Carotid blood flow	−2·9 ± 2·3**	−3·3 ± 6·9
	(16)	(6)
Carotid conductance	−0·4 ± 6·5	−2·6 ± 7·0
	(16)	(6)
Renal blood flow	3·6 ± 2·3	10·8 ± 6·9
	(12)	(8)
Renal conductance	6·3 ± 3·4	17·0 ± 9·6
	(12)	(8)

Values indicate percentage change induced in each variable by theophylline (left) and 8-phenyltheophylline (right) during air breathing.

Asterisks indicate significant difference between values recorded before and after drug: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Numbers in parentheses indicate number of animals in which values recorded.

significantly after either drug, but there was a tendency for carotid blood flow to decrease slightly after both drugs, this reaching significance after theophylline. The increase in the mean level of renal vascular conductance after 8-phenyltheophylline can be explained by particularly large increases in two out of the eight animals. There was no significant change in renal blood flow after either drug.

The effects of theophylline and 8-phenyltheophylline on the responses induced by hypoxia

In order to analyse the effects of the drugs on the responses induced by hypoxia we compared the percentage change induced in each variable before the drug with that induced after the drug, the percentage changes being calculated from the baseline levels before and after the drug respectively: these data are shown in Figs 2 and 3. However, because the drugs induced changes in the baselines of some of the

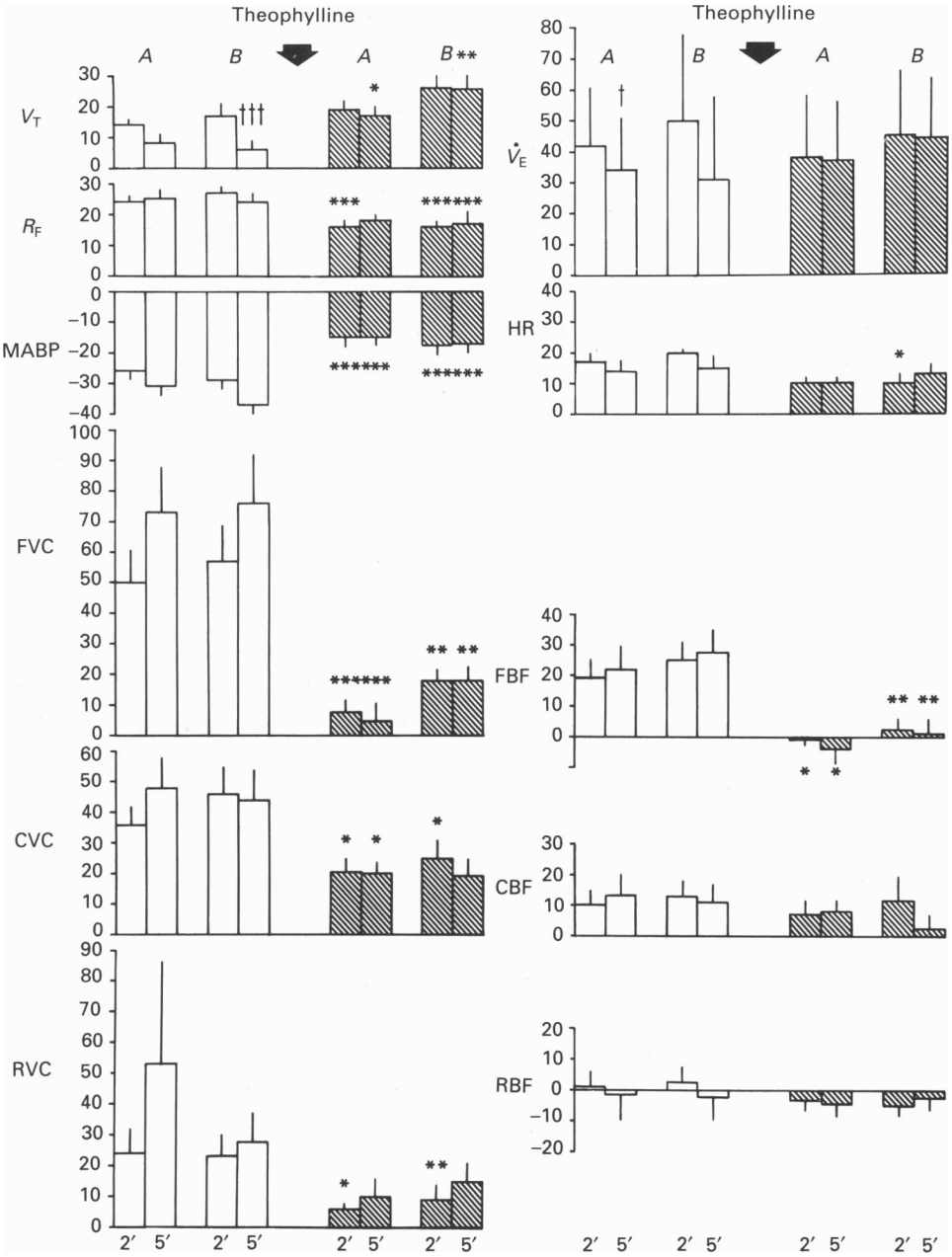

Fig. 2. Effects of theophylline upon mean respiratory and cardiovascular changes (given as percentage changes) induced by two different levels of systemic hypoxia: *A* and *B*, inspired O_2 8% O_2 and 6% O_2 respectively. $\dot{V}_E$ indicates respiratory minute volume, CVC and RVC indicate carotid and renal vascular conductance respectively, and CBF and RBF indicate carotid and renal blood flow respectively. All other abbreviations as in Fig. 1. Each column represents mean percentage change ± S.E.M. at the end of the 2nd and 5th minute indicated by 2′ and 5′ respectively. Number of animals in which each variable was

variables (Table 2), we have also compared (i) the absolute levels attained during hypoxia before and after the drug and (ii) the percentage changes induced by hypoxia before and after the drugs, with the change induced after the drug being considered as a percentage of the control level before the drug. These secondary analyses made no difference to any of the statements made below about the hypoxia-induced responses.

Both theophylline and 8-phenyltheophylline had similar, striking effects on the pattern of response evoked by hypoxia. Both antagonists abolished the secondary wane in tidal volume (Figs 2 and 3). Indeed, after theophylline, the increase in tidal volume at the 5th minute was significantly greater than that attained at the 5th minute before the drug (Fig. 2). Associated with this, the increase evoked in respiratory frequency was significantly smaller after both antagonists. The net results were that after theophylline, minute volume showed a similar percentage increase at the 2nd minute as before the drug, but much less tendency to wane between the 2nd and 5th minute. After 8-phenyltheophylline, the percentage increase in minute volume was significantly smaller than before the drug, but again it showed no tendency to wane. There were no significant differences between the levels of P_{a,O_2} and P_{a,CO_2} attained during hypoxia, before and after either theophylline or 8-phenyltheophylline. However, arterial pH reached significantly greater values after both antagonists (Table 1).

Both theophylline and 8-phenyltheophylline reduced the tendency for the hypoxia-induced tachycardia to wane between the 2nd and 5th minute (Figs 2 and 3). For the theophylline experiments this becomes more obvious if the animals that actually showed a secondary wane of the tachycardia before the drugs are considered as a separate group. Thus, in fifteen animals, before theophylline heart rate showed an increase of $7{\cdot}0 \pm 1{\cdot}5\%$ at the 2nd minute of 8% O_2 which waned to $3{\cdot}3 \pm 2{\cdot}9\%$ at the 5th minute ($P < 0{\cdot}05$, 2nd *vs.* 5th minute), while after theophylline heart rate increased by $4{\cdot}6 \pm 1{\cdot}5$ and $4{\cdot}8 \pm 1{\cdot}4\%$ at the 2nd and 5th minute respectively. Similarly, fourteen animals showed increases in heart rate of $8{\cdot}2 \pm 1{\cdot}4$ and $3{\cdot}3 \pm 2{\cdot}9\%$ at the 2nd and 5th minute of 6% O_2 before theophylline ($P < 0{\cdot}001$), and increases of $3{\cdot}0 \pm 1{\cdot}6$ and $4{\cdot}1 \pm 1{\cdot}2\%$ at the 2nd and 5th minute respectively after theophylline.

Both drugs greatly reduced the hypoxia-induced fall in mean arterial pressure (Figs 1, 2 and 3). Further, both drugs substantially reduced the hypoxia-induced increase in femoral vascular conductance and in femoral blood flow (Figs 2 and 3). This occurred even when the drug did not increase the baseline level of femoral conductance and blood flow (Table 2), as can be seen in Fig. 1.

The evoked increase in carotid vascular conductance was significantly smaller after theophylline (Fig. 2) and a similar trend was apparent after 8-phenyltheophylline, but did not reach statistical significance (Fig. 3). Neither drug had any significant effect on the changes in carotid blood flow induced by hypoxia; these still amounted to mean increases of $\sim 10\%$ (Figs 2 and 3).

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recorded is shown in Table 2. Open and hatched columns indicate changes before and after theophylline respectively. † indicates significant difference between changes at 2nd and 5th minute, * indicates significant differences between changes evoked before and after theophylline. 1, 2 and 3 symbols indicate significance at $P < 0{\cdot}05$, 0·01, 0·001 respectively.

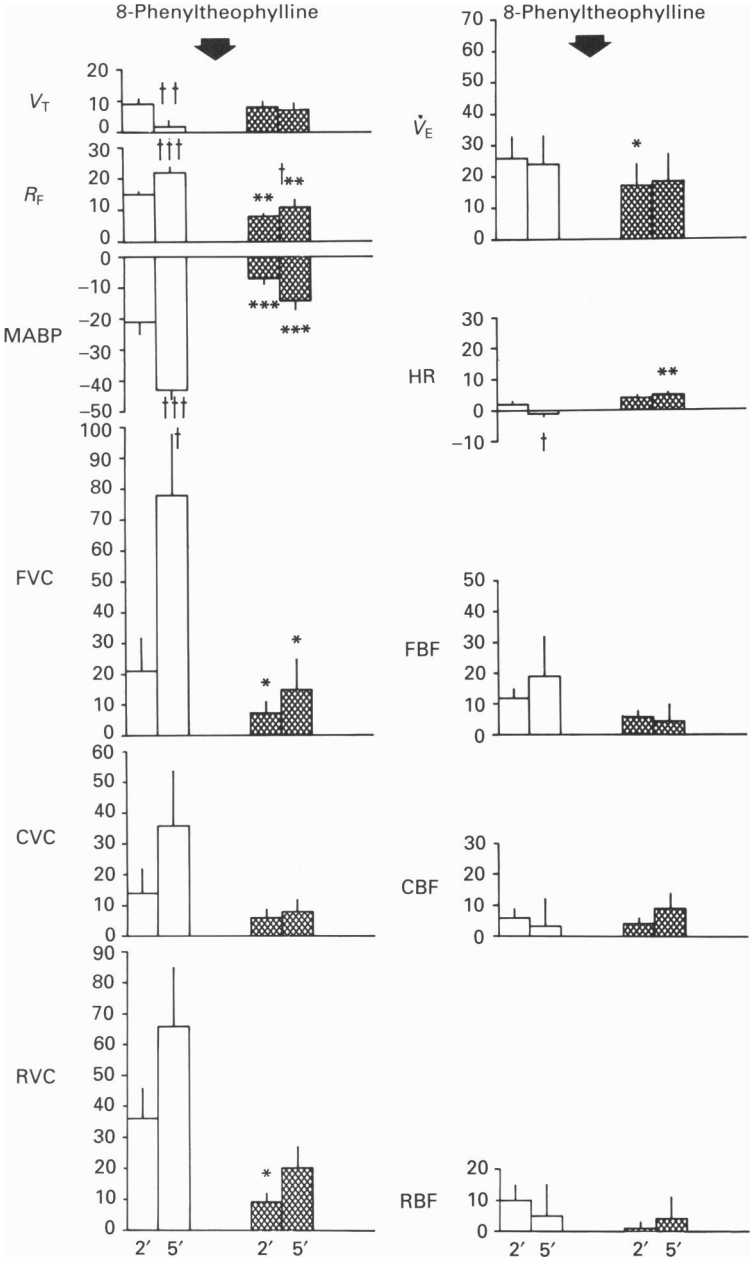

Fig. 3. Effect of 8-phenyltheophylline upon mean respiratory and cardiovascular changes induced by a moderate level of systemic hypoxia (see text). All abbreviations and symbols as in Fig. 2. All values given as percentage change. Number of animals in which each variable was recorded is shown in Table 2. Open columns, before and cross-hatched columns, after 8-phenyltheophylline.

Similarly, the increases in renal vascular conductance were significantly reduced both after theophylline and 8-phenyltheophylline (Figs 2 and 3). Neither theophylline nor 8-phenyltheophylline had a significant effect on renal blood flow, which still

remained more or less constant during hypoxia (Figs 1, 2 and 3). However, whereas before theophylline the mean increase in renal vascular conductance at the 5th minute of breathing 8% O_2 was greater than that at the 5th minute of breathing 6% O_2, this was not the case after theophylline (Fig. 2).

The effects of theophylline and of 8-phenyltheophylline upon responses evoked by adenosine were tested with the aim of assessing the degree of antagonism produced by this drug. However, interpretation of the results is complicated by the fact that both before and after the drug administration of adenosine evoked a respiratory gasp, as did injections of saline (cf. Glogowska, Richardson, Widdicombe & Winning, 1972). In the rat, a gasp is usually accompanied by a transient fall in arterial pressure, an increase in femoral vascular conductance and sometimes by a brief bradycardia (Marshall & Metcalfe, 1988*a*). These cardiovascular changes are qualitatively similar to the responses we expected to be evoked by activation of adenosine receptors (see Introduction). Thus, our finding that theophylline had no significant effect on the increase in femoral vascular conductance ($+35{\cdot}3 \pm 9{\cdot}7$ *vs.* $31{\cdot}2 \pm 5{\cdot}4$%, $n = 21$), nor on the bradycardia ($-7{\cdot}5 \pm 1$ *vs.* $-8{\cdot}9 \pm 2$%, $n = 19$) induced by adenosine, but significantly reduced the fall in arterial pressure ($-10{\cdot}6 \pm 1{\cdot}9$ *vs.* $+4{\cdot}5 \pm 17$%, $P < 0{\cdot}001$, $n = 25$), is equivocal. However, the fact that adenosine evoked a decrease in renal vascular conductance of $19{\cdot}2 \pm 5{\cdot}8$% before theophylline, but an *increase* of $12{\cdot}9 \pm 6{\cdot}4$% ($P < 0{\cdot}001$, $n = 12$) after theophylline, is fully consistent with blockade of the expected vasoconstrictor response to adenosine (see Introduction), particularly as a respiratory gasp is associated with a transient increase in renal vascular conductance (Marshall & Metcalfe, 1988*a*). The effect of 8-phenyltheophylline upon the pattern of response evoked by adenosine were similar. The evoked increase in femoral vascular conductance was $54{\cdot}5 \pm 11{\cdot}6$% before and $20{\cdot}9 \pm 7{\cdot}7$% ($P < 0{\cdot}01$, $n = 8$) after 8-phenyltheophylline, while the decrease in heart rate was $-10{\cdot}4 \pm 1{\cdot}9$% before and $-9{\cdot}09 \pm 3{\cdot}2$% ($n = 7$) after 8-phenyltheophylline. The change in renal vascular conductance was reversed from $-14{\cdot}9 \pm 2{\cdot}2$ to $+6{\cdot}1 \pm 5{\cdot}3$% ($P < 0{\cdot}01$, $n = 8$).

Neither drug had any significant effect on the isoprenaline-evoked increase in femoral vascular conductance, these amounting to $111{\cdot}3 \pm 8{\cdot}3$% before and $74{\cdot}7 \pm 9{\cdot}6$% ($n = 10$) after theophylline and to $125{\cdot}0 \pm 23{\cdot}4$% before and $116{\cdot}1 \pm 27{\cdot}1$% ($n = 7$) after 8-phenyltheophylline. However, both drugs significantly reduced the isoprenaline-evoked increase in heart rate, from $+12{\cdot}3 \pm 3{\cdot}9$% before to $0{\cdot}23 \pm 2{\cdot}8$% ($P < 0{\cdot}05$, $n = 9$) after theophylline and from $+19{\cdot}5 \pm 2{\cdot}1$% before to $13{\cdot}3 \pm 2{\cdot}3$% ($P < 0{\cdot}01$, $n = 8$) after 8-phenyltheophylline. It may be noted that the level of heart rate attained in response to isoprenaline before theophylline was equal to the baseline level of heart rate after theophylline (Table 1); 8-phenyltheophylline did not affect the baseline heart rate (Table 1).

DISCUSSION

The pattern of response evoked by 5 min periods of systemic hypoxia in the present study was essentially similar to that observed in previous studies on anaesthetized rats, viz. hyperventilation and tachycardia, both of which waned towards or below control levels, a fall in arterial pressure and vasodilatation in skeletal muscle and kidney (Marshall & Metcalfe, 1988*b*). The secondary decreases in

respiration and heart rate were far more obvious by the 5th minute of hypoxia than they had been at the end of the 3rd minute of hypoxia used in our previous studies. This accords with our previous conclusion that these effects are due to the local effects of hypoxia under central neural structures that regulate respiration and upon the heart, respectively. The present observation that the increase in femoral vascular conductance tended to be greater at the 5th minute than at the 2nd minute of hypoxia is consistent with this being mediated by the local dilator influence of tissue hypoxia (Marshall & Metcalfe, 1988*b*).

In the present study we also recorded blood flow in the common carotid artery having ligated all of its branches except those that enter the cranium (see Nosaka & Wang, 1972). As the fore- and mid-brain receive their blood supply mainly from the internal carotid via the Circle of Willis, our finding that carotid blood flow hardly changed during hypoxia is fully compatible with our recent observations on blood flow in cerebral hemispheres and cerebellum in the anaesthetized rat using radiolabelled microspheres (Marshall & Metcalfe, 1990). Further, the mean increase in carotid vascular conductance of 35–45% recorded in the present study when P_{a,O_2} fell to between 53 and 35 mmHg is within the range of changes we recorded for cerebral and cerebellar conductances (Marshall & Metcalfe, 1990) and is comparable to the 40% increase in vascular conductance of the whole brain of the rat that Morii *et al.* (1987) recorded when P_{a,O_2} was reduced to 45 mmHg. Thus, we have confidence that our recordings of blood flow in the internal carotid artery gave a good indication of forebrain blood flow.

The blocking action of theophylline and 8-phenyltheophylline

The doses of theophylline (15 mg kg^{-1}) and of 8-phenyltheophylline (10 mg kg^{-1}) used in the present study were based on those used in previous studies (Darnall, 1985; Wormald *et al.* 1989 and see below). We allowed a 15 min period after giving the drug and before testing cardiovascular responses, which seemed an optimum in view of the time dependence of the adenosine blocking actions of theophylline noted by Berne, Gidday, Hill, Curnish & Rubio (1987).

As described in the Results section, it was difficult to evaluate the degree of blockade produced by either of the antagonists because injection of the challenge dose of adenosine evoked a respiratory gasp. This is accompanied by short-lasting bradycardia, a fall in arterial pressure and vasodilatation in skeletal muscle (Marshall & Metcalfe, 1988*a*) which is the same pattern of change expected from stimulation of adenosine receptors (see Introduction). However, the renal vasoconstriction which would not have been expected to accompany a gasp (Marshall & Metcalfe, 1988*a*), but which is an expected effect of adenosine receptor stimulation (see Introduction), was abolished by the doses of theophylline and 8-phenyltheophylline we used. Moreover, others have shown that in the rat 1–50 mg kg^{-1} theophylline blocked the maintained 30–50% reduction in minute volume evoked by a stable adenosine analogue (Wessberg *et al.* 1985), while in the cat 6·8–13·6 mg kg^{-1} theophylline blocked the 30% reduction in arterial pressure evoked by a stable adenosine analogue (Eldridge, Millhorn & Kiley, 1985). Further, in the artificially ventilated rat, when adenosine could not evoke a gasp, 4 mg kg^{-1} 8-phenyltheophylline considerably reduced the 50% reduction in arterial pressure and 30% reduction in heart rate evoked by I.V. injection of 50 times the dose of adenosine used

in the present study (Kellet *et al.* 1989). Thus, it seems that the doses of theophylline and 8-phenyltheophylline we used were sufficient to produce effective blockade of the respiratory and cardiovascular effects of adenosine.

Theophylline is also able to inhibit cyclic AMP phosphodiesterase activity at concentrations in the millimolar range (Rall, 1981). It is highly improbable that millimolar concentrations were achieved at receptor sites in the present experiments. Nevertheless, we have considered the possibility of non-specific effects, whilst reviewing the influence of theophylline on the respiratory and cardiovascular variables during normoxia and upon the responses evoked by systemic hypoxia. Comparison with the effects of 8-phenyltheophylline is particularly valuable for it is more potent than theophylline as an adenosine antagonist and lacks the inhibitory effect on phosphodiesterase (Smellie *et al.* 1979).

Effects of theophylline and 8-phenyltheophylline in normoxia

Following both theophylline and 8-phenyltheophylline there was a small increase in ventilation, which in the case of 8-phenyltheophylline was associated with a significant rise in P_{a,O_2} and alkalosis. This is consistent with the idea that centrally released adenosine exerts a tonic inhibitory effect on respiration during normoxia (cf. Darnall, 1985; Eldridge *et al.* 1985; Wessberg *et al.* 1985).

After theophylline there was a significant tachycardia, as has been reported in the piglet (Darnall, 1985). This could be explained if sufficient adenosine is released even in normoxia to exert an inhibitory effect, either on the sino-atrial node, or presynaptically on the release of noradrenaline from sympathetic terminals (Collis, 1989), or to facilitate the influence of vagal activity (Verlato & Borgdorff, 1990). However, if this is the explanation, then it is surprising that 8-phenyltheophylline did not increase resting heart rate, particularly as it did in the rabbit (Verlato & Borgdorff, 1990). It is unlikely that the theophylline-induced tachycardia reflected an inhibitory effect upon cyclic AMP phosphodiesterase activity (see Collis, 1989), and a facilitated influence of sympathetic activity, which acts via β-adrenoreceptors and cyclic AMP, for theophylline did not facilitate the tachycardia evoked by isoprenaline. In fact both theophylline and 8-phenyltheophylline reduced the heart rate response to isoprenaline. The mechanisms responsible for this are not apparent. The effect of theophylline might be explained by its influence on the baseline level of heart rate (see Results) but 8-phenyltheophylline had no effect on the baseline heart rate.

Both drugs caused a substantial increase in femoral vascular conductance in some animals, although this effect did not reach statistical significance in the grouped data. If cyclic AMP phosphodiesterase activity had been inhibited, this might have induced vasodilatation by causing accumulation of cyclic AMP, but such an effect would not have been expected of 8-phenyltheophylline (Smellie *et al.* 1979; Collis, 1989). Moreoever, the vasodilator influence of isoprenaline in the femoral vascular bed, which is mediated via cyclic AMP, was not potentiated by either drug. Thus, we cannot explain our observations. We simply note that vasodilatation in skeletal muscle may have accounted for the significant fall in arterial pressure that occurred both after theophylline and 8-phenyltheophylline. A vasodilator influence of theophylline on dog skeletal muscle has been reported previously (Tabaie, Scott & Haddy, 1977). Moreover, Kellet *et al.* (1989) observed a significant fall in arterial

pressure in the rat following 8-phenyltheophylline, which, as there was no change in heart rate, probably reflected peripheral vasodilatation. By contrast, there was no vasodilatation in cat skeletal muscle following 8-phenyltheophylline (Poucher, Nowell & Collis, 1990).

Finally, carotid blood flow fell slightly, but significantly, after theophylline and tended to fall after 8-phenyltheophylline, the percentage change in carotid flow being similar to the percentage fall in arterial pressure. Thus, following these drugs carotid blood flow did not show good autoregulation to the fall in perfusion pressure. This is consistent with evidence that local release of adenosine in brain is causally involved in the cerebral vasodilator response to a fall in arterial pressure (see Berne *et al.* 1983) and with the view that adenosine exerts a tonic dilator influence on the cerebral circulation in normoxia (Morii *et al.* 1987).

Effects of theophylline and 8-phenyltheophylline on responses evoked by hypoxia

Both theophylline and 8-phenyltheophylline abolished the secondary fall in tidal volume during hypoxia. The fact that the increase in respiratory frequency was smaller after both drugs can be explained as a direct consequence of the increase in respiratory cycle length. These observations are consistent with published reports that theophylline or aminophylline can ameliorate the secondary respiratory depression caused by hypoxia in several species, including neonate pig, adult cat and man (Millhorn, Eldridge, Kiley & Waldrop, 1984; Darnall, 1985; Easton & Anthionsen, 1988; Javaheri, Teppema & Evers, 1988). There is much evidence that methylxanthines stimulate respiration predominantly by an action within the central nervous system, probably within the brain stem, on neuronal adenosine receptors (Eldridge *et al.* 1985). Accordingly, it is known that adenosine levels rise dramatically in rat brain tissue within the seconds of exposure to 10% O_2 (Winn *et al.* 1981), at levels of arterial hypoxia within the range induced in the present study.

Both theophylline and 8-phenyltheophylline also abolished the tendency for the hypoxia-induced tachycardia to wane during the period of hypoxia. This effect could not be explained by a change in the baseline level of heart rate, for no such change occurred after 8-phenyltheophylline, nor could it be explained by an inhibitory effect on phosphodiesterase activity (see above). Rather, our findings suggest that the secondary fall in heart rate is mediated by adenosine. This accords with evidence that during hypoxia adenosine levels in the heart are high enough to exert a negative chronotropic effect by a direct action on adenosine A_1 receptors on the sino-atrial node, by a presynaptic inhibitory effect on sympathetic transmission, or by potentiation of vagal activity (Bellardinelli, Files & West, 1988; Verlato & Borgdorff, 1990). A direct action provides at least a partial explanation for the present findings, as the secondary fall in heart rate was accentuated after vagotomy and when sympathetic transmission had been blocked with guanethidine (Marshall & Metcalfe, 1988*b*).

The better maintenance of the tachycardia following the adenosine antagonists must have helped to maintain arterial pressure during the later part of the hypoxic period. However, as the increase in femoral vascular conductance was considerably reduced at the 2nd and 5th minute of hypoxia following both theophylline and 8-phenyltheophylline, it seems that a major reason for the smaller fall in arterial pressure was a marked attenuation of vasodilatation in skeletal muscle. This could

not be explained simply by the increase in the baseline level of femoral vascular conductance that occurred in some animals (see above and Results). Indeed, in all animals a further increase in femoral conductance, to well above that evoked by hypoxia, could still be produced by isoprenaline. We have already argued above that neither theophylline nor 8-phenyltheophylline inhibited phosphodiesterase activity in our experiments. Indeed, if they had, then the hypoxia-induced dilatation in muscle might well have been potentiated for it is partly due to the β-receptor- and cAMP-mediated effect of catecholamines (Mian, Marshall & Kumar, 1990). Thus, the obvious conclusion is that adenosine makes a major contribution to the increase in femoral vascular conductance induced by systemic hypoxia.

Adenosine has been shown to be released from rat hindlimb muscles during hypoxic conditions created by perfusion with Krebs–Heinseleit solution rather than blood (Bockman, Berne & Rubio, 1975), from skeletal muscles of rat, cat and dog during muscle contraction performed under hypoxic conditions created by the maintenance of constant flow and during muscle contraction under free-flow conditions (Bockman *et al.* 1975; Bockman, Berne & Rubio, 1976; Bockman, Steffen, McKenzie, Yachnis & Haddy, 1982; Bockman & McKenzie, 1983). Moreoever, the use of adenosine receptor agonists and of drugs that interfere with the break-down of adenosine has indicated that adenosine contributes to the vasodilatation that occurs during muscle contraction under constant-flow and free-flow conditions (see Berne *et al.* 1983; Collis, 1989; Poucher *et al.* 1990). As far as we are aware, ours is the first evidence that adenosine contributes to the vasodilatation evoked in resting skeletal muscle by hypoxia *per se*. Since P_{a,O_2} fell to $\sim$ 55 and to $\sim$ 40 mmHg in the experiments involving 8-phenyltheophylline and theophylline respectively, our findings implicate adenosine in moderate, as well as in severe, hypoxia. The dilatation could be due to a direct action on adenosine receptors on the muscle blood vessels and/or to a presynaptic inhibition of the increased sympathetic activity that occurs during systemic hypoxia (see Marshall & Metcalfe, 1988*b*; Collis, 1989).

The fact that the hypoxia-induced increases in carotid vascular conductance were reduced both by theophylline and 8-phenyltheophylline is equivocal since the cerebral circulation is known to show good autoregulation of blood flow. Thus, this finding could simply reflect the fact that the hypoxia-induced fall in systemic arterial pressure was greatly reduced by both drugs. Previous findings that adenosine levels rise in rat brain within seconds of the onset of hypoxia (Winn *et al.* 1981) and that theophylline abolished the 40% increase in carotid vascular conductance induced in the rat when P_{a,O_2} was reduced to 45 mmHg for 5 min (Morii *et al.* 1987) implicated adenosine in its mediation. However, our findings cannot be directly compared with those of Morii *et al.* (1987) for under their experimental conditions, which included artificial ventilation so that P_{a,CO_2} remained constant, arterial pressure did not fall during hypoxia.

That the hypoxia-induced increase in renal vascular conductance was reduced both by theophylline and by 8-phenyltheophylline is similarly difficult to interpret since the kidney autoregulates well. However, whereas before theophylline the increase in renal vascular conductance evoked during 6% O_2 was less than that induced by 8% O_2, leading us to propose a vasoconstrictor influence on the kidney during more severe hypoxia (Marshall & Metcalfe, 1988*b*), this was not the case after theophylline, even though the levels of P_{a,O_2} attained during 8 and 6% O_2 were

comparable before and after the drug. This could be explained if locally released adenosine exerts a vasoconstrictor influence on the kidney during severe hypoxia (see Osswald *et al.* 1977).

In summary, the effects exerted by theophylline and by 8-phenyltheophylline upon the respiratory and cardiovascular response to systemic hypoxia in the present study can readily by explained by antagonism of the known effects of adenosine. They suggest that in the rat the secondary hypoventilation and bradycardia, the muscle vasodilatation and the consequent fall in arterial pressure are due in large part to the local actions of adenosine, synthesized as a result of tissue hypoxia. Inasmuch as the pattern of respiratory and cardiovascular response evoked by hypoxia in the neonate is comparable to that evoked in the rat (see Introduction), the present study raises the question of whether, in clinical use, the xanthine derivatives not only ameliorate respiratory depression (e.g. Darnall, 1985) but have similar effects on the accompanying cardiovascular changes as in the rat.

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