Postnatal development of the pattern of respiratory and cardiovascular response to systemic hypoxia in the piglet: the roles of adenosine

Basil Elnazir, Janice M. Marshall* and Prem Kumar

Department of Physiology, The Medical School, Vincent Drive, Birmingham B15 2TT, UK

1. In 3-day-old and 3-week-old spontaneously breathing piglets anaesthetized with Saffan, we have studied ventilatory and cardiovascular responses evoked by 5 min periods of hypoxia (breathing 10 and 6% O_2).

2. In 3-day-old piglets both 10 and 6% O_2 evoked an increase followed by a secondary fall in ventilation, a gradual tachycardia and a renal vasoconstriction, with an increase in femoral blood flow that was attributable to femoral vasodilatation. Arterial blood pressure rose initially but fell towards control values by the 5th minute of hypoxia.

3. The stable adenosine analogue 2-chloroadenosine (2-CA; 30 mg kg^{-1} I.V.) evoked bradycardia and renal vasoconstriction, but had no effect on femoral vasculature. These responses were blocked by the adenosine receptor antagonist 8-phenyltheophylline (8-PT; 8 mg kg^{-1} I.V.). 8-PT also abolished the secondary fall in ventilation evoked by 10 and 6% O_2 and the renal vasoconstriction evoked by 10% O_2, but had no effect on the tachycardia, or on the femoral vascular response.

4. By contrast, in 3-week-old piglets both 10 and 6% O_2 evoked a sustained increase in ventilation, tachycardia and a rise in arterial pressure with renal vasoconstriction, but no change in renal blood flow and substantial femoral vasodilatation with an increase in femoral blood flow. 2-CA evoked bradycardia and renal vasoconstriction, as in 3-day-old piglets, but also evoked pronounced femoral vasodilatation. 8-PT blocked these responses and the hypoxia-induced femoral vasodilatation, but had no significant effect on other components of the hypoxia-induced response.

5. We propose that there is postnatal development of the ventilatory and cardiovascular responses evoked by systemic hypoxia and of the role of locally released adenosine in these responses: at 3 days, adenosine released within the central nervous system and within the kidney is a major contributor to the secondary fall in ventilation and renal vasoconstriction, respectively, whereas at 3 weeks, adenosine makes little contribution to the ventilatory response, or renal vasoconstriction, but is largely responsible for hypoxia-induced vasodilatation in skeletal muscle.

It is well established that in the first few days after birth, the respiratory response to systemic hypoxia comprises an initial increase in ventilation followed by a return towards, or falling below, control values, and that in the 2–3 weeks after birth, the secondary fall in ventilation becomes less pronounced. The initial increase in ventilation has been attributed to the reflex effect of peripheral chemoreceptor stimulation, and the secondary fall to the central neural effects of hypoxia (e.g. Hanson, 1986). By contrast, relatively little is known of the postnatal development of the cardiovascular response to systemic hypoxia. The responses recorded have varied considerably between studies, which may partially reflect the fact that some species are more mature than others at birth (see Gootman *et al.* 1990). However, the results of different studies are also difficult to compare directly because the methods of inducing hypoxia, the levels and duration of hypoxia and other experimental conditions have varied between studies (see Gootman, Buckley & Gootman, 1979; Gootman, 1991). Nevertheless, the most common response evoked by severe hypoxia in the early newborn of several species seems to be bradycardia, or an initial tachycardia and secondary

* To whom correspondence should be addressed.

bradycardia accompanied by a fall in systemic arterial pressure with vasodilatation in the major peripheral vascular beds (Gootman *et al.* 1979; Gootman, 1991). This pattern of response has been demonstrated, for example, in the spontaneously breathing, conscious, 4- to 6-day-old lamb (Sidi, Kuipers, Teitel, Heymann & Rudolph, 1983) and in the artificially ventilated, 2- to 4-day-old piglet under Saffan anaesthesia (Gootman *et al.* 1990). It contrasts with the sustained tachycardia and rise in arterial pressure generally seen in mature mammals (Gootman *et al.* 1990; Marshall, 1994).

The adult rat apparently shows a pattern of response to hypoxia that is comparable to that of the neonate: a biphasic respiratory response, tachycardia and secondary bradycardia, a fall in arterial pressure and peripheral vasodilatation (Marshall & Metcalfe, 1988*b*). Moreover, in the rat, not only could the secondary fall in ventilation be attributed to the local effects of hypoxia within the central nervous system, as in the neonate (see above), but the secondary bradycardia, fall in arterial pressure and peripheral vasodilatation could be ascribed in large part to the local effects of hypoxia on the heart and peripheral vasculature. We therefore proposed that small mammals in general, whether newborn or the adults of small mammalian species, are more susceptible to the local effects of hypoxia, because they have a higher rate of O_2 consumption per unit body mass that is readily compromised by hypoxia (Marshall & Metcalfe, 1988*b*).

There is substantial evidence that adenosine that is locally released within the central nervous system is at least partly responsible for the secondary fall in ventilation in both neonates and adult animals (e.g. Millhorn, Eldridge, Kiley & Waldrop, 1984; Darnall, 1985; Easton & Anthonisen, 1988). Moreover, in the rat, locally released adenosine plays a part in the secondary bradycardia (Thomas, Elnazir & Marshall, 1994) and plays a major role in the muscle vasodilatation (Mian & Marshall, 1991; Neylon & Marshall, 1991; Marshall, Thomas, Turner, 1993; Thomas & Marshall, 1994; Thomas *et al.* 1994).

Thus, the aims of the present study were to establish the extent to which the respiratory and cardiovascular responses evoked by systemic hypoxia in the spontaneously breathing newborn piglet are comparable to those of the adult rat and to establish how this pattern of response changes over the postnatal period. For this purpose we used two different hypoxic mixtures (10 and 6% O_2), similar to those used by Sidi *et al.* (1983) in their study on conscious lambs. We further wished to establish whether, and to what extent, adenosine contributes to the respiratory and cardiovascular responses in the early and later postnatal period. The experiments were performed on piglets anaesthetized with Saffan, a short-acting steroid agent that can be given as a continuous infusion so as to achieve a stable level of anaesthesia with analgesia. Its particular virtue is that it preserves central neural control of the cardiovascular system more effectively than other commonly used anaesthetics (Timms, 1981; Marshall, 1987, 1994).

METHODS

Experiments were performed on two groups of piglets: eight 'newborn' piglets of postnatal age 3 days (1·3–2·0 kg) and six '3-week-old' piglets of postnatal age 19–21 days (5·5–6·5 kg). Each animal was tranquillized with azaperone at 2 mg kg^{-1} I.M. (Stresnil; Janssen, Wantage UK), and then anaesthesia was induced with 3–5% enflurane (Abbott, Anaquest Ltd) in oxygen delivered at 3·6 l min^{-1} via a face mask. When anaesthetized, the animal was intubated and anaesthesia was maintained with enflurane (3–5%) in O_2 while the right jugular vein was cannulated. The inhalation anaesthetic was then discontinued and anaesthesia was maintained with Saffan (Pitman-Moore, Uxbridge, Middlesex, UK): a priming dose of 4–8 mg kg^{-1} diluted with normal saline to 4 mg ml^{-1} (newborn group) or 8 mg ml^{-1} (3-week-old group) was given in small boluses. The animal was then given Saffan in these same concentrations by continuous I.V. infusion at 16–24 mg kg^{-1} h^{-1} total steroids during surgery (see Green, 1979) and at 7–12 mg kg^{-1} h^{-1} during the experimental period. The rate of infusion was adjusted in each animal. During surgery, there was no corneal reflex and no response to pinching the ear or pad of the trotter. During the experimental period, the corneal reflex was present and strong mechanical stimulation of a trotter with finger and thumb evoked a brisk, but short-lasting, carpo-pedal withdrawal reflex lasting 1–3 s with a transient rise in mean arterial blood pressure (ABP) of 10–15 mmHg and heart rate of 10–20 beats min^{-1}. The cardiovascular reflex lasted 8–15 s from onset and the complete somatic–cardiovascular reflex was stimulus locked: the test stimulus did not evoke crossed or long-spinal somatic reflexes, the cardiovascular changes were never prolonged and there were no spontaneous movements or changes in arterial pressure, heart rate or respiration in the absence of a test stimulus (see Figs 1 and 4 and Hilton & Marshall, 1982; Marshall, 1987).

Once anaesthesia was established and throughout the experiment, rectal temperature was continuously monitored and was maintained at 38·5–39·5 °C (see Darnall, Green, Pinto & Hart, 1991) by means of heating lamps placed under and over the operating table.

The animal was then prepared for recording respiratory and cardiovascular variables using techniques and apparatus that were essentially the same as those described before (Marshall & Metcalfe, 1988*a*; Thomas & Marshall, 1994). A respiratory flow head was attached to the end of a tracheal tube and was connected to an electrospirometer so that respiratory frequency (R_F) and tidal volume (V_T) could be recorded continuously. Minute volume ($\dot{V}_E$) was computed off-line. Pulsatile arterial pressure and thereby heart rate (HR) was recorded from a cannula in the left femoral artery. The right brachial artery was cannulated to allow blood samples to be taken for analysis of blood gases and pH. The accompanying basilic vein was cannulated to allow administration of drugs.

Femoral blood flow (FBF) was recorded from the right femoral artery by means of a cuff-type electromagnetic transducer. A stout ligature was tied around the ankle to ensure that the blood flow recorded was mainly that to the skeletal muscles of the hindlimb. The left hindlimb was raised and secured to a cross-frame so that

Table 1. Baseline values of respiratory and cardiovascular variables recorded in newborn and 3-week-old piglets before and after 8-PT

	Newborn		3 weeks old	
	Pre 8-PT	Post 8-PT	Pre 8-PT	Post 8-PT
Tidal volume (ml)	17·4 ± 1·2	23·6 ± 1·4**	37·9 ± 3·1	48·0 ± 3·5**
Respiratory frequency (breaths min^{-1})	41·3 ± 2·6	41·8 ± 2·8	43·0 ± 2·8	39·6 ± 2·8
Respiratory minute ventilation (ml min^{-1})	723·8 ± 44·5	970·7 ± 60·2**	1574·1 ± 91·2	1810·8 ± 65·8*
Mean arterial blood pressure (mmHg)	50·6 ± 0·9	52·1 ± 2·4	68·7 ± 1·8	71·2 ± 1·7
Heart rate (beats min^{-1})	190·4 ± 12·6	239·3 ± 14·5**	201·0 ± 6·1	231·6 ± 6·4**
Femoral blood flow (ml min^{-1})	27·5 ± 1·2	28·8 ± 1·6	47·9 ± 4·2	52·0 ± 5·8
Femoral vascular conductance (ml min^{-1} $mmHg^{-1}$)	0·54 ± 0·01	0·57 ± 0·01	0·69 ± 0·01	0·79 ± 0·01
Renal blood flow (ml min^{-1})	35·6 ± 5·9	29·9 ± 6·3	162·4 ± 25·9	157·6 ± 18·2
Renal vascular conductance (ml min^{-1} $mmHg^{-1}$)	0·70 ± 0·09	0·57 ± 0·06	2·3 ± 0·3	2·3 ± 0·3

* $P < 0·05$ and ** $P < 0·01$ significant difference between values recorded before and after 8-PT in either newborn or 3-week-old piglets.

it did not mechanically impede blood flow to the right limb. Renal blood flow (RBF) was recorded via a cuff-type transducer from the left renal artery which was approached retroperitoneally from the left flank. Zero flow was checked at intervals throughout, but always at the beginning and end of the recording period, before and after giving a drug (see below) and if the baseline level appeared to be drifting. For this purpose an occluding thread looped through polyvinyl tubing was placed distal to the probe so that the tube could be pushed along the ligature to occlude the vessel. Femoral and renal vascular conductance (FVC and RVC) were computed on-line by electronic division of blood flow by arterial pressure.

Throughout the experiment, the animal breathed room air or a hypoxic mixture, which was directed across the end of the respiratory flow head by an air pump at 2–3 l mm^{-1}. Hypoxic gas mixtures were prepared in PVC bags by adding 100% N_2 to atmospheric air. The desired mixture (10 or 6% O_2) was established by using a Nova Stat profile analyser (Stat 3, V. A. Howe, Waltham, MA, USA).

Protocol

The animal was allowed a 60 min equilibration period after completion of all surgery, whilst breathing air. The inspirate was then switched from air to 10% O_2 and from air to 6% O_2 for 5 min on at least two occasions for each gas mixture. All baselines were allowed to stabilize for at least 10 min between hypoxic periods. A blood sample (140 μl) was removed from the brachial artery immediately before the periods of hypoxia and in the 5th minute of each period of hypoxia; this was analysed for arterial P_{O_2} and P_{CO_2} (P_{a,O_2} and P_{a,CO_2}, respectively) and for arterial pH by using the Nova Stat analyser. For analysis of the cardiovascular and respiratory responses to hypoxia, we used the values recorded before and at the 2nd and 5th minute of the second period of breathing 10 or 6% O_2. The stable adenosine analogue 2-chloroadenosine (2-CA) was then given as a bolus over 15–30 s (30 μg kg^{-1} I.V.). This dose was found to give the maximum response that could be tolerated well; larger doses induced a long-lasting bradycardia and fall in ABP. After a period of up to 10 min to allow stabilization, the adenosine receptor antagonist 8-phenyltheophylline (8-PT; 8 mg kg^{-1} I.V.) was given via the basilar vein over 3–5 min and flushed in with saline; 10–15 min later the animal was again exposed to 5 min periods of breathing 10 and 6% O_2 and was given a second bolus injection of 2-CA (30 μg kg^{-1}). At the end of the experiment, the animal was killed by an overdose of pentobarbitone sodium.

Drugs

2-CA (Sigma) was dissolved in normal saline, in 1·5 ml for the newborn piglets and 3·0 ml for the 3-week-old piglets. 8-PT (Sigma) was dissolved in a mixture of ethylene glycol and 0·1 M NaOH (50 : 50 v/v), diluted with saline and then sonnicated for 5–10 min to ensure complete dissolution. The solution was administered in 1·5 ml (newborn piglets) or 3·0 ml (3-week-old piglets). Injection of the vehicle for 8-PT had no detectable cardiovascular or respiratory effect.

Statistical analysis of results

All results are expressed as means ± S.E.M. As indicated above, respiratory and cardiovascular variables were recorded before and at the 2nd and 5th minute of breathing 10 and 6% O_2. Values recorded before and at the 5th minute of hypoxia were compared by using Student's paired t test. Since we were interested in whether there was a secondary waning of the responses during hypoxia (see the introduction), comparisons were also made of the values recorded at the 2nd and 5th minute of hypoxia using the paired t test. In addition the paired t test was used to compare baseline values immediately before 8-PT and when baseline levels had stabilized after 8-PT. In all cases $P < 0·05$ was taken to be significant.

RESULTS

Newborn group

Baseline values of respiratory and cardiovascular variables before and after 8-PT are shown in Table 1. Table 2 shows blood gas values during air breathing and at the 5th minute of breathing 10 and 6% O_2 before and after 8-PT.

Responses evoked by hypoxia

Six of the eight newborn piglets showed similar patterns of responses to systemic hypoxia. The remaining two did not tolerate hypoxia well and since we were not able to take them through the whole protocol, they were not included in the statistical analyses; their responses are considered separately below.

Table 2. Blood gas values recorded during air breathing and hypoxia before and after 8-phenyltheophylline (8-PT) in newborn piglets (above) and 3-week-old piglets (below)

	Before 8-PT			After 8-PT		
	P_{a,O_2}	P_{a,CO_2}	pH_a	P_{a,O_2}	P_{a,CO_2}	pH_a
Newborn						
Air	76·8 ± 0·8	38·8 ± 1·8	7·356 ± 0·026	88·7 ± 5·2	26·5 ± 1·9*	7·418 ± 0·030*
10% O_2	27·0 ± 1·9	32·4 ± 1·1	7·391 ± 0·026	32·5 ± 3·4*	24·5 ± 2·1*	7·437 ± 0·038
6% O_2	20·9 ± 1·6	30·7 ± 1·2	7·412 ± 0·027	23·1 ± 2·2	23·5 ± 2·1*	7·461 ± 0·040
3 weeks old						
Air	69·6 ± 3·5	32·1 ± 1·6	7·364 ± 0·021	72·8 ± 6·9	25·1 ± 1·8*	7·452 ± 0·023**
10% O_2	33·3 ± 1·3	27·3 ± 3·0	7·403 ± 0·033	36·2 ± 3·1	22·1 ± 1·8	7·479 ± 0·024**
6% O_2	27·8 ± 0·6	25·5 ± 1·3	7·430 ± 0·029	29·2 ± 1·0	23·3 ± 1·2	7·475 ± 0·022*

Values of P_{a,O_2}, P_{a,CO_2} (mmHg) and arterial pH (pH_a) were recorded before and in the 5th minute of breathing 10 and 6% O_2 ($n = 6$ in each case). * $P < 0·05$, ** $P < 0·01$ significant differences between values before and after 8-PT.

Each of the group of six piglets showed an initial increase in V_T, R_F and $\dot{V}_E$ during exposure to 10 and 6% O_2. However, whereas R_F increased progressively so that it was greater than control values at the 5th minute, the increase in V_T waned so that by the 5th minute of hypoxia, both V_T and $\dot{V}_E$ were significantly lower than at the 2nd minute (Figs 1 and 2). Meanwhile, by the 5th minute mean P_{a,O_2} fell to an extent that was graded with the level of hypoxia, while P_{a,CO_2} fell and arterial pH rose (Table 2). ABP rose by the 5th minute of 10% O_2 (Figs 1 and 2), but fell back

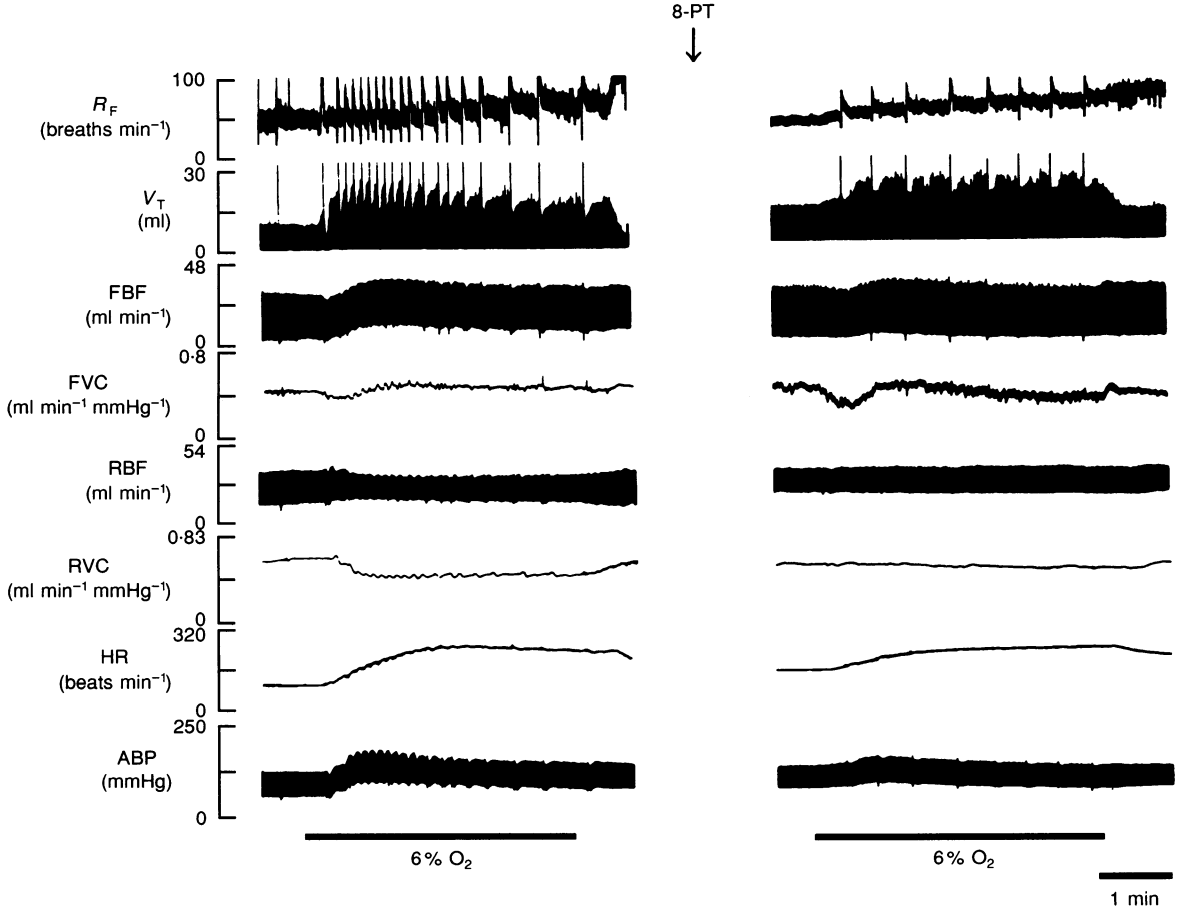

Figure 1. Respiratory and cardiovascular responses evoked by breathing 6% O_2 in a newborn piglet, before and after 8-PT (8 mg kg^{-1} I.V.)

Traces from top to bottom are respiratory frequency (R_F), tidal volume (V_T), femoral blood flow (FBF), femoral vascular conductance (FVC), renal blood flow (RBF), renal vascular conductance (RVC), heart rate (HR) and arterial blood pressure (ABP).

towards the control level between the 2nd and the 5th minute of 6% O_2. This was accompanied by a gradual tachycardia so that HR was greater than control at the 5th minute of 10 and 6% O_2. FVC showed no significant change, but FBF increased significantly by the 5th minute of 6% O_2 (Fig. 2). By contrast both RVC and RBF fell significantly by the 5th minute of 10 and 6% O_2, indicating renal vasoconstriction (Figs 1 and 2).

Superimposed upon these gradual changes there was an increase in the frequency of respiratory gasps during the period of hypoxia (Fig. 1). Each gasp comprised an additional inspiratory effort at the peak of a normal

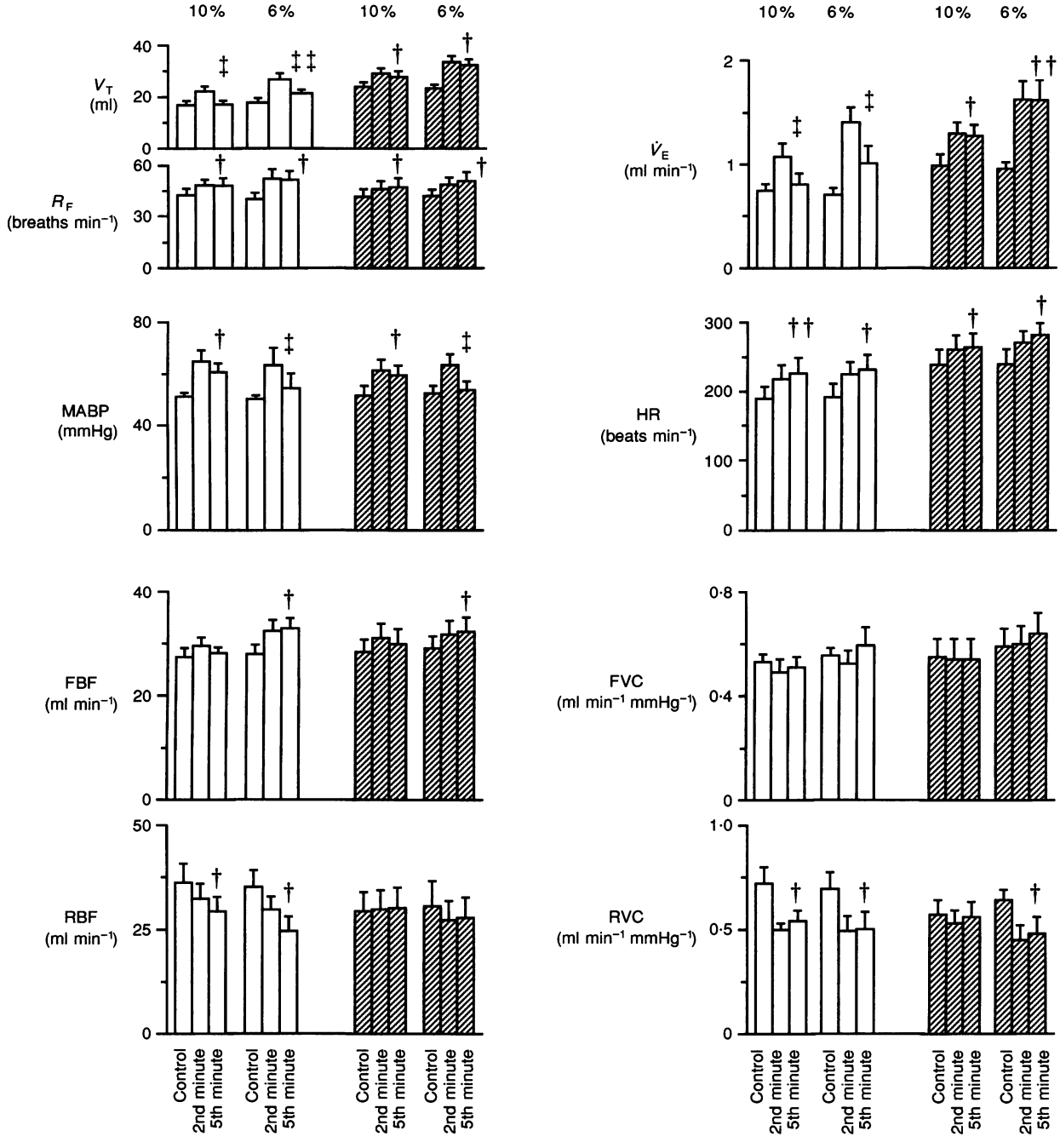

Figure 2. Mean respiratory and cardiovascular values recorded in newborn piglets during air breathing and during 10 and 6% O_2, before and after 8-PT (8 mg kg^{-1} I.V.)

Each column represents means ± S.E.M. recorded before and at the 2nd and 5th minute of breathing 10 and 6% O_2. Hatched columns indicate values recorded after 8-PT. Abbreviations of recorded variables as in Fig. 1; in addition $\dot{V}_E$, minute ventilation, MAPB; mean arterial blood pressure. † Significant difference between values recorded during air breathing and the 5th minute of 10 or 6% O_2; ‡ significant difference between values recorded at 2nd and 5th minute of breathing 10 or 6% O_2. In each case single and double symbols indicate $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively.

inspiration and was followed by a short-lasting period of increased R_F and reduced V_T. Each gasp was generally accompanied by a transient increase in FVC and RVC, bradycardia and a fall in ABP.

The two remaining piglets (see above) had low P_{a,O_2} values during air breathing (30–40 mmHg). When given 10 or 6% O_2 to breathe they initially showed a pattern of respiratory and cardiovascular response that was similar to that seen in the other animals. However, within 1 or 2 min, ABP and HR began to fall towards and then below control values. Meanwhile RVC fell gradually and FVC increased to above control values, indicating vasodilatation in limb muscle (Fig. 3). As these changes developed, so V_T fell to below control values and there were sometimes apnoeic episodes such that we terminated the period of hypoxia and the animal was artificially ventilated until normal ventilation returned. We did not attempt to take blood samples during the test period of hypoxia: both P_{a,O_2} and P_{a,CO_2} must have been changing continuously in association with the erratic respiration.

In these animals, respiratory gasps occurred more frequently during air breathing than in the other animals and gasping frequency became much greater during hypoxia; as can be seen from Fig. 3, transient cardiovascular changes generally accompanied each gasp, as described above. At postmortem examination these animals showed areas of grey hepatization in the lungs, indicative of an inflammatory process.

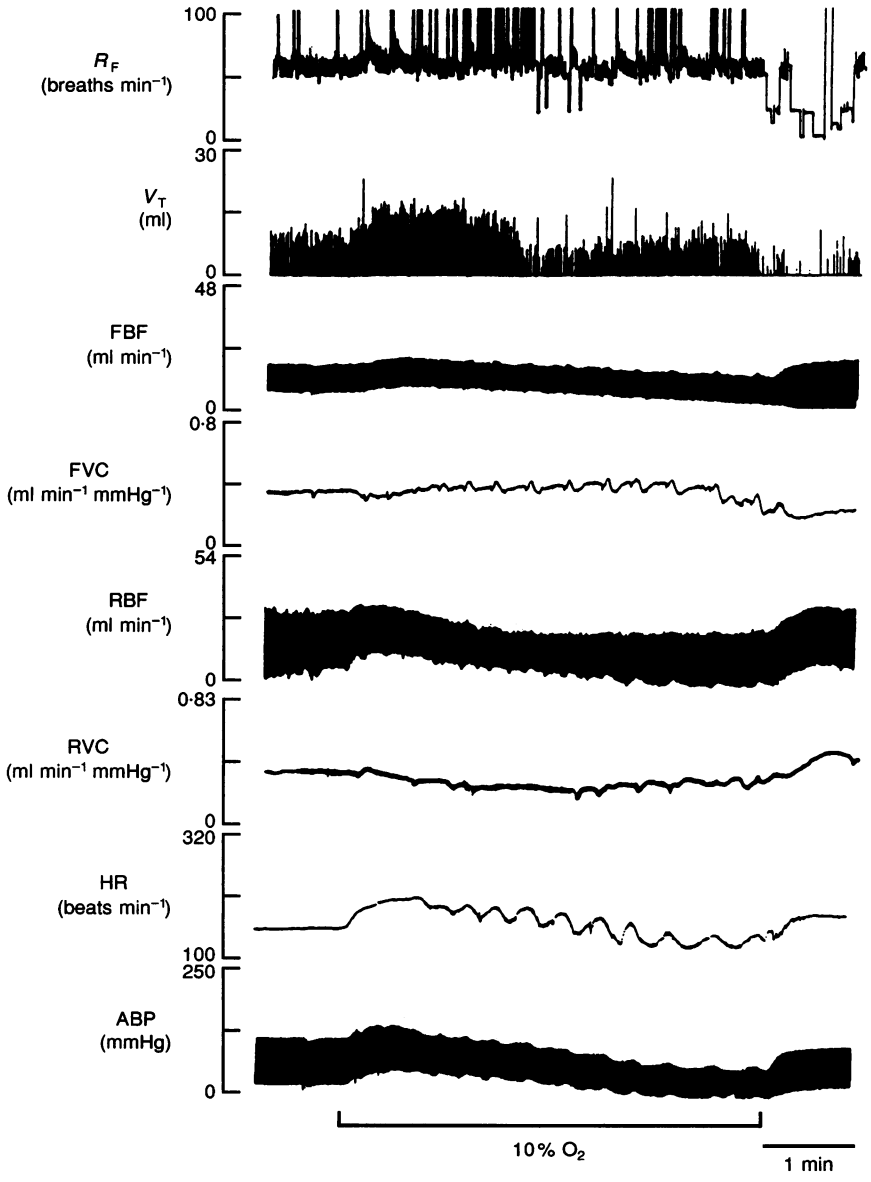

Figure 3

Respiratory and cardiovascular responses evoked in a newborn piglet whose P_{a,O_2} during air breathing was low (see text). Abbreviations as in Fig. 1.

Effects of 2-CA and of 8-PT

Before administering 8-PT, 2-CA (30 mg kg^{-1} I.V.) evoked an increase in R_F (6·9 ± 2·3% at peak from control level), a more sustained fall in HR (12 ± 1·2%) and ABP (24 ± 4%) and a fall in RVC (59 ± 11%) indicating renal vasoconstriction; FVC showed no consistent change (5·6 ± 2·1%).

Administration of the adenosine receptor antagonist 8-PT (8 mg kg^{-1}) seemed to cause the level of anaesthesia to become lighter. Thus, we gave a bolus injection of Saffan (2 mg kg^{-1}) and increased the infusion rate of Saffan until the level of anaesthesia was the same as before 8-PT as judged by the criteria described in Methods and using the experience we have had in manipulating the level of Saffan anaesthesia in the cat and rat (Hilton & Marshall, 1982; Marshall, 1987). V_T and thereby $\dot{V}_E$ stabilized at new higher baseline values than before 8-PT (Table 1). As a consequence, P_{a,CO_2} during air breathing fell, arterial pH rose and P_{a,O_2} tended to rise, though not significantly (Table 2). There was also a significant increase in baseline HR, but no change in the other cardiovascular variables. From these new baselines, 2-CA (30 mg kg^{-1}) administered at the end of the experiment had negligible effects on the respiratory and cardiovascular variables indicating that the adenosine receptors were effectively blocked. The vehicle for 8-PT had no effect when given alone.

During blockade of adenosine receptors, the respiratory responses evoked by hypoxia were changed compared with those evoked before 8-PT (Figs 1 and 2). Both 10 and 6% O_2 evoked sustained increases in $\dot{V}_E$ such that V_T and R_F were greater than control values at the 5th minute; there was no secondary fall in V_T between the 2nd and the 5th minute. Further, P_{a,CO_2} fell to a lower level during hypoxia after 8-PT than before (Table 2). The cardiovascular responses were similar to those seen before 8-PT except that the hypoxia-induced decrease in RVC was abolished during 10% O_2 and RBF did not decrease during either 10 or 6% O_2 (see Figs 1 and 2). As baseline RVC tended to decrease after 8-PT (Table 1 and Fig. 2), it is important to consider whether this relative vasoconstriction was responsible for the alternation of the renal vasoconstrictor response to 10% O_2. This seems not to be the case, since before 8-PT, 2-CA reduced RVC to 0·42 ± 0·05 ml min^{-1} mmHg^{-1}, which is substantially lower than induced by either 10 or 6% O_2 (Fig. 2).

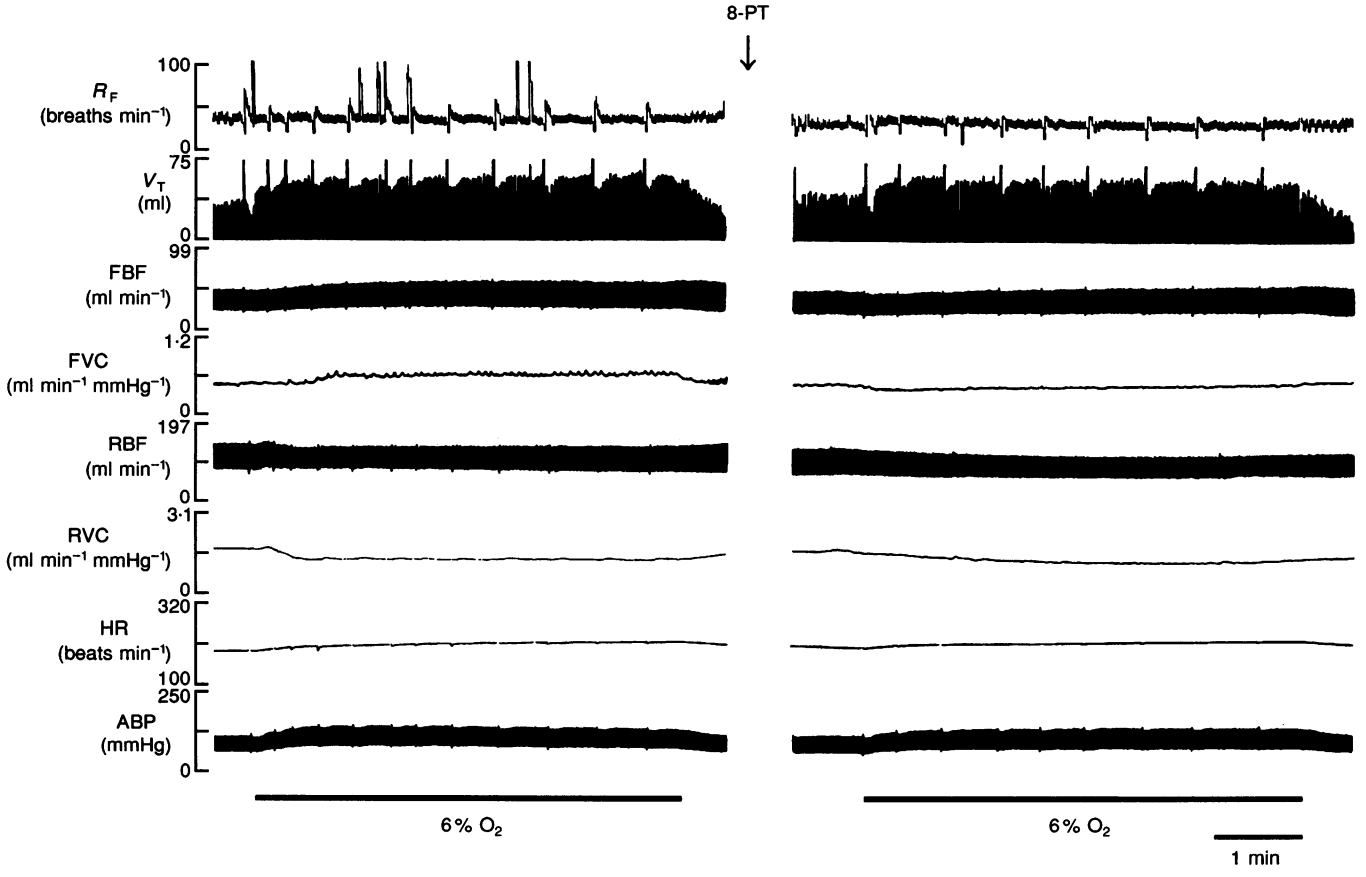

Figure 4

Respiratory and cardiovascular responses evoked in a 3-week-old piglet by 6% O_2 before and after 8-PT (8 mg kg^{-1} I.V.). Abbreviations as in Fig. 1.

3-week-old group

Baseline values of respiratory and cardiovascular variables before and after 8-PT are shown in Table 1, while blood gas values recorded during air breathing and hypoxia, before and after 8-PT, are shown in Table 2.

Responses evoked by hypoxia

Both 10 and 6% O_2 evoked sustained increases in V_T, R_F and thereby in $\dot{V}_E$, such that values recorded at the 5th minute were significantly different from control: there was no waning of V_T between the 2nd and 5th minute (Figs 4 and 5). Concomitantly, P_{a,O_2} and P_{a,CO_2} fell while arterial pH rose (Table 2). These changes were accompanied by a sustained increase in ABP and HR such that both were significantly different from control at the 5th minute. FVC also increased substantially by the 5th minute allowing an increase in FBF, whereas RVC decreased such that RBF remained constant (Figs 4 and 5).

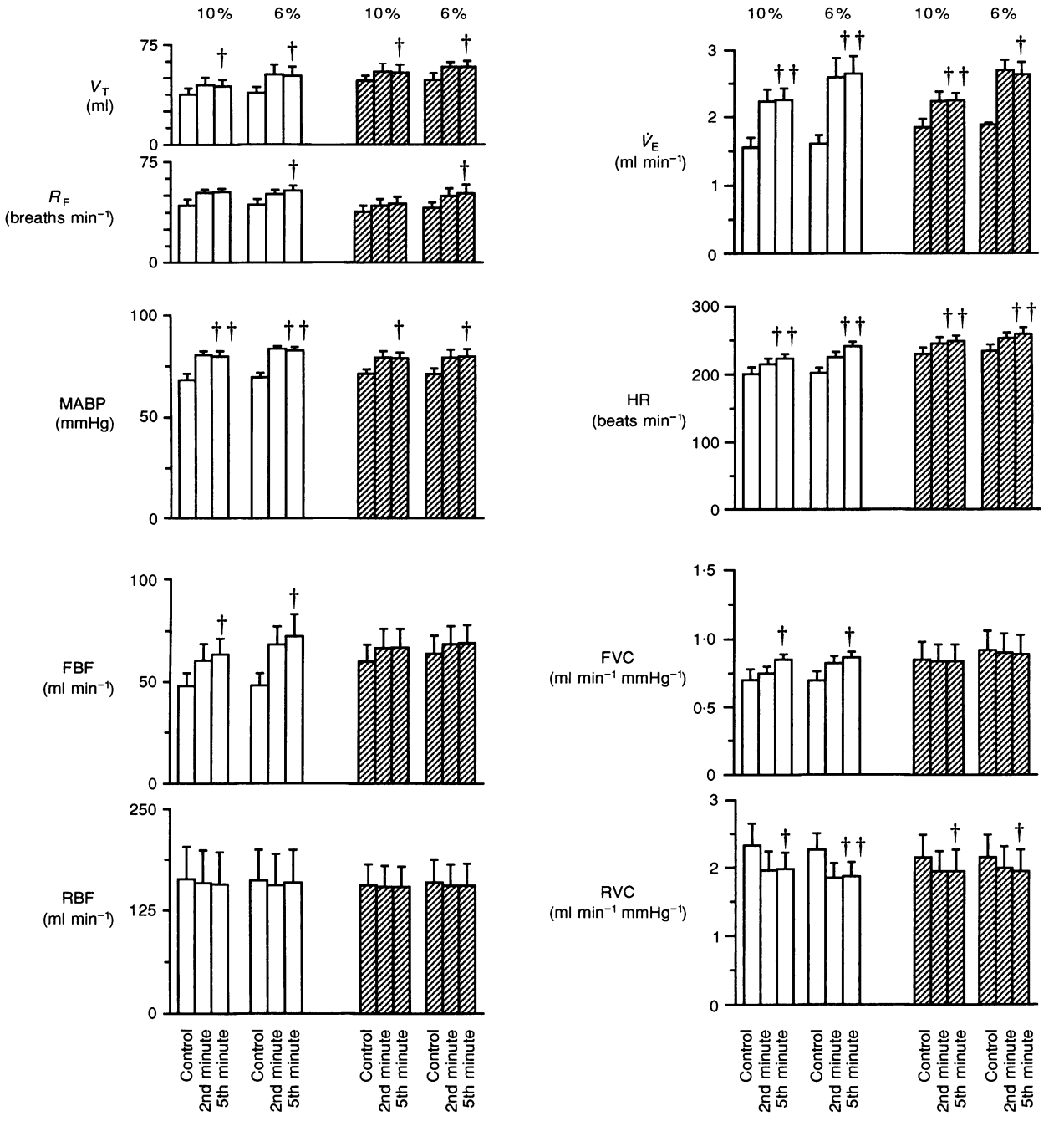

Figure 5. Mean respiratory and cardiovascular values recorded in 3-week-old piglets during air breathing and during 10 and 6% O_2 before and after 8-PT (8 mg kg^{-1} I.V.)

Columns show means ± S.E.M. before (open) and after (hatched) 8-PT. All abbreviations and symbols as in Fig. 2.

As in the newborn group, hypoxia induced an increase in the frequency of gasps and their associated cardiovascular changes (Fig. 4), but during 10 and 6% O_2 the gasping frequency was lower than in the younger group (compare Figs 1 and 4).

Effects of 2-CA and 8-PT

Before 8-PT, 2-CA (30 mg kg^{-1}) evoked an increase in R_F ($16 \pm 2{\cdot}6$% at peak), and a fall in ABP (-37 ± 12%), in HR ($-15 \pm 1{\cdot}6$%) and in RVC (-70 ± 20%) as in the younger group. However, in addition, there was an increase in FVC (47 ± 17%).

Administration of 8-PT caused a significant increase in the baseline values of V_T and $\dot{V}_E$ and a concomitant fall in P_{a,CO_2} and rise in arterial pH (Tables 1 and 2). HR increased, but there was no significant change in the other cardiovascular variables (Table 1). The respiratory and cardiovascular responses evoked by 2-CA were virtually blocked.

From these new baselines, 10 and 6% O_2 still caused sustained increases in V_T, R_F, $\dot{V}_E$ and HR (Figs 4 and 5). Further, there was still a decrease in RVC such that RBF remained constant. However, in contrast to the changes evoked before 8-PT, there were no significant increases in FVC or RBF by the 5th minute of 10 and 6% O_2 (Figs 4 and 5). Given that baseline FVC tended to increase after 8-PT (Table 1), it might be questioned whether the loss of the hypoxia-induced increase in FVC occurred simply because the muscle vasculature was maximally dilated. This was not the case: baseline FVC after 8-PT and immediately before 10 and 6% O_2 ($0{\cdot}85 \pm 0{\cdot}13$ and $0{\cdot}92 \pm 0{\cdot}14$ ml min^{-1} $mmHg^{-1}$, respectively) was lower than that achieved in response to 2-CA before 8-PT ($1{\cdot}01 \pm 0{\cdot}01$ ml min^{-1} $mmHg^{-1}$, respectively).

DISCUSSION

The results of the present study indicate that the pattern of respiratory and cardiovascular response evoked by moderate and severe systemic hypoxia in healthy, spontaneously breathing piglets changes substantially between 3 days and 3 weeks after birth. They also suggest that locally released adenosine makes important contributions to the respiratory and cardiovascular response, but that these contributions are very different at 3 days and 3 weeks.

Responses evoked in newborn piglets

The baseline levels of V_T and R_F, blood gases, ABP and HR recorded in the 3-day-old, Saffan-anaesthetized piglets of the present study were comparable to those recorded by others in conscious or lightly sedated piglets of a similar age (Darnall, 1985; Moss, Runold, Dahlin, Fredholm, Nyberg & Lagercrantz, 1987; Darnall *et al.* 1991; Rosen, Schecter, Mellins & Haddad, 1993). The levels of P_{a,O_2} and ABP were markedly lower than those expected in adult mammals, but well within the range expected in a newborn mammal. The animals were relatively hypocapnic even during air breathing, indicating hyperventilation. This may reflect the actions of Saffan anaesthesia and the surgical trauma. It seems unlikely that this hypocapnia affected the cardiovascular system significantly given the normal levels of ABP and HR (see Marshall, 1994).

Considering the pattern of response evoked by systemic hypoxia, the biphasic respiratory response (an initial increase in V_T and R_F, and secondary fall in V_T) was as expected in newborn mammals (e.g. Lawson & Long, 1983; Darnall, 1985). Given the close interrelationships between respiration and HR, at least in adult mammals (Daly, 1986; Marshall, 1994), it is perhaps surprising that HR showed a sustained increase during hypoxia, rather than a biphasic response. Indeed, as the primary reflex effect of hypoxic stimulation of the peripheral chemoreceptors is bradycardia in neonates, as in adult mammals (see Marshall, 1994), the initial increase in HR in hypoxia may have been secondary to the increase in ventilation and caused by one or more of the mechanisms that are known to exist in adult mammals. (Daly, 1986; Marshall, 1994). Certainly, Gootman *et al.* (1991) showed that in piglets of up to 2 weeks old, hypoxia increased cervical sympathetic activity and this activity was modulated in phase with central respiratory drive whether the vagi were intact or sectioned, indicating that central inspiratory drive can modulate sympathetic activity at this age. The influence of hypoxia of the central nervous system on cardiac sympathetic activity may also have contributed to the tachycardia (Downing, Mitchell & Wallace, 1963) and may have been the overriding influence that kept HR raised when ventilation fell towards control level between the 2nd and 5th minute of hypoxia.

A renal vasoconstrictor response to systemic hypoxia has been recorded previously in neonates (in newborn rabbits; Guoyon & Guignard, 1988). By contrast, as FBF increased significantly by the 5th minute of breathing 6% O_2, at a time when ABP was falling towards control, this suggests that vasodilatation must have occurred in skeletal muscle, even though the change in FVC failed to reach statistical significance; muscle vasodilatation was also recorded in the newborn lamb during 6% O_2 (Sidi *et al.* 1983). Muscle vasodilatation at the 5th minute of hypoxia was unlikely to have been a secondary effect of the increase in ventilation (Daly, 1986; Marshall, 1994), given that ventilation was returning to control values at this time.

Taking the cardiac and vascular responses together, it can be concluded that the initial increase in ABP during hypoxia was probably due to an increase in cardiac output associated with tachycardia and augmented by peripheral vasoconstriction occurring in the kidney and possibly elsewhere. Since tachycardia, and presumably cardiac output, was maintained until the end of hypoxia, the secondary fall in ABP during exposure to 6% O_2 may be ascribed to a net fall in total peripheral resistance, reflecting vasodilatation in skeletal muscle.

The respiratory gasps – each associated with transient bradycardia, peripheral vasodilatation and fall in ABP that were superimposed upon the gradual changes induced by hypoxia – were comparable to those seen in the adult rat and cat. They have been attributed to the primary reflex effect of stimulation of the rapidly adapting irritant receptors in the airways (Marshall & Metcalfe, 1988*a*, 1989). The whole of this reflex is apparently present in the newborn piglet and, as in the rat, it makes an important contribution to the pattern of response produced by hypoxia (Marshall & Metcalfe, 1988*a*).

Clearly, healthy, spontaneously breathing, 3-day-old piglets did not show the biphasic tachycardia and bradycardia, and fall in ABP we had expected from some previous studies on newborn mammals or adult rats (see the introduction). In fact, this pattern of cardiovascular response only occurred in the two piglets that had low P_{a,O_2} when breathing air and in whom respiration failed during experimentally imposed hypoxia. This raises the possibility that HR and ABP are more likely to fall in the newborn mammal when the level of hypoxia is particularly severe and/or when hyperventilation is weak or non-existent. The results of Gootman *et al.* (1990; and see the introduction) are consistent with that view, for the fall in ABP and the femoral vasodilatation with little change in HR that they recorded in 2- to 4-day-old, Saffan-anaesthetized piglets occurred when P_{a,O_2} was reduced from 187 to 33 mmHg under constant artificial ventilation. A comparable response pattern occurred in spontaneously breathing newborn lambs when the period of breathing 6% O_2 was extended beyond 10, to 20 min (Sidi *et al.* 1983).

We proposed on the basis of experiments on the rat (see Thomas & Marshall, 1994) that during spontaneous ventilation, the responses induced by hypoxia can become interdependent in a positive feedback fashion that ultimately leads to death: a fall in ABP resulting from bradycardia and vasodilatation limits blood flow to the brain so accentuating the cerebral hypoxia that leads to hypoventilation (see the introduction) and causing further hypoxia. The present observations on the hypoxic piglets accord with that proposal and with our finding that the very components of the response pattern to acute hypoxia that predispose towards the development of positive feedback were more pronounced in rats that had been chronically hypoxic from birth, so that such individuals may be at risk of sudden infant death syndrome (Thomas & Marshall, 1995).

The effects of 8-PT: the role of adenosine

The increase in R_F, decrease in HR and RVC evoked by the stable analogue of adenosine, 2-CA, were fully comparable with the responses evoked in the adult rat (Thomas *et al.* 1994). The increase in R_F can be attributed to the reflex effect of stimulating peripheral chemoreceptors (McQueen & Ribeiro, 1983), the decrease in HR to direct actions on adenosine receptors on the sino-atrial node, or on presynaptic receptors that potentiate the effects of vagal activity, or that reduce the effects of sympathetic activity (Olsson & Pearson, 1990; Verlato & Borgdorff, 1990) and the decrease in RVC can be ascribed to stimulation of adenosine receptors in the kidney (Spielman & Thompson, 1982). However, 2-CA had no effect on muscle vasculature, in direct contrast to the substantial muscle vasodilatation evoked by 2-CA in the adult rat (Thomas *et al.* 1994). This suggests that at 3 days, the density of adenosine receptors in skeletal muscle is low, or the adenosine receptors are not able to stimulate the appropriate second messenger systems. Accordingly, carotid arteries taken from newborn piglets showed a much lower sensitivity to the relaxing effect of adenosine than those of adult pigs (Laudignon, Arnada & Varma, 1990).

In order to block adenosine receptors we used 8-PT, a xanthine derivative that is more effective than theophylline (Williams, 1989) and does not have the inhibitory effect on phosphodiesterase activity, or other non-specific effects of theophylline (Smellie, Davis, Daly & Wells, 1979). Given at 8 mg kg^{-1} as in the present study, 8-PT remained at a stable concentration in plasma of newborn piglets for at least 1 h (Laudignon *et al.* 1990). Certainly, responses evoked by 2-CA were still blocked at the end of our experiments, ~1 h after giving 8-PT.

The fact that baseline V_T stabilized at a higher level after 8-PT is consistent with previous observations on the effect of theophylline on newborn piglets and human infants (Darnall, 1985; Milerad, 1987) and 8-PT on the adult rat (Thomas & Marshall, 1994). These results indicate that adenosine tonically inhibits respiration even during air breathing, by an action within the central nervous system. The fall in baseline P_{a,CO_2} and rise in arterial pH can be seen as the consequence of the increase in V_T. Since we were interested in the full effect of 8-PT on the hypoxia-induced respiratory and cardiovascular responses we made no attempt to correct this respiratory alkalosis. As baseline FVC and RVC were not changed by 8-PT, this is consistent with evidence that hypocapnia generally has a weak influence on peripheral vasculature (Marshall, 1994). That 8-PT caused an increase in the baseline HR could be attributed to the influence of hypocapnia on the sino-atrial node (Marshall, 1994), but would also be explained if locally released adenosine exerts a tonic inhibitory effect on HR during air breathing (see also Darnall, 1985). The apparent competition between 8-PT and the effects of the Saffan anaesthesia may be attributed to removal of a tonic inhibitory influence of locally released adenosine upon neurones of the cortex and other forebrain structures (Zimmerman, 1994).

During hypoxia, 8-PT abolished the secondary decrease in V_T, in accord with many previous studies on newborn mammals in which xanthine derivatives were used as adenosine antagonists (Darnall, 1985; Runold, Lagercrantz, Prabhakar & Fredholm, 1989) and with our own studies on

the adult rat (Thomas & Marshall, 1994). In the newborn piglet and adult rat, adenosine levels within the brain or cerebrospinal fluid have been shown to rise during systemic hypoxia (Winn, Rubio & Berne, 1981; Laudignon, Farri, Beharry, Rex & Aranda, 1991), consistent with the view that adenosine acts centrally to depress respiration. By contrast, the lack of effect of 8-PT on the tachycardia induced by systemic hypoxia indicates that adenosine was not released in sufficiently high concentrations to counteract the effect of the mechanisms that produce tachycardia. Although experiments on guinea-pig, rat and rabbit hearts *in vitro* indicate that locally released adenosine can exert a major effect on HR in hypoxia (Froldi & Belardinelli, 1990), agents that block adenosine receptors or increase the breakdown of adenosine did not consistently reduce the secondary bradycardia induced in the rat by systemic hypoxia (see Thomas *et al.* 1994; Thomas & Marshall, 1994).

On the other hand, since 8-PT blocked the decrease in RVC and FBF during 10% O_2, this indicates that locally released adenosine did contribute to the renal vasoconstriction induced by moderate hypoxia (see Spielman & Thompson, 1982). Similarly, theophylline blocked the renal vasoconstriction induced by systemic hypoxia in 3- to 12-day-old rabbit kittens (Gouyon & Guignard, 1988). However, as 8-PT had no effect on FVC or FBF during hypoxia, it seems locally released adenosine did not exert a dilator influence upon skeletal muscle vasculature. This agrees with our finding that 2-CA had no effect on muscle vasculature (see above) but contrasts markedly with our findings in the adult rat (see the introduction). In the piglet, hypoxia-induced muscle vasodilatation may be due to the direct influence of hypoxia on vascular smooth muscle, to potassium or other locally released vasodilator substances, or to the action of circulating catecholamines on β-adrenoreceptors, which are present in femoral circulation at birth (Buckley, Brazeau & Gootman, 1983; Marshall, 1995).

Responses evoked in older piglets

The fact that a secondary fall in V_T was no longer present during the 5 min periods of hypoxia in the 3-week-old piglets is consistent with much previous evidence (see the introduction and Moss *et al.* 1987). The hypoxia-induced tachycardia was similar in time course to that seen in the newborn piglets, but whether it was produced by the central effects of hypoxia or the secondary effects of the hyperventilation (see above), or whether the relative importance of these effects differed at 3 weeks and 3 days, cannot be deduced without further experimentation. In contrast to the newborn piglets, the renal vasoconstrictor response to hypoxia of the 3-week-old piglets was not associated with a change in RBF. Thus, there is no need to invoke any mechanism other than a myogenic response of the renal vasculature to the rise in ABP. On the other hand, the increase in FVC was much more pronounced at 3 weeks, indicating substantial muscle vasodilatation. Although direct comparisons cannot be made between the present study and that of Gootman *et al.* (1990), they too reported that artificially ventilated, 2-week-old piglets showed a much greater increase in FVC when P_{a,O_2} was reduced from 200 to 37 mmHg than did newborn piglets.

Hypoxia also evoked an increase in the frequency of respiratory gasps and their associated cardiovascular changes as in newborn piglets, but at a lower frequency. This is consistent with much evidence that gasping frequency is indirectly related to lung size (see Marshall & Metcalfe, 1988*a*, for references).

Effects of 8-PT: the role of adenosine

As in newborn piglets, 8-PT increased baseline levels of V_T and HR, suggesting a tonic influence of locally released adenosine upon these variables. Moreover, as in newborn piglets, 2-CA evoked an increase in R_F, bradycardia and renal vasoconstriction that were attenuated by 8-PT. However, 2-CA also evoked a substantial increase in FVC that was abolished by 8-PT. Thus, by 3 weeks adenosine receptors are present within skeletal muscle vasculature and are coupled to the appropriate second messenger systems as they are in adult mammals (see Laudignon *et al.* 1990; Marshall *et al.* 1993).

The fact that 8-PT had no effect on the sustained ventilatory response to hypoxia in the 3-week-old piglets is perhaps surprising since the concentration of adenosine in the cerebrospinal fluid of 1-month-old piglets has been shown to rise substantially during hypoxia (P_{a,O_2}: 27 mmHg; Laudignon *et al.* 1991). By this age, the stimulatory effect of the peripheral chemoreceptors upon ventilation can apparently overcome the depressive effect of centrally released adenosine, at least during hypoxia lasting 5 min. The lack of effect of 8-PT upon the hypoxia-induced tachycardia indicates that any further release of adenosine in the heart did not counteract the mechanisms that induce tachycardia (see above). As 8-PT also had no effect on the renal vasoconstrictor response to hypoxia, it seems that, in contrast to the newborn piglet, locally released adenosine made relatively little contribution to it. A similar disparity was noted by Gouyon & Guignard (1988) in newborn and mature rabbits. The fact that 8-PT completely abolished the hypoxia-induced femoral vasodilatation represents a further contrast with the newborn piglets and indicates that by 3 weeks, adenosine is released within skeletal muscle during hypoxia and acts on adenosine receptors to induce vasodilatation.

In summary, the present study on the spontaneously breathing piglet has shown that the respiratory and cardiovascular responses evoked by acute systemic hypoxia change during postnatal development. We have confirmed that at 3 weeks an increase in ventilation predominates and a secondary fall in ventilation is much less evident than at 3 days. However, we have also shown that while tachycardia occurs at both ages, strong renal vaso-

constriction occurs at 3 days, but not at 3 weeks, while muscle vasodilatation is weak at 3 days, but becomes pronounced by 3 weeks. Further, we have not only substantiated previous evidence that centrally released adenosine contributes to the secondary fall in ventilation at 3 days, but have demonstrated that locally released adenosine may be largely responsible for the renal vasoconstriction at 3 days and for the muscle vasodilatation at 3 weeks.

BUCKLEY, N. M., BRAZEAU, P. & GOOTMAN, P. M. (1983). Maturation of circulatory responses to adrenergic stimuli. *Federation Proceedings* **42**, 1643–1647.

DALY, M. DE BURGH (1986). Interactions between respiration and circulation. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol II, part 2, ed. CHERNIACK, N. S. & WIDDICOMBE, J. G., pp. 529–594. American Physiological Society, Bethesda, MD, USA.

DARNALL, R. A. (1985). Aminophylline reduces hypoxic ventilatory depression: possible role of adenosine. *Pediatric Research* **19**, 706–710.

DARNALL, R. A., GREEN, G., PINTO, L. & HART, N. (1991). Effect of acute hypoxia on respiration and brain-stem blood flow in the piglet. *Journal of Applied Physiology* **70**, 251–259.

DOWNING, D. E., MITCHELL, J. H. & WALLACE, A. G. (1963). Cardiovascular responses to ischaemia, hypoxia and hypercapnia of the central nervous system. *American Journal of Physiology* **204**, 881–887.

EASTON, P. A. & ANTHONISEN, N. R. (1988). Ventilatory response to sustained hypoxia after pretreatment with aminophylline. *Journal of Applied Physiology* **64**, 1445–1450.

FROLDI, G. & BELARDINELLI, L. (1990). Species-dependent effects of adenosine on heart rate and atrio ventricular nodal conduction-mechanism and physiological implications. *Circulation Research* **67**, 960–978.

GOOTMAN, P. M. (1991). Developmental aspects of reflex control of the circulation. In *Reflex Control of the Circulation*, ed. ZUCKER, I. H. & GILMORE, J. P., pp. 965–1027. Telford Press, Calwell, NJ, USA.

GOOTMAN, P. M., BUCKLEY, N. M. & GOOTMAN, N. (1979). Postnatal development of neural control of circulation. In *Review in Perinatal Medicine*, ed. SCARPELLI, E. M. & COSMI, E. V., vol. 3, pp. 1–92. Raven Press, New York.

GOOTMAN, P. M., GANDHI, M. R., STEELE, A. M., HUNDLEY, B. W., COHEN, H. L., EBERLE, L. P. & SICA, A. L. (1991). Respiratory modulation of sympathetic activity in neonatal swine. *American Journal of Physiology* **261**, R1147–1154.

GOOTMAN, P. M., GOOTMAN, N., BUCKLEY, B. J., PETERSON, B. J., STEELE, A. M., SICA, A. L. & GANDHI, M. R. (1990). Effects of hypoxia in developing swine. In *Chemoreceptors and Chemoreceptor Reflexes*, ed. ACKER, H., TRZEBSKI, A. & O'REGAN, R. G., pp. 15–163. Plenum Press, New York.

GOUYON, J. B. & GUIGNARD, J. P. (1988). Theophylline prevents the hypoxemia-induced renal haemodynamic changes in newborn and adult rabbits. *Kidney International* **33**, 1078–1083.

GREEN, C. J. (1979). *Animal Anaesthesia*. Laboratory Animals Ltd, London.

HANSON, M. A. (1986). Peripheral chemoreceptor functions before and after birth. In *Reproductive and Perinatal Medicine (III), Respiratory Control and Lung Development in the Fetus and Newborn*, ed. JOHNSON, B. M. & GLUCKMAN, P. D., pp. 311–330. Perinatology Press, Ithaca, NY, USA.

HILTON, S. M. & MARSHALL, J. M. (1982). The pattern of cardiovascular response to carotid chemoreceptor stimulation in the cat. *Journal of Physiology* **326**, 495–513.

LAUDIGNON, N., ARANDA, J. V. & VARMA, D. R. (1990). Effects of adenosine and its analogues on isolated internal carotid arteries from newborn and adult pigs. *Biology of the Neonate* **58**, 91–97.

LAUDIGNON, N., FARRI, E., BEHARRY, K., REX, J. & ARANDA, J. V. (1991). Influence of adenosine on cerebral blood flow during hypoxic hypoxia in the newborn piglet. *Journal of Applied Physiology* **68**, 1534–1541.

LAWSON, E. E. & LONG, W. A. (1983). Central origin of biphasic breathing pattern during hypoxia in newborns. *Journal of Applied Physiology* **55**, 483–488.

MCQUEEN, D. S. & RIBEIRO, J. A. (1983). On the specificity and type of receptor involved in carotid body chemoreceptor activation by adenosine in the cat. *British Journal of Physiology* **80**, 347–354.

MARSHALL, J. M. (1987). Analysis of cardiovascular responses evoked following changes in chemoreceptor activity in the rat. *Journal of Physiology* **394**, 393–415.

MARSHALL, J. M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiological Reviews* **74**, 543–594.

MARSHALL, J. M. (1995). Skeletal muscle vasculature and hypoxia. *News in Physiological Sciences* **10**, 274–280.

MARSHALL, J. M. & METCALFE, J. D. (1988*a*). Cardiovascular changes associated with augmented breaths in normoxia and hypoxia in the rat. *Journal of Physiology* **400**, 15–27.

MARSHALL, J. M. & METCALFE, J. D. (1988*b*). Analysis of the cardiovascular changes induced in the rat by graded levels of systemic hypoxia. *Journal of Physiology* **407**, 383–403.

MARSHALL, J. M. & METCALFE, J. D. (1989). Analysis of factors that contribute to cardiovascular changes induced in the cat by graded levels of systemic hypoxia. *Journal of Physiology* **412**, 429–448.

MARSHALL, J. M., THOMAS, T. & TURNER, L. (1993). A link between adenosine, ATP-sensitive K^+ channels and muscle vasodilatation in the rat in systemic hypoxia. *Journal of Physiology* **459**, 1–9.

MIAN, R. & MARSHALL, J. M. (1991). The role of adenosine in dilator responses induced in arterioles and venules of rat skeletal muscle in systemic hypoxia. *Journal of Physiology* **493**, 499–511.

MILERAD, J. (1987). Effects of theophylline on ventilatory responses to hypoxic challenge. *Archives of Diseases in Childhood* **62**, 1242–1246.

MILLHORN, D. E., ELDRIDGE, F. L., KILEY, J. P. & WALDROP, T. G. (1984). Prolonged inhibition of respiration following acute hypoxia in glomectomised cats. *Respiration Physiology* **57**, 331–340.

MOSS, I. R., RUNOLD, M., DAHLIN, I., FREDHOLM, B. B., NYBERG, F. & LAGERCRANTZ, H. (1987). Respiratory and neuroendocrine responses of piglets to hypoxia during postnatal development. *Acta Physiologica Scandinavica* **131**, 533–541.

NEYLON, M. & MARSHALL, J. M. (1991). The role of adenosine in the respiratory and cardiovascular response to systemic hypoxia in the rat. *Journal of Physiology* **440**, 529–545.

OLSSON, R. A. & PEARSON, J. D. (1990). Cardiovascular purinoceptors. *Physiological Reviews* **70**, 761–845.

ROSEN, C. L., SCHECTER, W. S., MELLINS, R. B. & HADDAD, G. G. (1993). Effects of acute hypoxia on metabolism and ventilation in awake piglets. *Respiration Physiology* **91**, 307–319.

RUNOLD, M., LAGERCRANTZ, N., PRABHAKAR, N. R. & FREDHOLM, B. B. (1989). Role of adenosine in hypoxia ventilatory depression. *Journal of Applied Physiology* **67**, 541–546.

SIDI, D., KUIPERS, J. R. G., TEITEL, D., HEYMANN, M. A. & RUDOLPH, A. M. (1983). Developmental changes in oxygenation and circulatory responses to hypoxemia in lambs. *American Journal of Physiology* **245**, H674–682.

SMELLIE, F. W., DAVIS, C. W., DALY, J. W. & WELLS, J. N. (1979). Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and brain phosphodiesterase activity. *Life Sciences* **24**, 2475–2482.

SPIELMAN, W. S. & THOMPSON, C. I. (1982). A proposed role for adenosine in the regulation of renal haemodynamics and renin release. *American Journal of Physiology* **242**, F423–435.

THOMAS, T., ELNAZIR, B. & MARSHALL, J. M. (1994). Differentiation of the peripherally mediated from the centrally mediated influences of adenosine in the rat during systemic hypoxia. *Experimental Physiology* **79**, 809–822.

THOMAS, T. & MARSHALL, J. M. (1994). Interdependence of respiratory and cardiovascular changes induced by systemic hypoxia in the rat: the roles of adenosine. *Journal of Physiology* **480**, 627–636.

THOMAS, T. & MARSHALL, J. M. (1995). A study on rats of the effects of chronic hypoxia from birth on respiratory and cardiovascular responses evoked by acute hypoxia. *Journal of Physiology* **487**, 513–525.

TIMMS, R. J. (1981). A study of the amygdaloid defence reaction showing the value of Althesin anaesthesia in studies of the functions of the fore-brain in cats. *Pflügers Archiv* **391**, 49–56.

VERLATO, G. & BORGDORFF, P. (1990). Endogenous adenosine enhances vagal negative chronotropic effect during hypoxia in the anaesthetised rabbit. *Cardiovascular Research* **24**, 532–539.

WILLIAMS, M. (1989). Adenosine antagonists. *Medical Research Reviews* **9**, 219–243.

WINN, H. R., RUBIO, R. & BERNE, R. M. (1981). Brain adenosine concentration during hypoxia in rats. *American Journal of Physiology* **241**, H235–242.

ZIMMERMAN, H. (1994). Signalling via ATP in the nervous system. *Trends in Neurosciences* **17**, 420–426.

Acknowledgements

B.E. was supported by a British Council Studentship. We also acknowledge the generous support of the Mary Crosse Trust Fund, administered by South Birmingham Health Authority.

Author's present address

B. Elnazir: Coombe Women's Hospital, Department of Paediatrics, Dolphin's Barn, Dublin, Eire.

Received 10 July 1995; accepted 20 November 1995.