Br. J. Pharmac. (1985), **86**, 69–77

The influence of blood gases on α_1- and α_2-adrenoceptor-mediated pressor responses in the pithed rat

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1 The influence of blood gases on α_1- and α_2-adrenoceptor-mediated pressor responses was studied in the pithed rat by varying the inspired gas mixture or the ventilation stroke volume.

2 Acidosis favoured the peak responses to the α_2-adrenoceptor agonist, xylazine, while alkalosis favoured the peak responses to the α_1-adrenoceptor agonist, phenylephrine. A combination of hypoxia and hypercapnia greatly depressed the α_1 response to phenylephrine whereas the α_2 response to xylazine remained relatively unaffected.

3 When Pa_{O_2} was varied in either acidotic or alkalotic conditions the response to the phenylephrine increased as Pa_{O_2} increased.

4 To prevent hypoxia in air ventilated rats, large stroke volumes were required. This caused alkalosis and hence decreased responsiveness to xylazine. Consequently, air ventilated pithed rats gave poorer responses to xylazine than did those ventilated on 100% O_2.

5 The results show that α_1- and α_2-adrenoceptor-mediated pressor responses can be differentially affected by blood gases. The relative contribution of α_1- and α_2-adrenoceptors to vascular tone may be either under- or over-estimated depending on the arterial blood gases.

Introduction

The pithed rat preparation has been employed to demonstrate the presence of α_1- and α_2-adrenoceptors in vascular smooth muscle (Docherty *et al.*, 1979; Docherty & McGrath, 1980 a, b). In a preliminary study of oxygen-ventilated rats (McGrath *et al.*, 1982), it was found that altering the artificial ventilation volume, and hence varying the blood gases, changed the susceptibility of the pressor response of adrenaline to α-blockers. In respiratory acidosis the α_1 antagonist, prazosin, was more potent than in alkalosis while the opposite was found with the α_2 antagonist, rauwolscine. Similarly, agonists that are 'selective' for these two receptor sub-types were affected differently by alteration of ventilation. Hyperventilation favoured the pressor response to phenylephrine (α_1) while hypoventilation favoured xylazine (α_2). Since O_2 tensions remained above physiological levels in these experiments these changes were attributed to alterations of the acid-base status.

These experiments could not exclude the possibility that artificially high Pa_{O_2} had distorted the physiological response nor could they distinguish between the simultaneous changes in Pa_{O_2} and Pa_{CO_2} that accompany alterations in the stroke volume. In order to separate these variables and to examine the relative significance of α_1- and α_2-mediated responses under more physiological levels of blood gases, we have now repeated these experiments using three further protocols. First, the animals were respired on air and the ventilation stroke volume was varied as before. Secondly, the stroke volume was kept constant and the inspired gas mixture was varied to keep the Pa_{O_2} constant at approximately physiological levels while CO_2 was added in order to alter acid-base status. Thirdly, the O_2 was varied against two different background levels of CO_2.

These results are discussed in relation to the physiological conditions which might be optimal for activation of the α-adrenoceptor sub-types.

Methods

Male Wistar rats (245–265g) were pithed under halothane anaesthesia by the method of Gillespie *et al.* (1970). Carotid arterial pressure was monitored and

Table 1 A comparison of arterial blood gases at the beginning and the end of concentration-response curves to either xylazine or phenylephrine

	pH		$Paco_2$		Pao_2	
	before	*after*	*before*	*after*	*before*	*after*
Mean	7.55	7.55	18.2	18.3	77.9	73.5
± s.e.mean	0.04	0.03	2.8	2.9	3.8	3.6
Paired *t* test	NS		NS		NS	

Rats were ventilated on air (60 strokes min^{-1}) with a stroke volume of 4.0 ml per stroke. Comparisons were made using Student's paired *t* test ($n = 5$). Table 1 shows that arterial blood gases did not significantly change during these experiments.

the heart rate was extracted from this by an instantaneous ratemeter. Drugs were injected via a jugular vein. Carotid arterial blood samples (0.6 ml) were taken for blood gas analysis (IL 213 blood gas analyser) approximately 5 min before administration of agonists. On several occasions, a blood sample was also taken at the end of the experiment to confirm that the arterial blood gases had not significantly altered during the experiment. Table 1 shows a comparison of the blood gases in air ventilated rats (4.0 ml per stroke) at the beginning and end of administration of either xylazine or phenylephrine. There were no significant changes in arterial blood gases during these experiments. Similarly, no significant changes in arterial blood gases were observed in rats ventilated by any of the other methods employed in this study.

The rats were ventilated with one of the following gas mixtures; (i) air, (ii) 100% O_2, (iii) 30% O_2 with varying CO_2 levels (0–4%) or (iv) varying O_2 levels with 0 or 4% CO_2. The ventilation rate was fixed at 60 strokes per min in all rats. Stroke volume was varied in rats ventilated with air or 100% O_2 (1.8–4.0 ml per stroke) thus altering acid-base status. Stroke volume was fixed at 3.5 ml in rats ventilated by methods (iii) and (iv): the remainder of the gas was N_2. pH and $PaCO_2$ values were within the ranges 7.73–7.10 and 10–66 mmHg, respectively. PaO_2 varied between 50 and 470 mmHg. Pressor responses were assessed as changes in diastolic arterial blood pressure.

The diastolic pressor responses to six increasing doses of α-adrenoceptor agonists, phenylephrine (α_1) and xylazine (α_2), were studied at different values of pH, $PaCO_2$ and PaO_2. One dose-response curve to one agonist was obtained in each rat. Each dose was administered in a fixed volume of 1 mg kg^{-1} followed by a similar volume of saline.

The results were analysed by Student's *t* test (paired or unpaired) and regression analysis. Most of the changes which we analysed appeared to fit a linear relationship between response and gas tension. However, in the case of the response to phenylephrine in alkalotic conditions (see Figure 5) the data suggested a quadratic relationship. In Figure 5 we have thus attempted to fit a curve of best fit choosing the model

$$y = a_o + a_1x + a_2x^2 + e$$

where y = response and x = gas tension. An estimate of the coefficients a_o, a_1 and the constant e was made by the regression analysis routine described in MINITAB (Ryan *et al.*, 1976). This procedure chooses the coefficients to minimise the mean of the squares of the difference between the measured values and those predicted by the equation. For this case the predicted equation was

$$y = 25.9 + 0.545x - 0.0013x^2$$

This fit (and the linear relations indicated in the other regression analyses) is being used in a purely descriptive manner and not to make statistical inferences about the underlying processes.

The drugs were dissolved in 0.9% w/v NaCl solution. Drugs used were (−)-phenylephrine hydrochloride (Sigma) and xylazine hydrochloride (Bayer).

Results

The effects of manipulating ventilation on the arterial blood gases and resting heart rate of the pithed rat

Arterial pH and $PaCO_2$ As shown in Figure 1 (a and b), increasing the ventilation stroke volume (1.8–3.5 ml for O_2; 2.5–4.0 ml for air) increased the arterial pH and decreased the $PaCO_2$. In air-ventilated rats, if the stroke volume were reduced below 2.5 ml, the rats became so hypoxic that the heart stopped. In O_2-ventilated rats, extremely low pH and high $PaCO_2$ levels could be achieved by reducing the stroke volume below 2.5 ml. The rats remained viable because they were still hyperoxic.

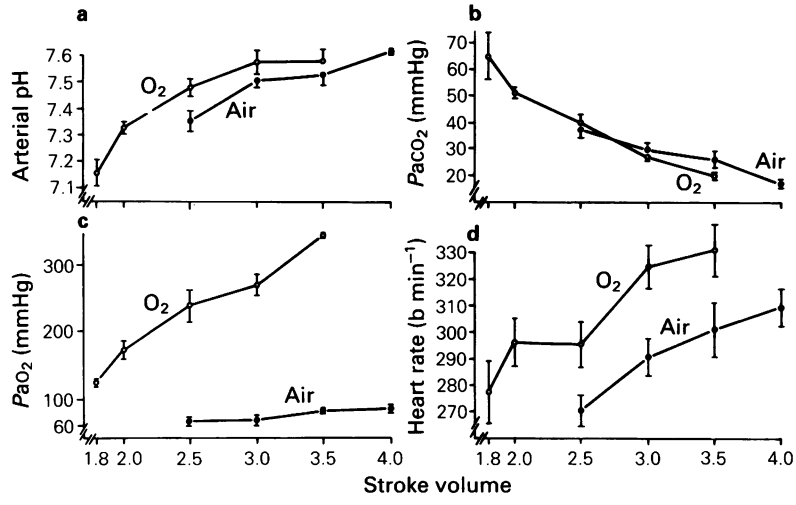


Figure 1 A summary of the effects of varying the ventilatory stroke volume (ml) on (a) arterial pH, (b) $Paco_2$, (c) Pao_2 and (d) heart rate in the pithed rat. Rats were ventilated either with air (●, $n = 21$) or with 100% O_2 (○, $n = 16$).

Pao_2 As shown in Figure 1 (c), increasing the stroke volume (2.5–4.0 ml) modestly increased the Pao_2 in air-ventilated rats. Approximately physiological Pao_2 could only be obtained if the rats were ventilated at a stroke volume of 4.0 ml. However, as shown in Figure 1 (a & b), this would produce a high pH and low $Paco_2$ levels. In contrast, O_2-ventilated rats were always hyperoxic, even at very low stroke volumes. Increasing the stroke volume produced marked increases in Pao_2. At the highest stroke volume studied, Pao_2 values in excess of 300 mmHg were obtained.

Heart rate In both air and O_2-ventilated rats, the basal heart rate increased with increasing stroke volume. Over the directly comparable range of stroke volumes (i.e. 2.5–3.5 ml) O_2-ventilated rats always had higher basal heart rates than air-ventilated rats (see Figure 1d).

The effects of blood gases on pressor responses to α-agonists

Phenylephrine Pressor responses to phenylephrine were biphasic consisting of a rapid initial increase which reached a peak 10–12 s after injection, followed by a slower secondary response with a peak at 22–30 s. Baseline was re-established at between 1.5 and 9 min according to the dose and the blood gas status (see below). A typical pressor response to phenylephrine is shown in Figure 2a. Responses were measured at the early peak, at 30 s and at 2 min. In the dose range

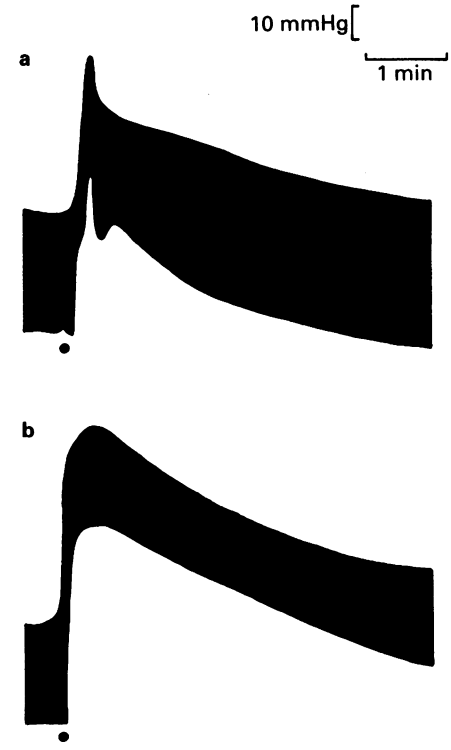


Figure 2 Shows typical pressor responses to (a) phenylephrine (3 μg kg^{-1}) and (b) xylazine (0.5 mg kg^{-1}) in the pithed rat.

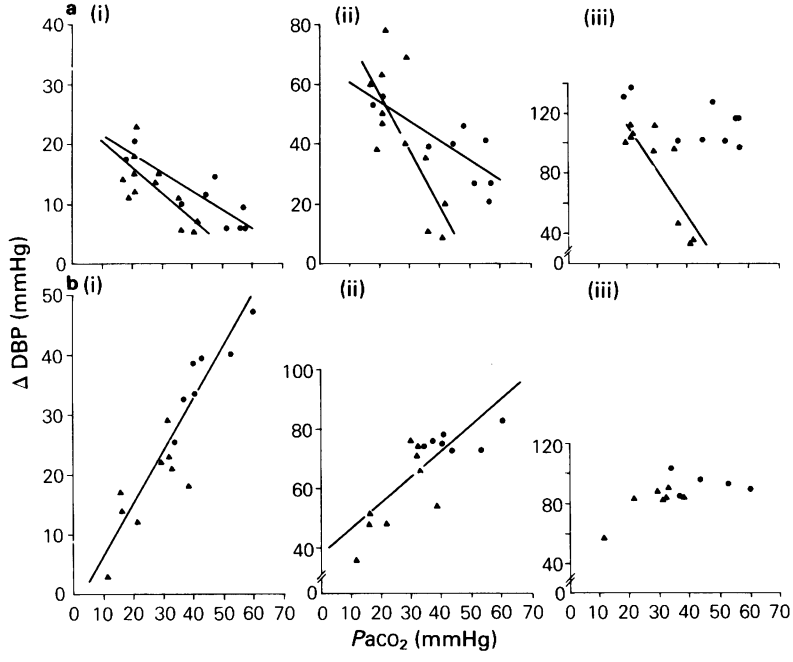


Figure 3 The effects of P_{aCO_2} on the peak change in diastolic arterial blood pressure (DBP mmHg) to (a) phenylephrine (i) 0.3 μg kg^{-1}, (ii) 3.0 μg kg^{-1}, (iii) 30 μg kg^{-1} and (b) xylazine (i) 0.05 mg kg^{-1}, (ii) 0.5 mg kg^{-1}, (iii) 5.0 mg kg^{-1}. Rats were respired on air (▲) or 100% O_2 (●) and ventilatory stroke volume varied. Regression lines are shown in (a) for air (n = 10–12) and O_2 (n = 9) groups; in (b) for all points combined (air, n = 7–9; O_2, n = 5–7). For phenylephrine (30 μg kg^{-1}) on O_2 and for the combined data on xylazine (5 mg kg^{-1}) the correlation is not significant since the responses are maximal.

tested, all components of the response were blocked by prazosin (1 mg kg^{-1}) (Flavahan & McGrath 1981a, b) and are, thus, considered to be mediated by α_1-adrenoceptors.

Air At every dose of phenylephrine studied, the responses decreased with increasing P_{aCO_2} over the range 19–42 mmHg (Figure 3a, i–iii) (further increases in P_{aCO_2} were not possible since reducing

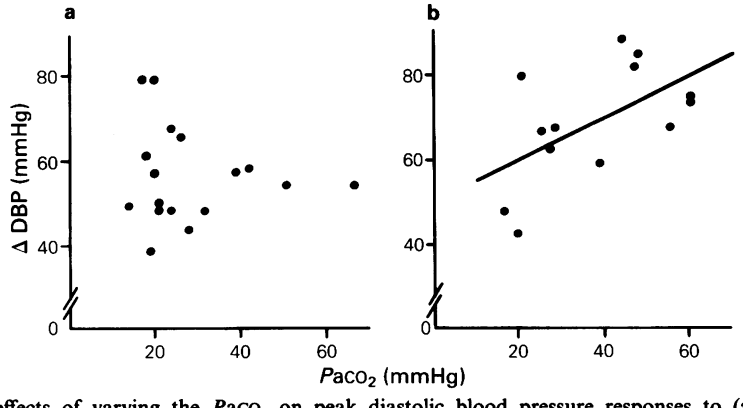


Figure 4 The effects of varying the P_{aCO_2} on peak diastolic blood pressure responses to (a) phenylephrine (3.0 μg kg^{-1}; n = 17) and (b) xylazine (0.5 mg kg^{-1}; n = 13). Rats were ventilated at a constant stroke volume with 30% O_2 to produce normal P_{aO_2} levels. The % of inspired CO_2 was varied in order to vary the P_{aCO_2}.

stroke volume made the rats so hypoxic that the heart stopped). This relationship was similar whether the early peak or 30 s response was measured. At doses falling in the steepest part of the dose-response curve (e.g. 3 $\mu g\,kg^{-1}$), the correlation was clearer for peak than for 30 s (r, peak = 0.79, $0.001 < P < 0.01$; r, 30 s = 0.51, NS, where r = regression coefficient). Therefore Figure 3a shows the peak responses. No significant correlation was found when the response was plotted against PaO_2. The responses were short-lived on air compared to those obtained when respiring with O_2 or a 'normoxic' mixture. Responses at 2 min we detectable only with 10 and 30 $\mu g\,kg^{-1}$ and hypocapnic conditions.

O_2 The preparation was viable over a wider range of $PaCO_2$ levels than could be obtained in air-ventilated rats (18–57 mmHg). The responses decreased with increasing $PaCO_2$ but not as markedly as with air ventilation. Peak responses are shown in Figure 3(a) since the correlation was better (e.g. r, peak = 0.79, $0.001 < P < 0.01$; r, 30 s = 0.67, $0.01 < P < 0.05$ at 3 $\mu g\,kg^{-1}$). With the highest dose of phenylephrine (30 $\mu g\,kg^{-1}$), peak responses did not decline with increasing $PaCO_2$ but responses measured at 30 s did. This may indicate that the 'peak' is maximal and therefore is insensitive to change whereas the declining phase, measured at 30 s, remains sensitive. No significant correlation was found between PaO_2 and response. The response persisted beyond 2 min at doses of 3 $\mu g\,kg^{-1}$ or greater. This prolonged part of the response declined with increasing $PaCO_2$.

'Normoxic' Rats ventilated with a gas mixture containing 30% O_2 and a varying amount of CO_2 (0–4%) had responses to phenylephrine of similar magnitude to those in O_2-ventilated rats (Figure 4a) but there was no significant correlation between the response and $PaCO_2$.

Varying O_2 under acidotic and alkalotic conditions The O_2 content of the inspired gas was varied (15–100%) at constant CO_2 levels of either 0% CO_2 (alkalotic conditions) or 4% CO_2 (acidotic conditions). Under both alkalotic conditions ($PaCO_2$ = 19.0 ± 0.9 mmHg; pH = 7.53 ± 0.02, n = 13) and acidotic conditions ($PaCO_2$ = 52.0 ± 1.1 mmHg; pH = 7.26 ± 0.01, n = 12) responses increased with increasing PaO_2 (Figure 5). However in alkalotic rats, responses reached an optimum at a PaO_2 of around 200 mmHg. Any further increase in PaO_2 produced a reduction in response to phenylephrine.

Xylazine Pressor responses to xylazine were biphasic but less markedly so than were those to phenylephrine. The pressure rose sharply for 12 to 15 s and then continued to rise more slowly to a peak at 30 s or more.

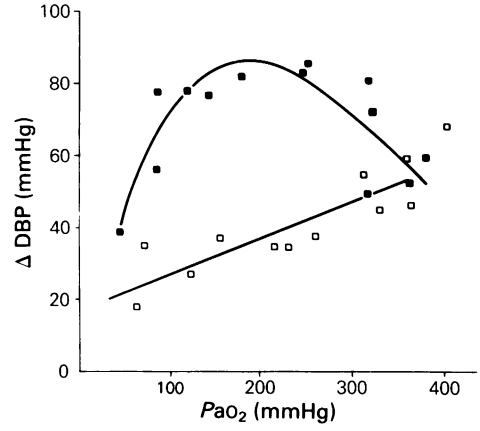


Figure 5 The effects of PaO_2 on the peak diastolic blood pressure response to phenylephrine (3.0 $\mu g\,kg^{-1}$). Rats were ventilated at 3.5 ml per stroke with either 0% CO_2 (■), producing alkalosis, or 4% CO_2 (□), producing acidosis. The inspired % of O_2 was varied at both levels of CO_2. The top line is a line of best fit (13 experiments; quadratic relationship – see methods) using the regression analysis routine in MINITAB (Ryan *et al.*, 1976). The bottom line was obtained from regression analysis (12 experiments).

Baseline was regained between 3–13 min depending on dose. At high doses (> 0.5 mg kg^{-1}) the response remained at a plateau for several minutes before declining. A typical pressor response to xylazine is shown in Figure 2b. Responses were measured at 30 s, at the peak (between 30 s and 5 min according to dose) and at 5 min. At high doses of xylazine the initial peak is sensitive to prazosin and therefore contains an α_1-component. The remainder of the response is resistant to prazosin but susceptible to the α_2 antagonist, rauwolscine (Flavahan & McGrath, 1981a, b). However, in the dose range employed in this study, the pressor response to xylazine is not significantly affected by prazosin (Grant & McGrath, unpublished observations).

Air At each dose of xylazine the response increased with increasing $PaCO_2$. This relationship was similar whether the response was measured at 30 s or at the peak but did not hold at 5 min. Figure 3b shows peak responses (e.g. r, peak = 0.71, r, 30 s = 0.8 ($0.001 < P < 0.01$ for both) and r, 5 min = 0.28, not significant, for 0.1 mg kg^{-1} xylazine). There was no correlation with PaO_2.

O_2 Ventilation with O_2 allowed continuation of the $PaCO_2$-response relationship into the hypercapnic range. At low doses of xylazine (0.01–0.05 mg kg^{-1})

responses continued to increase with increasing $PaCO_2$ over the range studied (34–60 mmHg). At higher doses ($>0.5\,mg\,kg^{-1}$) responses reached a plateau level at $PaCO_2 = 35$ mmHg. The correlation was similar at each of the three time intervals after injection (e.g. r, peak = 0.84; r, 30 s = 0.82, r, 5 min = 0.96 ($0.001 < P < 0.01$) for $0.1\,mg\,kg^{-1}$ xylazine) (see Figure 3b). There was no significant correlation of responses with PaO_2.

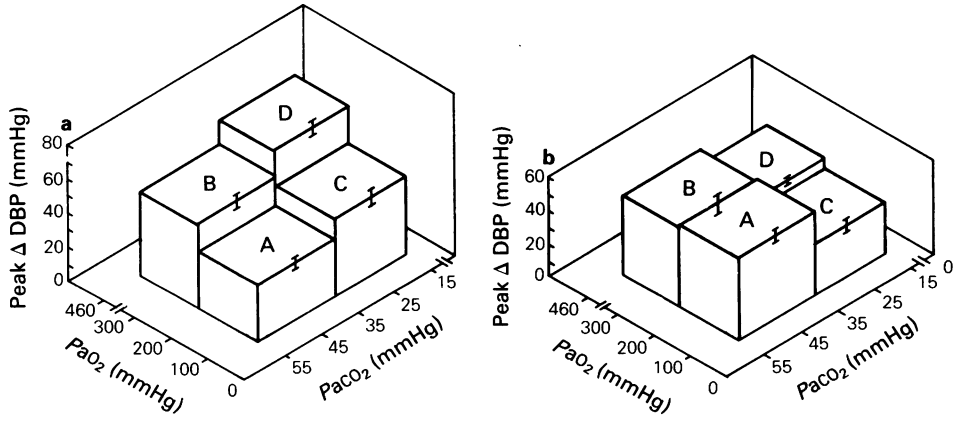
Comparison of air and oxygen On air ventilation, since the responses varied with $PaCO_2$, responses to low doses of xylazine were widely scattered. Nevertheless, if the 'air' and 'O_2' groups are compared, the responses on 'air' are significantly smaller at the lower doses ($P < 0.001$ for $0.05\,mg\,kg^{-1}$; $0.001 < P < 0.01$ for $0.5\,mg^{-1}kg^{-1}$; $0.01 < P < 0.05$ for $5\,mg\,kg^{-1}$). This was not the case with any dose of phenylephrine ($0.05 < P$ for 0.3, 3.0 and $30\,\mu g\,kg^{-1}$).

'Normoxic' When PaO_2 was held constant at approximately physiological levels ($PaO_2 = 100$ mm Hg) and the $PaCO_2$ was varied (0–4%), the responses followed the same relationship as when ventilation was varied on air or 100% O_2. Figure 4b shows the peak responses for $0.5\,mg\,kg^{-1}$ xylazine ($r = 0.62$, $0.01 < P < 0.05$). That is, responses increased with increasing $PaCO_2$.

Discussion

The results show that, (i) increased $PaCO_2$ decreases responses to phenylephrine whereas those to xylazine are increased, (ii) increased PaO_2 increases responses to phenylephrine whereas those to xylazine are unaffected. Thus the responses to phenylephrine in air-ventilated rats show a more marked reduction to increased CO_2, indicating synergism between hypercapnia and hypoxia in depressing the response. This becomes apparent when all the data are grouped in ranges of $PaCO_2$ and PaO_2 and plotted as a 3-dimensional histogram (Figure 6). This shows that, within the physiological range of blood gases, responses to phenylephrine are depressed by decreasing O_2 or increasing CO_2. In the case of xylazine there is no reduction as PaO_2 decreases but increasing the $PaCO_2$ produces increases in response in each range of PaO_2.

This corroborates and extends the earlier interpretation from the pressor responses to adrenaline that respiratory acidosis favours α_2- and diminishes α_1-mediated pressor responses in the pithed rat (McGrath *et al.*, 1982). The earlier experiments did not detect the additional effect of PaO_2 since they employed ventilation with 100% O_2 in an attempt to maintain PaO_2 at supra-physiological levels even in the face of considerable reductions in ventilation stroke volume. With air ventilation, the additional depressant effect



Box	*n*
A	11
B	16
C	30
D	12

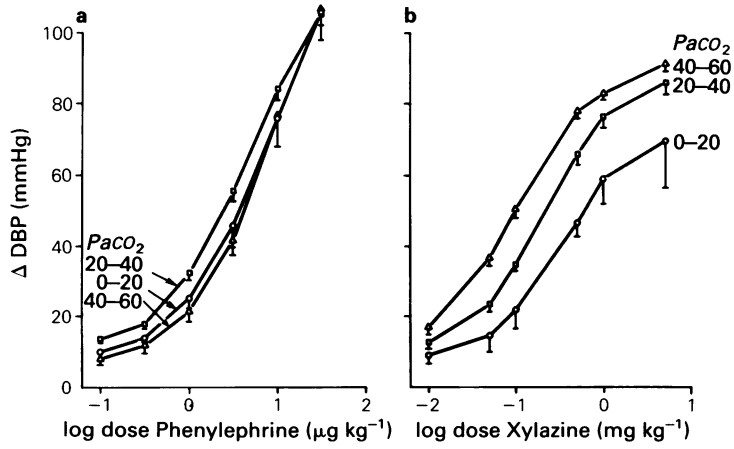
Box	*n*
A	11
B	4
C	13
D	3

Figure 6 Three dimensional histograms of the mean peak diastolic pressor responses obtained within 'ranges' of PaO_2 and $PaCO_2$ to (a) phenylephrine ($3.0\,\mu g\,kg^{-1}$) and (b) xylazine ($0.1\,mg\,kg^{-1}$). Vertical bars indicate s.e.mean.

of low Pa_{O_2} on the response to phenylephrine emerged. With xylazine, any depression by hypoxia was offset since responses were potentiated by hypercapnia. Consequently, in conditions of hypoxia with hypercapnia the phenylephrine response was greatly depressed whereas the response to xylazine was still near to its optimum.

When the Pa_{CO_2} was elevated by addition of CO_2 to the inspired gas and the Pa_{O_2} was maintained at physiological levels, the effect on the response to xylazine remained the same as with altered ventilation, increasing with Pa_{CO_2}. This suggests that for xylazine, the effect of CO_2 alone is unequivocal. However, in the case of phenylephrine, when O_2 was kept constant at normal 'physiological' levels, there was no longer a clear depression as Pa_{CO_2} increased. When Pa_{CO_2} was held constant and Pa_{O_2} was varied, and in either acidotic or alkalotic conditions, the response to phenylephrine increased as the O_2 levels increased. This suggests that around physiological levels, Pa_{O_2} was more critical for the response to phenylephrine but that, at supraphysiological Pa_{O_2}, Pa_{CO_2} had a greater influence.

It was clear in preliminary experiments that rats ventilated with air gave poor responses to xylazine compared to those found when ventilating with O_2. The reasons for this became apparent when the effects of blood gases were analysed. To keep the preparation 'viable', i.e. not hypoxic, on air, large stroke volumes were necessary. This caused alkalosis and, hence, decreased the response to xylazine. If stroke volume was reduced to achieve 'normal' pH and Pa_{CO_2}, the rats became hypoxic and the preparation was non-viable. The basis of this blood-gas imbalance seems to be a straightforward diffusion problem in the lungs, presumably as a result of the massive sympathetic discharge on pithing which produces, simultaneously, large increases in cardiac output and peripheral resistance and leads to pulmonary congestion. This diffusion problem can be circumvented by ventilating with pure O_2 or a mixture where air is supplemented with O_2. A comparison of dose-response curves for phenylephrine and xylazine at different levels of Pa_{CO_2} (Figure 7) shows that the response to xylazine will be relatively underestimated and the response to phenylephrine overestimated under the type of alkalotic conditions likely to be found in 'over ventilated', air-ventilated, pithed rats. Conversely, when 100% O_2 is employed, rats can remain viable even at Pa_{O_2} = 100 mmHg with an 'under-ventilation' which induces severe acidosis; in these conditions the relative effect of xylazine might be overestimated. 'Physiological' blood gases were mimicked by ventilating the rats with 40% O_2, 60% N_2 at 2.5 ml per stroke and 60 strokes min^{-1}.



c Summary of ED_{50} mmHg values with increasing P_aCO_2

ED_{50} (mmHg) ($\mu g\ kg^{-1}$)	$Paco_2$ (mmHg) 0–20	$Paco_2$ (mmHg) 20–40	$Paco_2$ (mmHg) 40–60
Xylazine	603 (4)	219 (15)	96 (9)
Phenylephrine	3.8 (11)	2.5 (33)	4.2 (24)
Potency ratio $\frac{Phe}{Xyl}$	159	88	23

Figure 7 The effects of varying the Pa_{CO_2} on the log dose-response curve to phenylephrine (a) and xylazine (b) in the pithed rat. Experiments were separated according to Pa_{CO_2} in the ranges (i) 0–20 mmHg, (ii) 20–40 mmHg and (iii) 40–60 mmHg. The table shows the effect of increasing Pa_{CO_2} on the relative potencies of the two agonists (c). The figures in parentheses in the table are the number of animals in the group.

These would seem to be the optimal conditions for studying physiological adrenergic mechanisms in the pithed rat.

The choice of phenylephrine and xylazine as mimics of the α_1- and α_2-stimulation by adrenaline is based not only on their receptor selectivities but also in the time courses of the responses which they initiate. Adrenaline has a biphasic response in which the first, rapid, transient phase is mainly α_1 and the second, slower-onset, more prolonged phase is mainly α_2 (Flavahan & McGrath, 1981c). Phenylephrine mimics the first phase and has a poor second phase. Xylazine has a dominant second phase, which is similar to or slightly more prolonged than an equivalent response to adrenaline (Flavahan, 1983). We have, therefore, tried to use these agents to mimic adrenaline's α_1 and α_2 responses without recourse to antagonists, which is the alternative approach (Flavahan & McGrath, 1981c; McGrath *et al.*, 1982).

This similarity to adrenaline is not shared by all 'α-adrenoceptor' agonists. For example, where a bolus of an agonist can produce a prolonged α_1-mediated pressor response, the relationship established here for blood gases no longer holds. This can be demonstrated with amidephrine which produces an extremely persistent α_1-mediated pressor response that is potentiated by respiratory acidosis (Flavahan, 1983). In contrast to the short-lasting α_1-response to adrenaline or phenylephrine but similar to the second phase of the response to xylazine, this prolonged α_1-response is susceptible to calcium entry blockers (Flavahan & McGrath, 1982). This provides further support for the proposal that it is the excitation contraction coupling rather than the receptor *per se* that is the critical element influenced by blood gases. It also emphasises the need to consider the time course of responses in pithed rats which never represent an equilibrium but rather show a series of linked events reflecting the rise and fall of the blood concentration of the drug (Docherty & McGrath, 1980c).

The earlier results, which showed opposite effects of acidosis on α_1- and α_2-mediated responses to adrenaline, suggested a simple functional distinction between the two subtypes of α-adrenoceptor which might enable them to play distinct physiological roles; α_2 but not α_1 could survive a combination of hypoxia and hypercapnia. Hence α_1 could be overridden in ischaemic conditions, while α_2 could survive and become the dominant mediator of vasoconstriction, perhaps, on the arterial side, as a final means of sacrificing certain beds to preserve circulation to vital areas and, on the venous side, to maintain venoconstriction and hence sustain circulating volume. The present study extends this by showing that, with short term responses, oxygen tension is the main influence on α_1 and carbon dioxide (pH) on α_2. These observations provide clues to the excitation-contraction coupling processes involved in the two responses. However, it should be borne in mind that it is only transient α_1-responses that are differently affected by blood cases. This applies also to the influence of calcium entry blockers and angiotensin II (Flavahan & McGrath, 1982; O'Brien *et al.*, 1985).

It remains possible that increased pH might affect the ionisation and hence, the distribution of the agonists. This could be the basis of the increase in the prolonged part of the responses to adrenaline, amidephrine and xylazine in acidotic conditions. We have no evidence on which to assess this.

This work was supported by the Wellcome Trust (J.C. McG), S.E.R.C. (T.L.G and J.W.O'B) and the Medical Research Funds of the University of Glasgow. We are grateful to Bayer for the gift of xylazine.

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(Received December 21, 1984.
Revised April 26, 1985.
Accepted May 9, 1985.)