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Blockade by nifedipine of responses to intravenous bolus injection or infusion of α_1- and α_2-adrenoceptor agonists in the pithed rat

[1]J.C. McGrath & [2]J.W. O'Brien

Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

1 Nifedipine was tested against pressor responses in the pithed rat to ten agonists with varying selectivity for α_1- and α_2-adrenoceptors, injected as a bolus or infused intravenously: i.e. amidephrine, azepexole, cirazoline, indanidine, M7, methoxamine, noradrenaline (NA), oxymetazoline, phenylephrine and xylazine. Nifedipine, administered before the agonists, inhibited responses initiated by all agonists, usually for both the bolus and infusion responses.

2 With a bolus, blockade was significantly greater against the more prolonged, secondary components of the pressor responses. This demonstrates that calcium-entry occurs during the secondary component of the α-adrenoceptor-mediated response and can be initiated by either α_1- or α_2-adrenoceptor subtypes.

3 The time courses of responses to infusion varied. Selective α_1-adrenoceptor agonists, with the exception of indanidine, did not produce a stable pressor response during the 20 min infusion time but α_2-adrenoceptor agonists did. Nifedipine reduced responses to infusion with no preference for α_1- or α_2-agonists. Phenylephrine and NA produced pressor responses which reached a peak and then declined during the remainder of the infusion.

4 The levels of NA in arterial and venous plasma were measured by h.p.l.c. during the infusion of NA. Arterial NA levels rose throughout the infusion whereas venous levels remained relatively unaffected. The absolute levels of plasma NA suggest that a large proportion of intravenously administered NA is removed in the pulmonary circulation and the remainder is removed in the systemic circulation with negligible recirculation.

5 The consequences of these results, for assessment of the mechanisms of action of adrenoceptor agonists and calcium entry blockers, are discussed.

Introduction

The pithed rat has been used to demonstrate the two subtypes of α-adrenoceptor which mediate pressor responses and are presumably located on the smooth muscle of resistance arteries and/or arterioles (Drew & Whiting, 1979; Docherty & McGrath, 1980a). The responses mediated by agonists which are selective for the two receptors can be differentially modulated by a variety of factors including blood gases, angiotensin converting enzyme inhibitors and calcium channel blockers (Flavahan & McGrath, 1981; Van Meel *et al.*, 1981; McGrath *et al.*, 1982; Grant & McGrath, 1984; O'Brien *et al.*, 1985).

[1] Author for correspondence.

[2] Present address: Inveresk Research International, Inveresk Gate, Musselburgh, East Lothian.

Part of the reason for this differentiation is that the pressor responses to bolus injections have more than one phase and modulating factors affect these to different extents. It is difficult to make direct comparisons between such findings in pithed rat and similar types of experiment *in vitro* since the *in vivo* responses to bolus injections never attain equilibrium and are multiphasic. For example, it was originally suggested that the α_1-adrenoceptor-mediated responses were relatively resistant to calcium channel blockers compared with those mediated by α_2-adrenoceptors (Van Meel *et al.*, 1981). However, when a wider range of agonists was tested this no longer remained true and it became apparent that different time courses of the response played a part in this (Flavahan & McGrath, 1982; Timmermans *et al.*, 1983).

We have now compared the effects of the calcium

channel blocker, nifedipine, against responses to both bolus injections and constant intravenous infusion in the hope of establishing an equilibrium with infusion which might be more comparable with *in vitro* results. A group of agonists with varying selectivity for the two receptor subtypes were used. Having established the general properties of responses to bolus injections and infusions of agonists, we then used the same methods to analyse the pressor effects of the physiological agonist noradrenaline (NA).

Preliminary communications of these results have been published (O'Brien & McGrath, 1984; Grant *et al.*, 1984b).

Methods

Male Wistar rats (245–265 g) were pithed under halothane anaesthesia by the method of Gillespie *et al.* (1970), following tracheal cannulation. Carotid arterial pressure was monitored (Elcomatic EM 752) via a heparinized cannula (200 u ml^{-1} saline) and the heart rate was extracted from the pressure wave by an instantaneous rate meter (Lectromed 4522). Heart rate and blood pressure were displayed on a u.v. oscillograph (SE Labs 6150).

The temperature of the rat was maintained at 37°C using a tungsten lamp and the rectal temperature was monitored with a thermometer. The rats were ventilated using a Harvard Rodent Respirator at a rate of 60 strokes min^{-1}, 2 ml per stroke, with 100% oxygen in order to maximize any prolonged α-adrenoceptor-mediated responses (Grant *et al.*, 1984a; 1985). This makes them slightly acidotic (arterial pH = 7.33; PCO_2 = 50 mmHg) but well oxygenated (PO_2 = 180 mmHg) (Grant *et al.*, 1985). The rats were allowed to stabilize for 20–30 min before administering drugs.

The right jugular vein was cannulated for drug injections and infusions (McLennan DS 201 digital syringe). Injections were administered in a fixed volume of 1 ml kg^{-1} followed by a similar volume of 0.9% saline and the agonists infused in a volume of 1.5 ml kg^{-1} h^{-1}. Nifedipine (0.3 mg kg^{-1}) was injected intra-arterially (via the right carotid artery into the aorta) to avoid cardiac depression, 15 min before the agonist. Propranolol (1 mg kg^{-1}) was injected 5 min before any drug that produced β-adrenoceptor-mediated heart rate effects, i.e. noradrenaline (NA) and phenylephrine. All doses for either bolus injections or infusions were calculated to produce an approximately 60 mmHg rise in the diastolic blood pressure (ensuring first that this response was submaximal).

In 'bolus' experiments, both pre- and post-nifedipine injections, were carried out in the same rat, since responses were consistently reproducible. However, for infusion experiments, separate rats were used for controls and for post-nifedipine infusions since responses were not always reproducible. For example, in a first series of experiments with cirazoline, nifedipine was injected into the rat when the first infusion response had returned to baseline and the infusion was repeated. However, very little blockade of the peak response was seen (15.1 ± 2.6%; n = 3: mean ± s.e.mean). To test whether a blockade had been counteracted due to the response to the second infusion being 'potentiated', two infusions were given to the same rat with 30 min between them (the usual time taken for the first response to wane and nifedipine to be administered, in the previous experiments) but without any antagonist. It was found that on the second administration the peak pressor response was potentiated by 24 ± 6% (n = 3). Therefore, all future experiments involving infusions were carried out in separate rats for the control and antagonist.

The responses (Δ diastolic blood pressure mmHg) were measured every 30 s (and at the first 15 s for α_1-adrenoceptor agonists) and the mean responses were plotted. The results were analysed, (a) by comparing the change in diastolic blood pressure at various times and (b) by comparing the area under the curve (mmHg min) between control and nifedipine-blocked experiments. This was done using a digitizing tablet linked to an Apple IIe micro-computer (see Moss, 1981). For the bolus experiments, the area was measured from t = 0 min (when the drug was injected) to the time taken for the response to return to baseline. Some drug responses (e.g. indanidine) did not return to the baseline so their responses were measured until they returned to a constant value above the baseline, the same time being used whenever comparison of with and without nifedipine was made. For the infusion responses the area was measured from t = 0 min (when the infusion began) to the end of the infusion at t = 20 min.

Plasma noradrenaline analysis.

Blood samples (2 ml) were taken from the pithed rats which were infused for 20 min with either 0.9% saline (control) or 1 μg kg^{-1} min^{-1} NA. The samples were taken from either the left carotid artery or the left jugular vein and placed immediately into heparinized tubes, rotamixed for 2 min and placed on ice to await centrifugation. The blood was taken either: (1) 30 min after pithing i.e. just before infusion began, (2) 5 min into the infusion i.e. where the peak blood pressure response to NA infusion is seen, (3) at 20 min just as the infusion was stopped. The cannulae for the venous samples were inserted via the left jugular vein but the samples were withdrawn from the level of the ascending vena cava: the infusions of agonists into the right jugular vein go directly into the right atrium and so do not mix with the blood taken for the venous samples.

When the samples had been collected they were spun at 4000 *g* in a Gallenkamp Angle Head Centrifuge for 20 min and the plasma was decanted into 1 ml EDTA tubes (Teclab) and rotamixed before storing overnight at − 12°C. The plasma was thawed out and 1 ml placed in 10 ml conical tubes with 20 mg of chromatographic alumina (Basic WB-5, Sigma) and 5 ml of Tris-EDTA buffer (composition: pH 8.6, 12 g Tris base, 2 g EDTA per 100 ml). Two hundred and fifty μl of 2 ng ml^{-1} (control experiments) or 20 ng ml^{-1} (NA experiments) of 3,4-dihydroxybenzylamine hydrobromide (DHBA) were added to each tube as an internal standard. The tubes were capped and rotamixed for 30 min after which the eluate was aspirated off and the alumina washed 4 times with ice-cold distilled water. The NA was then eluted with 250 μl of 0.1 M perchloric acid added to the tubes which were then rotamixed for a further 30 min (adapted from Eriksson & Persson, 1982).

A reverse phase, ion-pairing h.p.l.c. system was then used to measure the levels of NA in the plasma samples; 100 μl of the above eluate was injected onto an Altex Ultrasphere IP reverse phase column, with a 90% phosphate buffer (pH 3.0) as the mobile phase. Levels of NA present in the two types of experiments (saline or NA infused) were calculated by constructing a standard curve to NA, i.e. known amounts of NA were added to the conical tubes and put through the whole extraction process along with the internal standard DHBA (both dissolved in 0.1 M perchloric acid). These samples were then injected onto the column immediately before the plasma. The height of the NA peak was measured and expressed as a ratio over the DHBA peak (this allows for any fluctuation in recovery between extraction processes) and this was plotted against initial NA concentration. Then, when the plasma sample peaks were also expressed as a ratio over their DHBA peaks, a value could be read off the standard curve for the actual concentration of NA in 1 ml of plasma.

Statistical analysis

Statistical comparisons were made using Student's *t* test for experiments carried out on separate rats. For paired data on the same rat, a paired *t* test was used.

Drugs

Drugs and compounds used were: (−)-amidephrine hydrochloride (Bristol-Myers*); azepexole (BHT 933; Thomae*); cirazoline hydrochloride (Synthelabo*); 3,4-dihydroxybenzylamine hydrobromide (Sigma); methoxamine hydrochloride (Burroughs Wellcome); M7 (2-N-dimethylamino-5,6,dihydroxy-1,2,3,4-tetrahydronaphthalene; Syntex*); nifedipine (Bayer*); noradrenaline bitartrate (Koch-Light); oxymetazoline hydrochloride (Merck*); (−)-phenylephrine hydrochloride (Sigma); (±)-propranolol hydrochloride (Sigma); indanidine (Siegfried 101/75; Siegfried Zofingen*); sodium octylsulphonate (Fisons); xylazine hydrochloride (Bayer*).

Drugs were dissolved in 0.9% saline except for nifedipine which was dissolved as follows: 10 mg in 1 ml of cremophor heated to approximately 75°C and diluted to the desired concentration in distilled water. We are grateful to the suppliers asterisked for generous gifts of the compounds.

Results

Nifedipine (0.3 mg kg^{-1} did not significantly alter resting diastolic blood pressure. It produced a transient depressor response of 10.3 ± 1 mmHg, which returned to baseline after 3.7 ± 0.2 min ($n = 14$). It inhibited α_1- and α_2-adrenoceptor-mediated responses, for both the bolus and the infusion (Table 1). Generally, the transient, first phase of the bolus response was nifedipine-resistant as was the response in the initial few min of the response to infusion (Figures 1–3).

The inhibition of the bolus response by nifedipine was demonstrated more clearly if the area under the curve was measured rather than the inhibition of the peak. For the infusion response the inhibition of the area under the curve or the inhibition of the peak response gave a similar indication of the extent of blockade (Figure 4a). The outstanding feature of this comparison was that those responses which are least affected by nifedipine appear to be virtually unaffected if 'peak height to bolus' was used as an index. When the inhibitions of the areas under the curves for the infusion and bolus are expressed as percentages and correlated (Figure 4b), the bolus response is seen to be blocked to a greater extent. Thus blockade is quite clear against the response to a bolus provided that the response is integrated, but measuring the peak gives a poor index of blockade.

Most of the α_1-agonists (except perhaps indanidine, Figure 1b) did not achieve and maintain a plateau during the course of the infusion. In contrast, all the α_2-agonists rapidly achieved and maintained a plateau. The most unusual infusion responses were those to phenylephrine and NA (Figure 3a and b). Both these agonists produced a peak response which steadily declined after approximately 8 min of infusing the drug. No other agonist tested displayed this phenomenon.

Measurement of noradrenaline levels in the plasma of the pithed rat

Arterial or venous blood samples were taken from rats

Table 1 Responses to (a) bolus injections and (b) infusions of α-adrenoceptor agonists before and after nifedipine (0.3 mg kg^{-1}) in the pithed rat

(a) *Bolus injections*

Agonist	*Selectivity**	*Dose* (μg kg^{-1})	*Area under curve % inhibition by nifedipine*	*Peak response*			$t_{1/2}$			n
				Control (mmHg)	*Nifedipine* (mmHg)	*% inhibition by nifedipine*	*Control* (min)	*Nifedipine* (min)	*% inhibition by nifedipine*	
Amidephrine	α_1	10	26	58 ± 3	54 ± 1	6	6.0 ± 1.0	2.0 ± 0.6	67	3
Methoxamine	α_1	40	31	60 ± 5	55 ± 3	8	1.8 ± 0.5	1.1 ± 0.1	39	4
Oxymetazoline	α_1/α_2	0.05	45	64 ± 3	64 ± 4	0	5.0 ± 0.5	2.3 ± 0.6	54	4
Noradrenaline	α_1/α_2	1	46	76 ± 8	61 ± 12	21	1.9 ± 0.4	1.1 ± 0.2	42	5
Phenylephrine	α_1	3	54	75 ± 14	72 ± 14	4	2.5 ± 0.5	1.0 ± 0.2	60	5
Indanidine	α_1	500	55	63 ± 6	45 ± 7	29	10.1 ± 0.9	3.0 ± 0.8	70	5
M7	α_2	10	57	57 ± 2	40 ± 4	30	4.2 ± 0.5	2.5 ± 0.3	40	6
Azepexole	α_2	250	66	55 ± 4	39 ± 7	29	3.8 ± 0.1	1.8 ± 0.4	53	4
Cirazoline	α_1	1	68	70 ± 5	50 ± 7	29	1.1 ± 0.1	0.4 ± 0.1	64	6
Xylazine	α_2	100	77	51 ± 4	24 ± 5	53	3.7 ± 0.2	1.5 ± 0.1	59	5

(b) *Infusions*

Agonist	*Selectivity**	*Dose* (μg kg^{-1} min^{-1})	*Was plateau achieved in 20 min?*	*Area under curve % inhibition by nifedipine*	*Maximum response*			n
					Control (mmHg)	*Nifedipine* (mmHg)	*% inhibition by nifedipine*	
Amidephrine	α_1	1·	NO	19	56 ± 8	42 ± 8	25	4
Methoxamine	α_1	25·	NO	30	63 ± 5	37 ± 1	41	4
Oxymetazoline	α_1/α_2	0.5	NO	34	64 ± 3	44 ± 7	31	6
Noradrenaline	α_1/α_2	1·	NO	31	58 ± 5	38 ± 4	34	6
Phenylephrine	α_1	3·	NO	59	54 ± 5	25 ± 1	54	5
Indanidine	α_1	250·	YES	22	58 ± 7	45 ± 7	22	7
M7	α_2	6·	YES	52	69 ± 5	32 ± 3	54	5
Azepexole	α_2	100·	YES	50	47 ± 2	24 ± 3	49	5
Cirazoline	α_1	1·	NO	58	57 ± 6	20 ± 2	65	6
Xylazine	α_2	250·	YES	56	71 ± 3	31 ± 2	56	5

Agonists are placed in the order of increasing susceptibility to nifedipine judged by the most sensitive index, % inhibition of area under curve (bolus).
*References for selectivity of agonists: Docherty & McGrath (1980a,b), McGrath *et al.* (1982), Timmermans *et al.* (1982) and O'Brien *et al.* (1985).

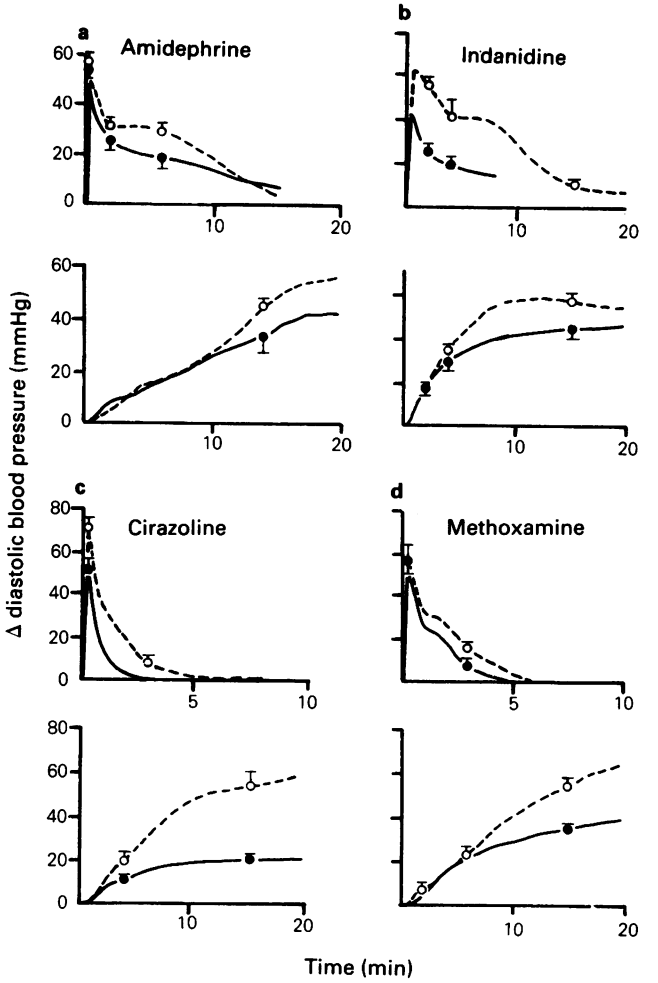

Figure 1 Effects of nifedipine (0.3 mg kg^{-1}) on the time courses of the pressor responses in pithed rats to bolus injections (upper graphs) and infusions (lower graphs) of: (a) amidephrine (10 μg kg^{-1}) and (1 μg kg^{-1} min^{-1}); (b) indanidine (500 μg kg^{-1}) and (250 μg kg^{-1} min^{-1}); (c) cirazoline (1 μg kg^{-1}) and (1 μg kg^{-1} min^{-1}); (d) methoxamine (40 μg kg^{-1}) and (25 μg kg^{-1} min^{-1}). The mean changes in diastolic blood pressure (n = 6) produced by the agonist have been plotted against time (min). Responses were measured 15 and 30 s after the initial administration and thereafter at 30 s intervals. All points are plotted but, for clarity, symbols are shown only at selected times. (O---O) Control responses; (●——●) responses after nifedipine. Vertical lines represent s.e.mean.

which had been infused with NA as above. The 2.0 ml samples were taken before the infusion (after the stabilization period), during the infusion (t = 5 min, when NA was exerting its peak pressor effect) and at the end of the 20 min infusion (see Figure 3c); either arterial or venous samples were taken. As a control, other rats were infused with 0.9% saline (the NA vehicle) at the same rate for 20 min and blood samples taken at the same time intervals (n = 4).

Due to the size of the samples needed, at first different rats were used for the arterial and venous samples and only two samples were taken per rat (n = 4), i.e. either 2 arterial or two venous samples were taken; one before and one after infusion. In 3 further rats in which 4 samples were taken from each rat (i.e. venous and arterial: before and after infusion), the NA levels lay within the same ranges. Therefore results from the two protocols were pooled.

Table 2 shows the pooled data. The level of NA in arterial plasma was still at a maximum when the blood

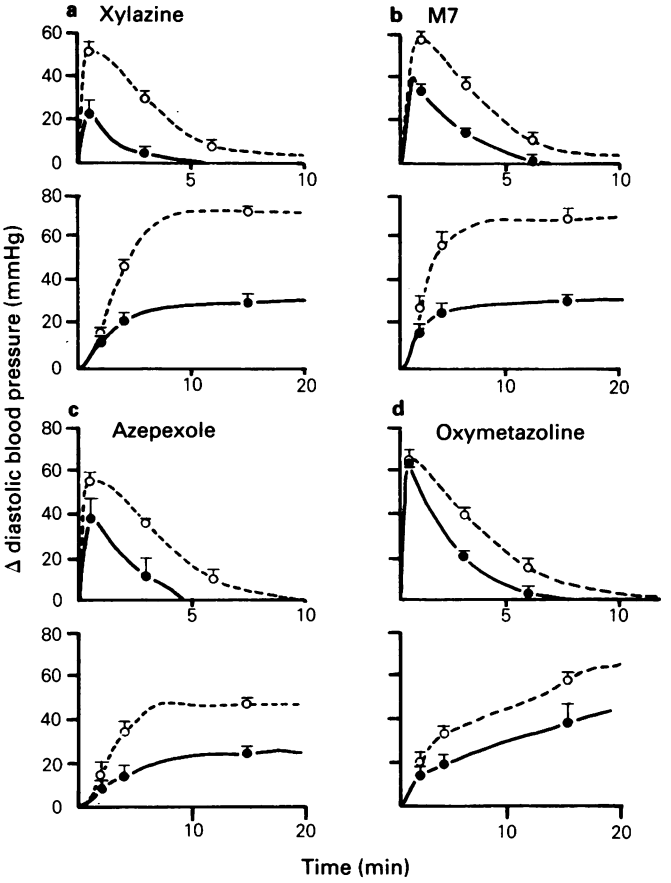

Figure 2 Effects of nifedipine (0.3 mg kg^{-1}) on the time courses of the pressor responses in pithed rats to bolus injections (upper graphs) and infusions (lower graphs) of: (a) xylazine (100 μg kg^{-1}) and (250 μg kg^{-1} min^{-1}); (b) M7 (10 μg kg^{-1}) and (6 μg kg^{-1} min^{-1}); (c) azepexole (250 μg kg^{-1}) and (100 μg kg^{-1} min^{-1}); (d) oxymetazoline (0.05 μg kg^{-1}) and (5 μg kg^{-1} min^{-1}). The mean changes in diastolic blood pressure ($n = 6$) produced by the agonist have been plotted against time (min). Responses were measured 15 and 30 s after the initial administration and thereafter at 30 s intervals. All points are plotted but, for clarity, symbols are shown only at selected times. (O---O) Control responses; (●——●) responses after nifedipine. Vertical lines represent s.e.mean.

pressure response was on the decline. No significant increase in the level of venous NA was found during or at the end of the infusion.

Discussion

The pressor response of the pithed rat to bolus injections of agonists has provided a useful model for the demonstation of subtypes of α-adrenoceptors. However, when it has been used to assess the effects of non-competitive or physiological antagonists of the response, a complication has arisen due to the biphasic nature of the response. In general α-adrenoceptor agonists produce a rapid rise in blood pressure which reaches a peak within 30 s and declines rapidly at first but is followed by either a much slower decline or a distinct second response which appears as a shoulder on the decline of the first phase.

These two phases are affected differently by physiological factors such as blood Po_2 or pH (Grant *et al.*, 1985; O'Brien *et al.*, 1985). Since different agonists elicit these phases in varying proportions, it can sometimes appear that responses to particular groups of drugs are selectively affected by some factor under study. If the agonists in such a group happen to have some property in common then it is possible to be misled into making a wrong correlation. For example,

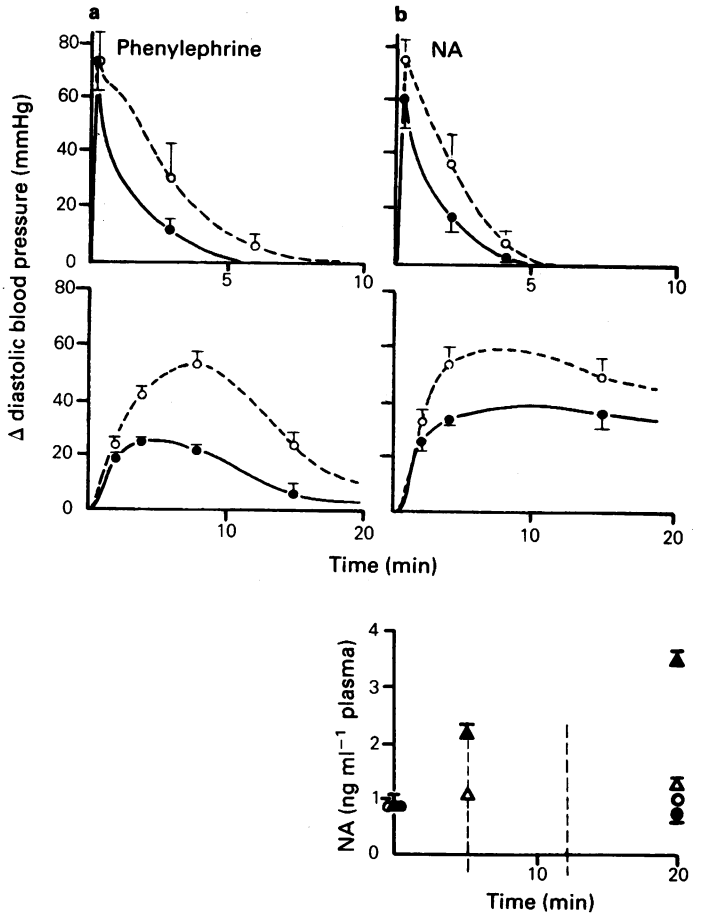

Figure 3 Effects of nifedipine (0.3 mg kg^{-1}) on the time courses of the pressor responses in pithed rats to bolus injections (upper graphs) and infusions (lower graphs) of: (a) phenylephrine (3 μg kg^{-1}) and (3 μg kg^{-1} min^{-1}); (b) noradrenaline (NA; 1 μg kg^{-1}) and (1 μg kg^{-1} min^{-1}). The mean changes in diastolic blood pressure ($n = 6$) produced by the agonist have been plotted against time (min). Responses were measured 15 and 30 s after the initial administration and thereafter at 30 s intervals. All points are plotted but, for clarity, symbols are shown only at selected times. (O---O) Control responses; (●——●) responses after nifedipine. Vertical lines represent s.e.mean.

Additionally, in (b) the bottom graph shows the concentration of NA (ng ml^{-1} plasma) before, during and after the infusion of 1 μg kg^{-1} min^{-1} of NA in the pithed rat. (O, ●) Control levels (saline infused), ((●) arterial, (O) venous); (△, ▲) levels with NA infusion ((▲) arterial plasma levels, (△) venous plasma levels). The vertical dashed lines indicate the average time at which the peak pressor response for NA was reached (left) and the time at which the pressor response began to decline (right). Vertical lines represent s.e.mean, ($n = 4$ for each condition).

when we initially found that responses to phenylephrine were decreased in respiratory acidosis and responses to xylazine were increased, we wrongly concluded that this was related to the selectivity of these two compounds for α-adrenoceptor subtypes (Flavahan & McGrath, 1981). Further investigation using a larger series of compounds revealed that responses to another α_1-adrenoceptor agonist, amidephrine, were increased in acidosis (Flavahan & McGrath, 1982). In addition, in this example it was also clear that the effects of pH correlated with the time course of the responses to the agonists rather than with receptor subtype.

Preliminary investigation of the effects of the calcium channel blocker, nifedipine, indicated a similar phenomenon; the second, slower component of pressor responses being more susceptible to blockade. We have tried to illuminate this by comparing the changes

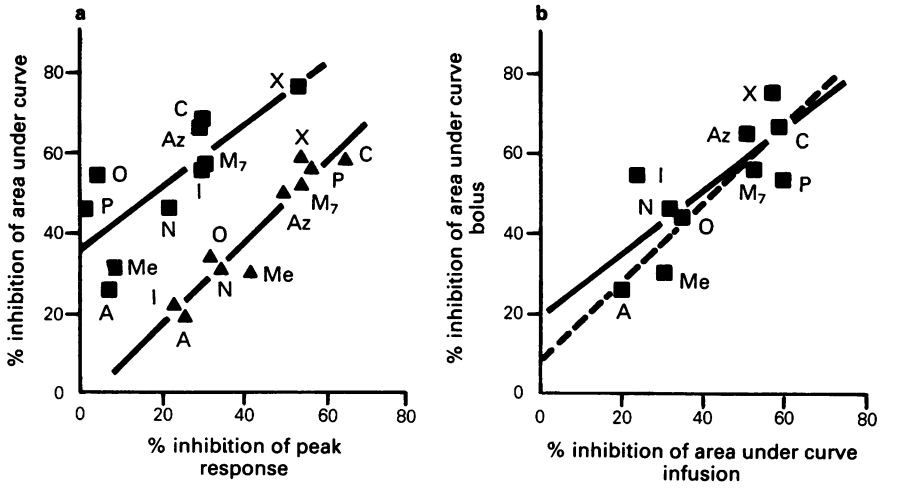

Figure 4 Comparison of different indices of inhibition by nifedipine (0.3 mg kg^{-1}) of pressor responses in the pithed rat. (a) % inhibition of the peak change in diastolic blood presure response versus % inhibition of area under the curve for the bolus responses (■) and the infusion responses (▲). Regression analysis produced a significant relationship for the bolus responses $r = 0.8$, $0.001 < P < 0.01$ and for the infusion responses $r = 0.95$, $P < 0.001$. (b) Correlation of the % inhibition of the area under the curve for the infusion and bolus responses. Regression analysis produced a significant relationship $r = 0.77$, $0.001 < P < 0.01$ (continuous line). Excluding indanidine the regression line passed nearer to the origin (broken line) $r = 0.88$, $0.001 < P < 0.01$. Key to abbreviations used: X = xylazine; C = cirazoline; Az = azepexole; M7; I = indanidine; N = noradrenaline; P = phenylephrine; O = oxymetazoline; Me = methoxamine; A = amidephrine.

in the peak response and the area under the curve, since the latter should reveal, and the former conceal, any blockade. This showed that there were quantitative differences but no clear qualitative differences in susceptibility to nifedipine among a diverse group of α-adrenoceptor agonists. All responses were attenuated but those compounds which produced a sharply delineated first phase showed no change in the height of the peak. Thus, resistance to nifedipine resided only in this initial component. The one correlation which existed with α-adrenoceptor subtypes was that the compounds which had the most resistant 'peaks' were all α_1-adrenoceptor agonists (amidephrine, methoxamine, oxymetazoline and phenylephrine) and all of these except for oxymetazoline are selective and are phenylethanolamines. However, other α_1-adrenoceptor selective compounds (cirazoline and indanidine) were no more resistant than were several α_2-adrenoceptor-selective compounds.

Since the nifedipine-resistant response appeared to be a non-equilibrium phenomenon, we decided to investigate infusions of agonists, which produce res-

Table 2 Concentrations of noradrenaline (NA; ng ml^{-1} plasma) found in the arterial and venous blood of the pithed rat

	NA			
	Control		*NA infused*	
	Arterial blood	*Venous blood*	*Arterial blood*	*Venous blood*
Before infusion	0.76 ± 0.04	0.80 ± 0.20	0.76 ± 0.04	0.80 ± 0.20
5 min infusion	—	—	2.20 ± 0.30	1.10 ± 0.20
20 min infusion	0.79 ± 0.17	1.14 ± 0.20	3.40 ± 0.40	1.30 ± 0.05

Samples were tested in both control (saline infused) and NA infused (1 μg kg^{-1} min^{-1}) animals before and during infusions (5 and 20 min). Data are expressed as mean values ± s.e.mean ($n = 4$, for each condition) and represent concentrations of NA in ng ml^{-1} plasma.

ponses closer to equilibrium. This eliminated the initial rapid, transient components. Responses to most agonists either steadily increased throughout infusion or reached equilibrium. Exceptions to this were NA and phenylephrine whose responses declined with prolonged infusion (see below). The response to every agonist was reduced by nifedipine so that 'blockade' was registered whether this was measured as change in peak response or area under the curve (Figure 4a). No particular group of compounds stood out as 'resistant', e.g. the most and least resistant were both selective α_1-adrenoceptor agonists, indanidine and cirazoline, respectively. If the area under the curve was used as an index of response there was a good correlation between the inhibitions detected with a bolus or an infusion. The compound deviating most from this was indanidine, which was unusually sensitive when given by bolus injection. However, it had by far the longest lasting response of any compound given by this route and so may not be directly comparable in this simple way (Figure 4b).

It should be noted that, for most compounds, the initial part of the response to infusion, even though of slow onset, was resistant to nifedipine. This would be consistent with there being a source of Ca^{2+}, either intracellular or extracellular but through another channel, which can sustain a finite amount of vascular contraction before nifedipine-sensitive channels are called into play.

The non-sustained nature of the pressor response to infusions of phenylephrine and NA was similar to an earlier observation with NA (Gillespie & Muir, 1967). Each response showed a marked decline after 10 min of infusion. According to the plasma NA levels found, the decline in response, for this catecholamine at least, is not due to the removal of the drug from the blood. Williams *et al.* (1984) have now discovered that phenylephrine is an endogenous substance and can be found in various animal tissues and in animal and human urine samples. It is interesting that it is only the natural phenylethanolamines which, when infused, display the decline in pressor response. This decline may represent some negative feedback mechanism with the vascular effector system or may involve the production or some independent factor. Whatever the receptor system involved, it is interesting that the non-physiological agonists do not replicate the phenomenon.

Measurement of plasma NA showed: (1) that the peak pressor response to NA was attained after 5 min when the concentration of NA in the arterial plasma had not yet reached its maximum (N.B. the pressor response to the infusion itself was sum-maximal). (2) When the pressor effect was waning the concentration of NA was still rising in the arterial blood. (3) The concentration of NA in the venous plasma showed very little change from control values throughout the infusion. (1) and (2) Show that there is some form of tachyphylaxis. (3) Shows that NA does not pass through the systemic vascular beds to recirculate.

A large proportion of the NA infused into the jugular vein seems to have been lost in its passage through the heart and pulmonary bed to the carotid arterial sampling site. From the Fick Principle, with our rate of infusion and the arterial levels found, cardiac output would need to be approximately 100 to 150 ml min^{-1} if no NA was lost. Since the best estimates of this in pithed rats of this size, obtained by three separate methods, are approximately 15 to 25 ml min^{-1} (e.g. Gerold & Haeusler, 1983; Kaufman & Vollmer, 1985; Hiley & Thomas, 1987), this suggests that at least 75% of intravenously infused NA is lost before it reaches the arterial side of the systemic circulation.

This rapid loss of NA, first 75% in the lungs and then the remainder in systemic beds, is in marked contrast to clonidine, which is present in plasma up to 40 min after bolus injection of a sub-maximal pressor dose (Docherty & McGrath, 1980b). High levels of clonidine were found in the venous blood, indicating that the drug was re-circulating. The pressor response to clonidine lasts no longer than those to many of the agonists tested here so it is likely that many of them recirculate. Conversely, the short-lived α-adrenoceptor agonists may all experience a strong 'first pass' effect and have very low venous concentrations. This implies that only the longer lasting α-adrenoceptor agonists could exert an α-adrenoceptor-mediated venomotor action, which by raising venous return could increase cardiac output. In a recent, independent study, also in pithed rats, Hiley & Thomas (1987) have found that our 'shortest-lived' agonist, cirazoline, can raise total peripheral resistance without significantly raising stroke volume, whereas two of our longest acting agonists, azepexole and xylazine can raise stroke volume without significantly increasing total peripheral resistance. This may mean that long-lived, recirculating agonists will raise blood pressure by a combination of resistance (pre-capillary tone) and cardiac output (post-capillary tone). Since blood pressure is proportional to both, then the influence on blood pressure of actions at the two sites will be geometric rather than additive and a small change in each will produce a disproportionately large change in blood pressure.

This, rather than any real difference in the excitation process may be the reason for prolonged responses being more susceptible to blockade or to facilitatory agents than are responses due to transient acute increases in resistance. It would also be likely that only re-circulating drugs would build up high concentrations in the tissues. This may be another contributing factor to the difference in the duration of response produced. Furthermore, in this pithed rat model,

several other factors may combine with the differential distribution of the agonists to produce individual profiles of susceptibility to blockade. For example, (i) differences in distribution of α-adrenoceptor subtypes between pre- and post-capillary vessels, (ii) differences in the agonist potency series at these different sites even at the same receptor (such as can arise when receptor reserve varies) or (iii) different sensitivity of the excitation-contraction coupling processes in the different vessels to drugs producing 'physiological' antagonism, such as might arise from the uneven distribution of different Ca^{2+} channels.

It is of some interest that nifedipine did not produce a maintained lowering of blood pressure on its own, although it did attenuate responses to agonists. Since vascular tone in the pithed rat (mainly venous) seems to be maintained by a high circulating level of angiotensin II (Kaufman & Vollmer, 1985; Grant & McGrath, unpublished) and since the pressor response to intravenous infusion of exogenous angiotensin II is blocked by the dose of nifedipine used in the current study (Grant & McGrath, 1984), we expected that nifedipine would reduce arterial blood pressure by blocking the effects of endogenous angiotensin: blocking the venomotor effect of endogenous angiotensin II reduces venous return and hence cardiac output, thus decreasing arterial blood pressure. Consequently, any vasopressor response caused by an increase in peripheral resistance, to whatever stimulus, should be reduced since such responses are proportional to the initial arterial blood pressure. Since nifedipine did not decrease arterial blood pressure, analysis of the pressor responses to agonists is simplified, at least in this respect, but we have to conclude, either that the effects of the endogenous angiotensin II (AII) are relatively resistant to nifedipine, or that the loss of AII-induced venomotor tone has been compensated by some other action of nifedipine, possibly on the heart. Whatever the explanation, this situation contrasts with the effects of angiotensin converting enzyme inhibitors which readily lower arterial blood pressure in this model by decreasing cardiac output (via decreased venomotor tone; Kaufman & Vollmer, 1985) and may point to important differences between the haemodynamic effects of Ca^{2+} entry blockers and angiotensin converting enzyme inhibitors, particularly in pathophysiological situations where cardiac output or venomotor tone are disturbed.

We conclude that antagonism of pressor responses to bolus injections of agonists in the pithed rat can be most comprehensively analysed if the two phases of the response are separately considered, the first from its height and the second from the area under the curve. Responses to infusions show essentially similar characteristics and may be useful in quantitative studies of equilibrium phenomena. We also conclude that there is no evidence in this model that calcium channel blockers (represented by nifedipine) have differential effects against responses to particular subtypes of the α-adrenoceptor.

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