Br. J. Pharmac. (1983), **80**, 315–322

Non-competitive antagonism of the α-adrenoceptor-mediated fast component of contraction of rat aorta, by doxazosin and prazosin

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1 α-Adrenoceptor antagonists have been compared for their effects on dose-response curves of fast and slow components of contraction of the rat aorta to noradrenaline (NA).

2 All agents caused a competitive antagonism of the slow component of contraction to NA. The order of potency was: prazosin > WB4101 = doxazosin > tiodazosin > phentolamine > corynanthine > trimazosin > rauwolscine.

3 For the fast component, doxazosin, prazosin, tiodazosin and WB4101 caused some depression of the maximum response. Doxazosin (25 nM) and prazosin (25 nM) produced a complete and unsurmountable antagonism of the maximum fast component. Phentolamine, corynanthine, trimazosin and rauwolscine all competitively antagonized the fast component.

4 The degree of antagonism of the fast component by prazosin and its analogues appeared to be directly related to the potency of individual agents for the slow component. WB4101, which was equipotent with doxazosin and more potent than tiodazosin was less effective than either in reducing the fast component.

5 The antagonism of the fast component by prazosin or doxazosin was easily reversed by washing and prevented by phentolamine (2.5 μM).

6 Neither prazosin nor doxazosin in concentrations of up to 2.5 μM had any effect on contractions of the aorta to 5-hydroxytryptamine (5-HT, 0.25–250 μM) or caffeine (20 mM).

7 It is concluded that the ability of some α-adrenoceptor antagonists to produce a non-competitive antagonism of the fast component of contraction is (a) dependent upon blockade of α-adrenoceptors; (b) unrelated to selectivity for α_1-adrenoceptors; (c) related to potency and structure.

8 EGTA (3.0 mM) caused a selective suppression of the slow component of contraction to NA. Both doxazosin and prazosin caused a non-competitive antagonism of EGTA-resistant contractions to NA whereas corynanthine showed competitive antagonism. These observations, together with those above imply that prazosin and doxazosin non-competitively antagonize α-adrenoceptor-induced release of calcium in the rat aorta, but competitively antagonize α-adrenoceptor-induced calcium entry.

Introduction

The contractile response of rat aorta to concentrations of noradrenaline (NA) of 10 nM and above is biphasic; the initial fast component is believed to be mediated by release of an intracellular source of activator calcium and the slow component by entry of extracellular calcium (Godfraind & Kaba, 1972).

In a preliminary investigation (Downing, Wilson & Wilson, 1981), we found that the α_1-adrenoceptor antagonist, prazosin, caused a non-competitive inhibition of this fast component of contraction and a coincident competitive inhibition of the slow component. The present experiments were undertaken in order to extend and confirm these observations and to investigate the possibility that this effect of prazosin is related to its ability to block selectively α_1-adrenoceptors. We have compared prazosin with five selective α_1-adrenoceptor antagonists, three of which are structurally related to prazosin: doxazosin (UK33274), tiodazosin and trimazosin (Timmermans, Kwa, Karamat & van Zweiten, 1980; Buynis-

ki, Pircio, Schurig & Campbell, 1980), and two which are structurally unrelated: corynanthine and WB4101 (McGrath 1982). We have also included in this study one selective α_2-adrenoceptor antagonist rauwolscine (McGrath 1982) and the non-selective α-adrenoceptor antagonist, phentolamine.

Methods

Male Wistar rats (200–280 g) were killed by stunning and cervical dislocation. The thoracic aortae were quickly removed and helical strips approximately 1.5–2.0 mm. wide and 20–25 mm long were prepared by the method of Furchgott & Bhadrakom (1953) and mounted in 10 ml organ baths containing physiological salt solution (PSS) maintained at 37°C and gassed with 5% CO_2 in O_2. Contractions were recorded by means of Pioden isometric transducers (type UF1) connected to an Ormed MX412 pen recorder. The resting tension of each preparation was adjusted to 1.0 g and 1 h equilibration was allowed before administration of 1 μM NA which was removed by washing after 5 min contact and a further 1 h equilibration was allowed before starting construction of dose response curves (DRC). This procedure is essentially that of Ruffolo, Rosing, & Waddell (1979) and ensures minimal changes in sensitivity to further additions of agonist.

Effects of agonists and antagonists

Non-cumulative dose-response curves were constructed for the fast and slow components of contraction to NA. The fast component was measured at 15s after addition of NA and the slow component was measured at maximum development of tension (5–15 min). A minimum of 5 min was allowed between successive doses, measured from the time of return to baseline tension following washout. Curves were constructed prior to, and 40 min after, the addition of each antagonist. The responses to antagonists have been calculated as a percentage of the maximum control responses to NA alone, which have been separately expressed as 100% for each component of contraction (fast and slow).

In some experiments, EGTA 3.0 mM was added to the PSS and left in contact with the preparation for 2 min, with one wash at 1 min, before eliciting a response to NA. After a response to NA had been obtained, the EGTA was removed by washing along with the NA. A minimum of 15 min equilibration in normal PSS was then allowed before any re-application of EGTA. Using this procedure it was found that the EGTA-resistant responses to NA could be elicited without decrement for 6 to 8 h.

Drugs and solutions

The following drugs were used: caffeine hydrochloride (B.D.H.), corynanthine (Roth), doxazosin mesylate (Pfizer), EGTA- sodium salt (BDH), 5-hydroxytryptamine creatinine complex (BDH), noradrenaline bitartrate (Sigma), phentolamine mesylate (CIBA), prazosin hydrochloride (Pfizer), propranolol hydrochloride (I.C.I.), rauwolscine hydrochloride (Roth), tiodazosin levulinate (Bristol), trimazosin hydrochloride (Pfizer), WB4101 hydrochloride (2[2'6'-dimethoxy)-phenoxyethylamino] methylbenzodioxan) (Amersham).

All drugs were obtained as pure powders. Prazosin and its analogues were dissolved in aqueous solutions containing 5% glucose and 5% glycerol. Corynanthine and rauwolscine were prepared from 1.0 mM stock solutions containing an equimolar concentration of ascorbic acid. All other drugs were dissolved in PSS. The composition of the PSS was (mM): NaCl 118.4, KCl 3.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 2.2, $NaHCO_3$ 24.9 and glucose 10.0. Propranolol (1.0 μM) was added to the PSS solution throughout all experiments to reduce the likelihood of β-adrenoceptor stimulation. EDTA (10 μM) and ascorbic acid (50 μM) were also included in all experiments to reduce the degradation of NA in the bath.

Table 1 Dissociation constant ($-\log K_B$) and relative order of potency of α-adrenoceptor antagonists on the slow component of contraction of rat isolated aorta to noradrenaline.

Drug	*Concentration*	$-\log K_B$	n	*Relative potency*
Prazosin	0.3–25 nM	9.77 ± 0.03	28	1
WB4101	5–750 nM	*9.26–0.04	19	3
Doxazosin	0.3–25 nM	*9.20 ± 0.05	18	4
Tiodazosin	5–25 nM	8.95 ± 0.06	18	7
Phentolamine	0.25–25 μM	7.45 ± 0.05	21	210
Corynanthine	0.25–25 μM	7.30–0.03	16	300
Trimazosin	0.5–50 μM	7.07 ± 0.03	18	500
Rauwolscine	0.25–25 μM	6.73 ± 0.05	16	1070

Dissociation constant values are mean ± s.e.
* No significant difference ($P > 0.05$)

Statistical analyses

Results are presented as the mean together with the standard error of the mean (s.e.). Where appropriate, comparisons of means has been made by application of Student's *t* test for unpaired data. Agonist dose-ratios were obtained from the concentrations causing 50% of the maximum effect in the absence and presence of each concentration of antagonist. Unpaired control DRCs ($n = 12$) were performed to correct for changes in sensitivity in those experiments where antagonists were used (Furchgott, 1972). Antagonists were considered competitive if there was 10% or less reduction in the maximum response and

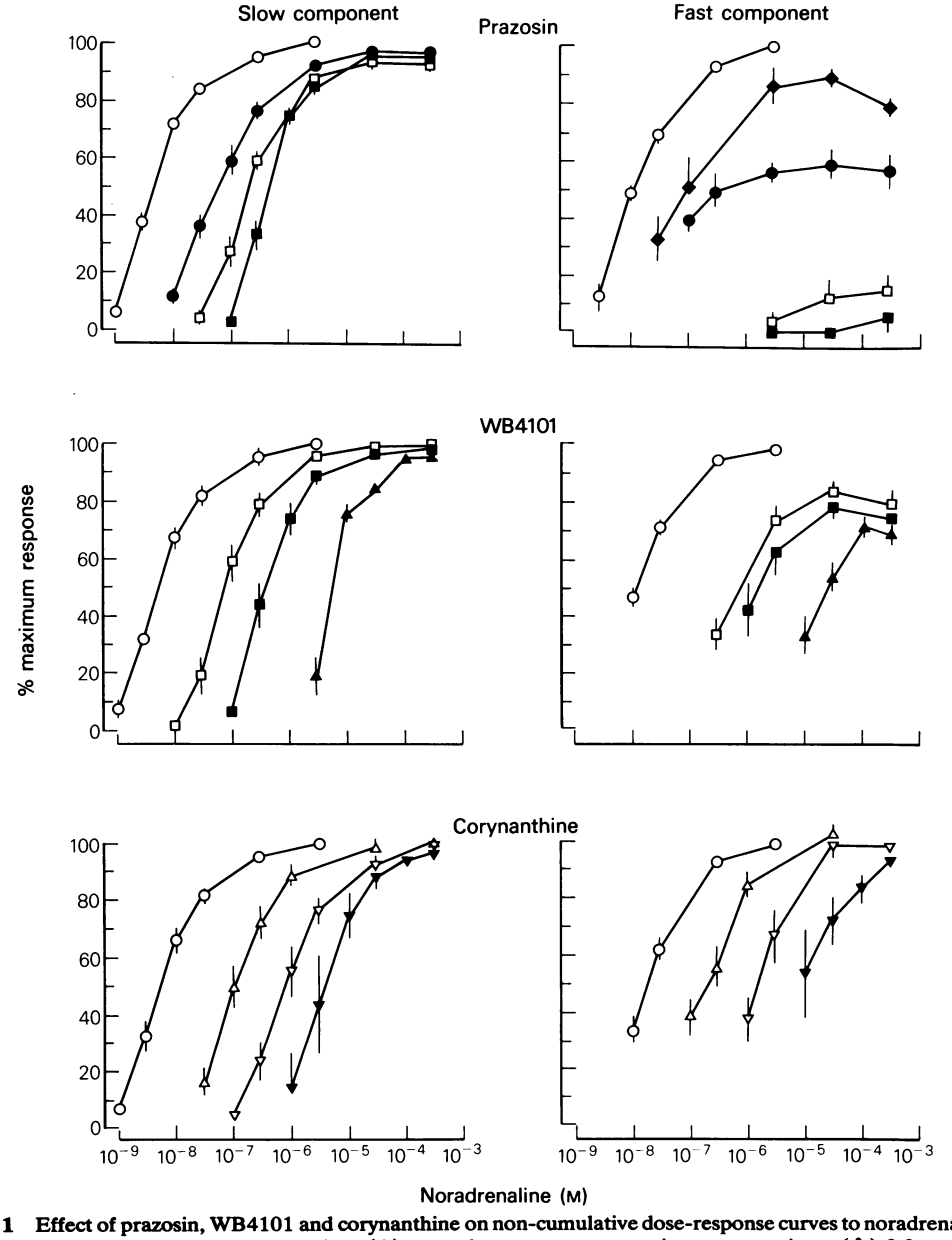

Figure 1 Effect of prazosin, WB4101 and corynanthine on non-cumulative dose-response curves to noradrenaline for slow and fast components of contraction: (○) control responses; antagonist concentrations: (◆) 0.3 nM; (●) 1 nM; (□) 5 nM; (■) 25 nM; (△) 0.25 μM; (▲) 0.75 μM; (▽) 2.5 μM; (▼) 25 μM. Each point and vertical bar represents the mean and s.e.mean ($n > 6$).

if the 95% confidence limits for the slope of the Schild plot (Arunlakshana & Schild, 1959), drawn by linear regression, overlapped unity. The dissociation constant ($-\log K_B$) was calculated for each antagonist on the slow component and the relative order of potency determined by the method of Furchgott (1972).

Results

Comparison of the effects of adrenoceptor antagonists on dose-response curves for fast and slow components of contraction to noradrenaline

Concentrations of NA of 10 nM and above produced a biphasic contraction of the aorta, consisting of an initial fast component which reached a maximum in about 15s followed by a slow rise to maximum tension which was attained between 5 and 15 min (see record (a) Figure 3). The maximum obtainable fast component to NA (3.0 μM) was (39.2 ± 0.7%, $n = 22$) of the total contraction. Non-cumulative DRCs have been constructed for both fast and slow components (see methods) and the effects of all adrenoceptor antagonists have been separately assessed on both. The EC_{50} for the slow responses was 4.8 ± 0.5 nM ($n = 18$). All agents, in the concentrations used (see Table 1), caused a parallel shift to the right of the DRCs for the slow response with little reduction in maximum response. The order of potency (see methods) for all drugs tested was prazosin > WB4101 = doxazosin > tiodazosin > phentolamine > corynanthine > trimazosin > rauwolscine (Table 1).

The effects of individual agents on the DRCs for the fast response were usually either (a) a parallel shift to the right with little depression of maximum; seen with phentolamine, trimazosin, corynanthine and rauwolscine, or (b) a parallel shift to the right with some depression of maximum response; seen with WB4101, or (c) a marked depression of the maximum response with a small non-parallel shift to the right; seen with prazosin and doxazosin. Tiodazosin had effects intermediate between those of prazosin and WB4101.

Representatives of agents having these different profiles are shown in Figure 1, which compares the effects of prazosin, WB4101 and corynanthine (all α_1-selective) on the DRCs for the fast and slow components of contraction. The effects of prazosin, its analogues and WB4101 on the depression of the maximum obtainable fast component are shown in Figure 2. It is seen that only the two most potent prazosin analogues (prazosin and doxazosin) were able to supress the fast response completely. The least potent prazosin analogue, trimazosin, produced

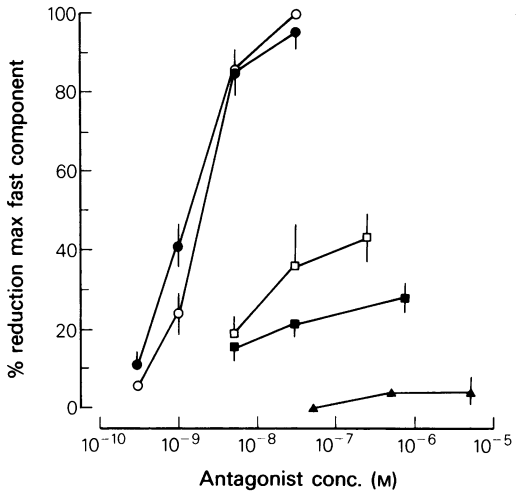

Figure 2 Effect of prazosin (●); doxazosin (○); tiodazosin (□); WB4101 (■) and trimazosin (▲) on the maximum fast component of contraction of rat isolated aorta to noradrenaline. Each point and vertical bar represents the mean and s.e.mean ($n > 6$).

no significant depression of the maximum fast component ($P < 0.05$). Tiodazosin having intermediate potency, had effects on the maximum fast component

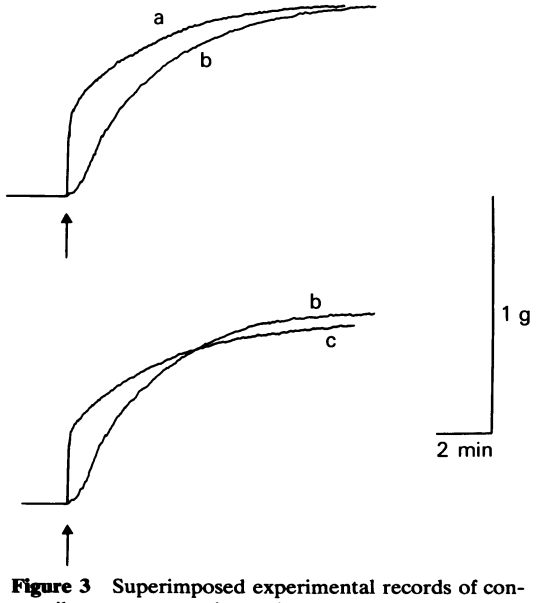

Figure 3 Superimposed experimental records of contractile responses of rat isolated aorta to noradrenaline(NA) (↑). (a) Control response to 3.0 μM NA; (b) response to 0.3 mM NA in the presence of prazosin (25 nM); (c) response to 0.3 mM NA in the presence of prazosin (25 nM) and phentolamine (2.5 μM).

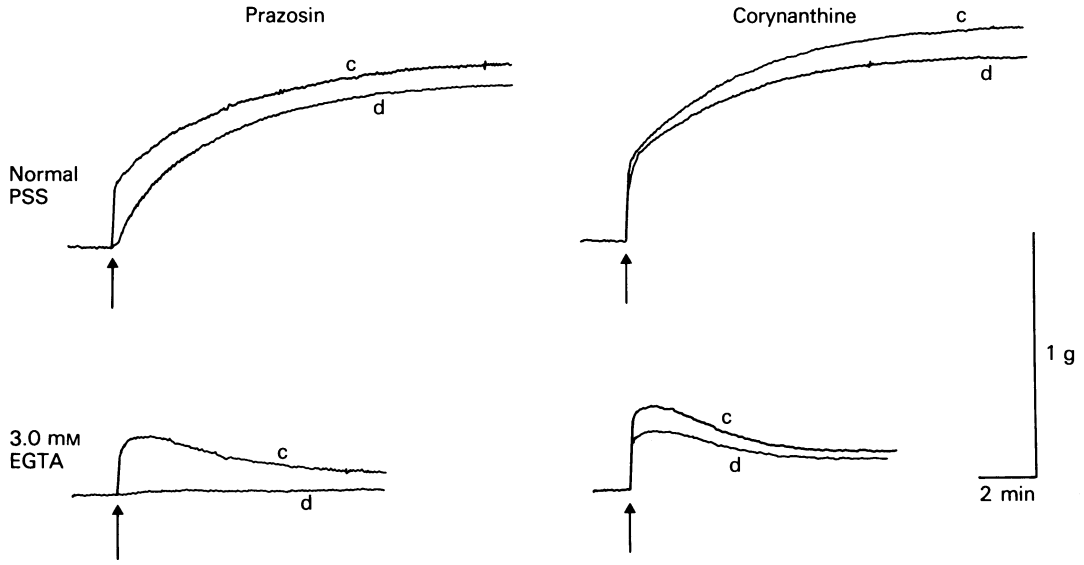

Figure 4 Superimposed experimental records of contractile responses of rat isolated aorta to noradrenaline (NA) (3.0 μM) (↑) in the absence (normal PSS) and the presence of 3.0 mM EGTA, to show the effects of prazosin (5.0 nM) and corynanthine (0.25 μM); c = control; d = with drug.

intermediate between those of prazosin and trimazosin. On the other hand, the structurally unrelated agent, WB4101, which was more potent than tiodazosin (Table 1), was less effective in depressing the fast component.

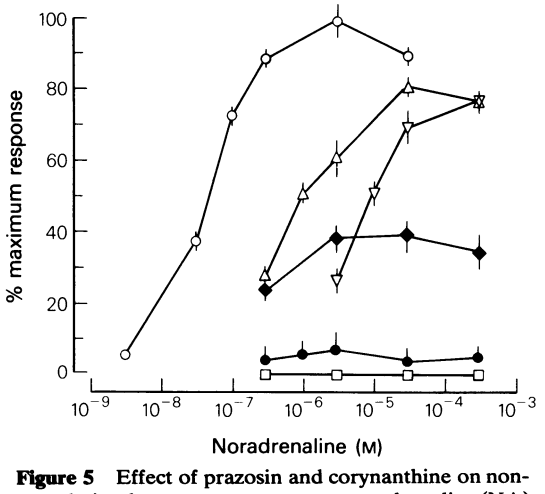

Figure 5 Effect of prazosin and corynanthine on non-cumulative dose-response curves to noradrenaline (NA) in the presence of 3.0 mM EGTA: (○) control NA; (◆) prazosin 0.3 nM; (●) prazosin 1.0 nM; (□) prazosin 5.0 nM; (△) corynanthine 0.25 μM; (▽) corynanthine 2.5 μM. Each point and vertical bar represents the mean and s.e.mean ($n = 6$).

On the nature of the depression of the fast component by prazosin and doxazosin

The suppression of the fast component achieved by doxazosin or prazosin (25 nM) was unsurmountable with concentrations of NA of up to 0.3 mM (Figure 3). If phentolamine was given at the same time as prazosin then the non-competitive blockade of the fast component of contraction by prazosin was prevented (Figure 3). Similar observation have been made with doxazosin (25 nM). The effect of both agents on the fast response, in these concentrations, was completely reversed by washing for 120 min.

Comparison of prazosin with corynanthine on contractions mediated by intracellular calcium release

Following 2 min exposure to 3.0 mM EGTA, contractile responses to 60 mM KCl were abolished. In the presence of EGTA, small, slowly developing responses only were observed with concentrations of NA below 10 nM. With concentrations of NA of 10 nM and above, rapidly developing contractions were observed ($EC_{50} = 29 \pm 2.6$ nM; $n = 40$) which were not maintained (Figure 4). The effect of prazosin and corynanthine on the form of the responses to NA both in the absence and presence of EGTA is shown in Figure 4. DRCs showing the effects of prazosin and corynanthine on the EGTA-resistant contractions (Figure 5) are similar to those of these two agents on the fast response in normal PSS (cf.

Figure 1), i.e. a non-competitive antagonism by prazosin and a competitive antagonism by corynanthine. Although some reduction of the maximum response was seen with corynanthine in the presence of EGTA (Figure 5), the term competitive is used since the effects are clearly different from those of prazosin. By comparison of Figure 1 and Figure 5, it may be seen that the EGTA-resistant contractions were more sensitive to the depressant action of prazosin and corynanthine than was the fast component in normal PSS. Doxazosin had effects on the EGTA-resistant contractions similar to those described for prazosin.

Contractions of the aorta to 5-HT (0.25–250 μM) were biphasic and those to caffeine (20 mM) resembled the EGTA-resistant responses except that relaxation was faster and more complete. Neither doxazosin or prazosin in concentrations of up to 2.5 μM had any effect on the form or amplitude of contractions of the aorta to either 5-HT or caffeine.

Discussion

Cohen, Wiley & Slater (1979) demonstrated a competitive blockade of NA-induced contractions by prazosin in rat isolated aorta. These investigators used cumulative DRCs in which the fast component of contraction cannot be separately assessed.

In a preliminary study (Downing *et al.*, 1981) using non-cumulative DRCs, we found that prazosin showed a 'non-competitive' antagonism of the fast component of contraction and a competitive antagonism of the slow component of contraction to NA. We suggested that this action of prazosin was unlikely to be related to its selectivity for α_1-adrenoceptors. This view is now strengthened by the observations that corynanthine and trimazosin, both selective α_1-adrenoceptor antagonists (Constantine & Hess, 1981; McGrath, 1982), did not selectively reduce the fast component but had a profile of action similar to that shown by the non-selective α-adrenoceptor antagonist, phentolamine, i.e. competitive suppression of both fast and slow responses. On the other hand, both of the other α_1-adrenoceptor antagonists tested (WB4101 and tiodazosin) did cause some selective reduction of the fast component. For the prazosin analogues the ability to depress the fast component appeared related to potency (Figure 2). Of the two α_1-adrenoceptor antagonists tested which were structurally unrelated to prazosin, (WB4101 and corynanthine) it is interesting to note that the most potent agent (WB4101) caused some depression of the maximum response suggesting that potency and structure are important factors in determining the ability of these agents to show this effect.

The fact that phentolamine was able to reverse completely the effects of prazosin and doxazosin on the fast component is seen as evidence that these agents are acting on the same receptor as phentolamine. In view of these observations it seems unnecessary to invoke the possibility of different receptors for the fast and slow responses on this preparation. It seems more likely that the ability of prazosin and doxazosin to reduce selectively the fast component is probably dependent on the way in which they combine with the receptor.

Godfraind, Miller & Socrates (1982), on the basis of agonist selectivity and action, have suggested that selective stimulation of α_2-adrenoceptors produces only the slow component of contraction in rat aorta. The inability of α_2-adrenoceptor agonists to induce a fast component of contraction may be related to their low efficacy in this preparation. The most effective α_2-agonist used by Godfraind *et al.* (1982) produced a maximum (slow) contraction to a single addition which was less than 50% of that achieved by NA. In our experiments, the EC_{50} (NA) for the slow component (4.8 ± 0.5 nM) was less than the threshold concentration of NA required (10 nM) for observing a clear biphasic response in normal PSS, or a clear fast response in the presence of EGTA. Therefore any agonist of low efficacy on this preparation would be expected to produce only a slow component of contraction.

Constantine, McShane, Scriabine & Hess (1973) suggested that prazosin might have a component of action which was distal to the postsynaptic α_1-adrenoceptor. Such a hypothesis could explain the observed action of prazosin on the fast component if it were able to interfere directly with intracellular calcium release. This seems unlikely in view of the ease with which the effects of prazosin can be reversed by phentolamine. We have been unable to demonstrate any difference in the rate of recovery of the fast and slow components of the NA response following the removal of prazosin by washing (unpublished observations). Furthermore, prazosin had no effect on the contractions of the aorta to 5-HT or caffeine. The observations of Yamashita, Takagi & Hotta (1977) suggest that both these agents release calcium from intracellular sites in this preparation. Taken together these observations make it unlikely that prazosin has sites of action other than the α-adrenoceptor.

The observations made with doxazosin and prazosin against the EGTA-resistant contractions, indicates that these agents probably non-competitively antagonize α-adrenoceptor-mediated release of intracellular calcium on this preparation. The non-competitive reduction of the fast component in normal PSS is a reflection of this effect. The differences in sensitivity of the fast component in normal PSS and the EGTA-resistant responses to prazosin and

corynanthine may be due to an increase in calcium sequestration (from inside to outside) which would be expected to occur in the presence of EGTA.

Vascular muscles from different vascular beds appear to utilise intracellular and extracellular sources of activator calcium to different degrees (Vanhoutte, 1978). Thus prazosin and doxazosin would be expected to show a competitive antagonism of contractions to NA in vessels that have a strong dependence upon extracellular calcium for activation and a non-competitive antagonism of responses of vessels to NA that are more dependent on an intracellular source of activator calcium. We have recently shown (Downing, Wilson & Wilson, 1983) that prazosin produces a non-competitive antagonism of NA-induced pressor responses in the perfused rat mesenteric bed. NA-induced pressor responses in this preparation have been shown to be resistant to the removal of extracellular calcium (Adeagbo & Okpaku 1980), suggesting that calcium from intracellular sites is more important in mediating these responses to NA. In the rat longitudinal portal vein, which is very sensitive to the removal of extracellular calcium (Altura, Altura & Carella, 1981), prazosin competitively antagonizes NA induced contractions (Harris, Swamy, Triggle & Waters, 1980).

The ability of these agents to cause a non-competitive suppression of contractions of vascular muscle due to α-adrenoceptor mediated intracellular calcium release may help in better understanding their known haemodynamic actions in the rat. The significance of these observations to other species remains to be determined, although it would appear that the rat aorta is different from the rabbit in that prazosin is equi-effective in blocking calcium entry and release in the latter (Cauvin, Loutzenhiser, Hwang & Van Breemen; 1982).

These observations query the suitability of prazosin as a selective α_1-adrenoceptor antagonist in experiments where the aim is to determine the relative roles of α_1- and α_2-adrenoceptors in excitation-contraction coupling.

We are grateful to Pfizer for gifts of prazosin and its analogues and to Bristol for a gift of tiodazosin. V.G.W. was supported by a SERC studentship.

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(*Received March 31, 1983.*
Revised May 5, 1983.)