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Investigation of the subtypes of α_1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen

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1 The subtypes of α_1-adrenoceptor mediating contractions to exogenous noradrenaline (NA) or phenylephrine in rat vas deferens, spleen and aorta, and mediating contractions to endogenous NA in rat vas deferens have been examined.

2 In rat vas deferens, the competitive antagonists prazosin, WB 4101, benoxathian and 5-methyl-urapidil inhibited contractions to NA with pA_2 values of 9.26, 9.54, 9.02 and 8.43, respectively. The irreversible antagonist chloroethylclonidine (CEC) (100 μM) failed to affect contractions to NA.

3 In rat vas deferens in the presence of nifedipine (10 μM), contractions to NA were significantly attenuated and under these conditions, CEC (100 μM) significantly reduced the maximum response to NA.

4 In rat spleen, the competitive antagonists prazosin, WB 4101 and benoxathian inhibited contractions to phenylephrine with pA_2 values of 9.56, 8.85 and 7.60, respectively, and 5-methyl-urapidil had a K_B of 6.62. CEC (100 μM) significantly reduced the maximum contraction to phenylephrine.

5 In rat aorta, the competitive antagonists, prazosin, WB 4101, benoxathian and 5-methyl-urapidil inhibited contractions to NA with pA_2 values of 9.45, 9.21, 8.55 and 8.12, respectively. CEC (100 μM) produced an approximately parallel shift in the potency of NA, without significantly reducing the maximum response.

6 In epididymal portions of rat vas deferens in the presence of nifedipine (10 μM), the isometric contraction to a single electrical pulse was significantly reduced by CEC (100 μM), and by the competitive antagonists prazosin, WB 4101, benoxathian and 5-methyl-urapidil at concentrations of 1 nM.

7 In prostatic portions of rat vas deferens, the α_1-adrenoceptor agonist, amidephrine, produced concentration-dependent increases in the isometric contraction to a single electrical stimulus and the maximum increase in the evoked response produced by amidephrine was unaffected by CEC (100 μM).

8 Contractions of rat vas deferens produced by NA (and amidephrine) are mediated predominantly by α_{1A}-adrenoceptors as shown by the high potency of α_{1A}-adrenoceptor selective antagonists and the lack of effect of CEC. A small CEC-sensitive response, particularly in epididymal portions, was revealed in the presence of nifedipine. Contractions of rat spleen are mediated by α_{1B}-adrenoceptors since α_{1A}-selective antagonists showed low potency and CEC significantly reduced the maximum contraction to phenylephrine. Contractions of rat aorta to NA are mediated by non-α_{1A}, non-α_{1B}-adrenoceptors, due to the high potency of the α_{1A}-selective antagonists and sensitivity to CEC.

9 The noradrenergic contraction of epididymal portions of rat vas deferens in the presence of nifedipine is CEC-sensitive, but the α_{1A}-selective antagonists showed high potency, suggesting that this response is mediated by non-α_{1A}, non-α_{1B}-adrenoceptors.

10 In conclusion, at least three subtypes of functional α_1-adrenoceptors have been demonstrated in these studies.

Keywords: Rat vas deferens; rat spleen; rat aorta; α_{1A}-adrenoceptors; α_{1B}-adrenoceptors; prazosin; WB 4101; benoxathian; 5-methyl-urapidil

Introduction

α_1-Adrenoceptors have been subdivided into α_{1A}- and α_{1B}-adrenoceptor subtypes based on affinities of a series of ligands for binding sites in rat cortex, hippocampus, vas deferens and spleen (Morrow & Creese, 1986; Han *et al.*,1987; Gross *et al.*,1988; see Docherty, 1989), and based on the ability of the alkylating agent chloroethylclonidine (CEC) to inactivate the α_{1B} but not the α_{1A}-subtype. Based on this classification, it has been suggested that contractions of rat vas deferens to exogenous noradrenaline (NA) or adrenaline are mediated predominantly by α_{1A}-adrenoceptors (Han *et al.*, 1987; Hanft & Gross, 1989), whereas contractions to NA in rat spleen are mediated predominantly by α_{1B}-adrenoceptors (Han *et al.*, 1987).

Molecular cloning techniques have revealed the existence of at least 3 genes coding for α_1-adrenoceptors, α_{1A} (rat: Lomasney *et al.*, 1991), α_{1B} (hamster: Cotecchia *et al.*, 1988; rat: Voigt *et al.*, 1990), α_{1C} (bovine: Schwinn *et al.*, 1990), although controversy exists as to whether the clone termed α_{1A} is indeed an α_{1A} or a novel α_{1D}-subtype (rat: Perez *et al.*, 1992).

However, although contractions of the rat spleen have been used as a model for α_{1B}-adrenoceptors and contractions of the rat vas deferens to NA have been used as a model for α_{1A}-adrenoceptors, there is evidence for the presence of an additional subtype of α_1-adrenoceptor involved in contractions to nerve stimulation in rat vas deferens. Mallard *et al.* (1992) have demonstrated that the α_1-adrenoceptor response to a single stimulus is sensitive to the alkylating agent CEC, and these authors have suggested that an α_{1B}-adrenoceptor mediates this response.

The subtype of α_1-adrenoceptor present in the rat aorta has been variously classified as α_{1B} (Han *et al.*, 1990) both α_{1A} and α_{1B} (Tian *et al.*, 1990; Piascik *et al.*, 1991) or atypical (Muramatsu *et al.*, 1991; Aboud & Docherty, 1992; Oriowo & Ruffolo, 1992). In the latter four studies, CEC failed to

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reduce the maximum response to NA, but caused a parallel shift in the concentration-response curve.

The objects of this study were to re-examine the α_1-adrenoceptor subtypes present in rat aorta, spleen and vas deferens in the light of current knowledge of α_1-adrenoceptor subtypes.

Some of these results have been published in abstract form (Aboud & Docherty, 1992) or in tabular form in a review article (Docherty, 1989).

Methods

Male Wistar rats (200–300 g) were obtained from Trinity College Dublin, and aorta, spleen and vas deferens were employed as outlined below.

Rat aorta

Aortic rings of 3–5 mm in length were gently rubbed to remove the endothelium, and attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs-Henseleit solution of the following composition: (mM): NaCl 119, $NaHCO_3$ 25, D-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03, ascorbic acid 0.28. Additionally, cocaine (3 μM), propranolol (3 μM) and indomethacin (10 μM) were present.

Tissues were contracted with NA (10 μM), exposed to acetylcholine (ACh, 10 μM) to test for absence of endothelium-dependent relaxations (EDR), and washed. Bathing fluid was then changed every 15 min for the next hour. Tissues were then contracted with NA administered cumulatively in 0.5 log unit increments beginning with 1 nM. Once a maximum response to NA had been obtained, tissues were washed and bathing fluid was changed every 15 min for 120 min, for the last 60 min of which tissues were incubated with various concentrations of competitive antagonists (prazosin, WB 4101, 5-methyl-urapidil or benoxathian) or vehicle (one tissue from each animal received vehicle). Tissues were then contracted with NA in the continuing presence of antagonist or vehicle. Antagonist potency was expressed as the dissociation constant K_B from the equation $K_B = [B]/(DR-1)$, where [B] is the concentration of antagonist and DR is the agonist dose-ratio produced by the antagonist, or as a pA_2 value. Antagonist pA_2 values were obtained from the x-intercept of the plot of log (agonist DR − 1) against log antagonist concentration, where the slope was not significantly different from negative unity (Arunlakshana & Schild, 1959).

In another series of experiments, tissues were incubated with CEC (10 or 100 μM) for 30 min, beginning 60 min after wash-out of the first NA concentration-response curve. After washing for another 45 min, a concentration-response curve to NA was repeated.

Rat spleen

Spleens were bisected transversely into two portions, and experiments were carried out as for the rat aorta except that phenylephrine was used as the contractile agent (except for a few experiments employing NA, as outlined in the results section).

Rat vas deferens

Whole vas deferens, or prostatic or epididymal portions were obtained.

In investigations of contractions produced by exogenous agonists, experiments were carried out as described for rat aorta, employing NA as agonist, except for the following differences. Tissues were pre-exposed to NA (10 μM), and following 60 min exposure to antagonist or vehicle, a single agonist concentration-response curve was obtained per tissue. In experiments carried out in the presence of nifedipine (10 μM), nifedipine was added during the 45 min washout after a 30 min exposure to CEC (100 μM).

In investigations of contractions produced by nerve stimulation, cocaine was omitted from the Krebs-Henseleit solution and tissues were placed between platinum electrodes and stimulated every 5 min with a single stimulus (0.5 ms pulses, supramaximal pulses) to produce isometric contractions. In investigations of the ability of the α_1-adrenoceptor agonist, amidephrine, to increase the isometric twitch response of prostatic portions, tissues were stimulated with a single electrical pulse every 5 min. When consistant control twitches had been obtained in tissues pre-exposed to CEC (100 μM) or vehicle for 30 min followed by 45 min wash, amidephrine was added in 0.5 log unit increments and a stimulus was obtained after 5 min exposure to each amidephrine dose.

In experiments investigating the ability of competitive antagonists to inhibit the isometric twitch in epididymal portions, nifedipine (10 μM) was present to block the non-noradrenergic component of the twitch, and antagonists were added in three cumulative concentrations at 15 min intervals. An isometric twitch was obtained following 15 min exposure to each antagonist concentration, or following exposure to the vehicle.

Drugs

The following drugs were used: acetylcholine chloride (Sigma, Poole, U.K.): amidephrine hydrochloride (gift: Bristol-Myers Squibb, Wallingford, CT, U.S.A.); benoxathian hydrochloride (Research Biochemicals, Natick, MA, U.S.A.); chloroethylchlonidine (Research Biochemicals); cocaine hydrochloride (Sigma); corticosterone (Sigma); 5-methyl-urapidil (gift: Byk Guilden, Konstanz); nifedipine (Sigma); noradrenaline bitartrate (Sigma); phenylephrine hydrochloride (Sigma); prazosin hydrochloride (gift; Pfizer, Sandwich, U.K.); propranolol hydrochloride (Sigma); WB 4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride: Research Biochemicals).

Drugs were dissolved in distilled water, except for corticosterone (100% ethanol).

Statistics

Values are arithmetic mean ± s.e.mean, or geometric mean and 95% confidence limits. The 95% confidence limits were calculated from the standard deviation, except in the case of pA_2 values (95% limits of the intercept of Schild plot). Antagonist EC_{50} K_B or pA_2 values were compared between tissues, and were compared with the effects of vehicle by Student's t test for unpaired or paired data, where appropriate, and by ANOVA analysis. Slopes and elevation of Schild regressions were compared by covariance analysis (see Snedecor & Cochran, 1980).

Results

Rat aorta

NA produced isometric contractions with a pD_2 of 7.50 ± 0.15 (mean and 95% confidence limits, −log M) and a maximum contraction of 1.02 ± 0.18 g ($n = 15$). Prazosin, WB 4101, benoxathian and 5-methyl-urapidil produced concentration-dependent shifts in the potency of NA without reducing the maximum response. Schild plots were constructed from the effects of a range of concentrations of each antagonist, and since the slope of these regressions did not differ significantly from negative unity, pA_2 values were calculated (see Figure 1 and Table 1).

The irreversible antagonist CEC (10 and 100 μM) failed to reduce significantly the maximum response to NA but produced parallel shifts in the potency of NA (Figure 2). The

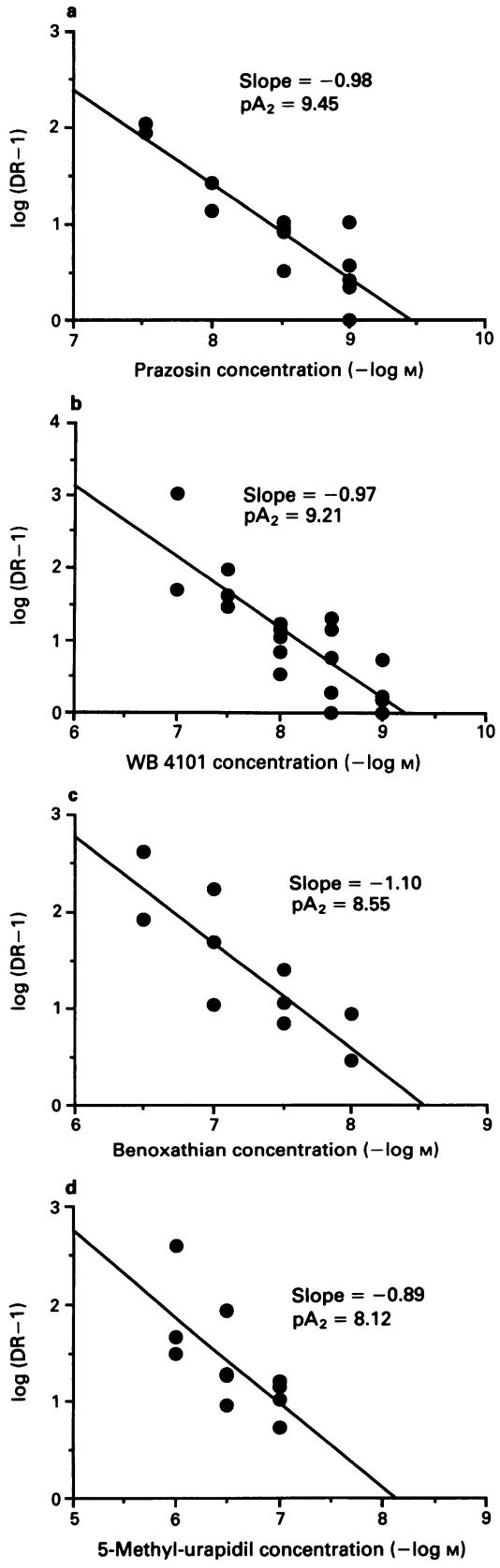

Figure 1 pA_2 values obtained for the competitive antagonists prazosin (a), WB 4101 (b), benoxathian (c) and 5-methyl-urapidil (d) against contractions to noradrenaline in rat aorta. Each point represents an individual experiment. Full details of pA_2 values are shown in Table 1.

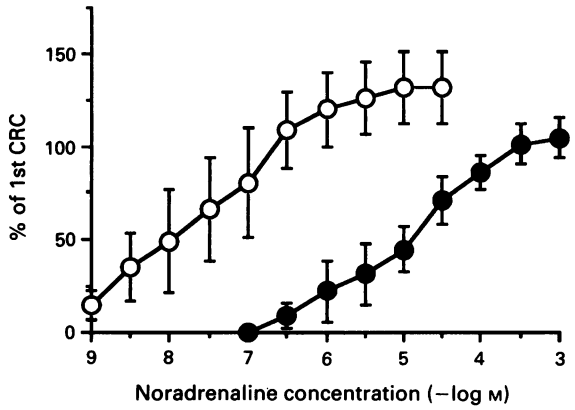

Figure 2 Effects of chloroethylclonidine (CEC, 100 μM) or vehicle on contractions to noradrenaline in rat aorta. Responses in the presence of CEC (●) or vehicle (○) are expressed as a percentage of the maximum response obtained in the first (control) concentration-response curve. Vertical bars represent s.e. of mean from at least 4 experiments.

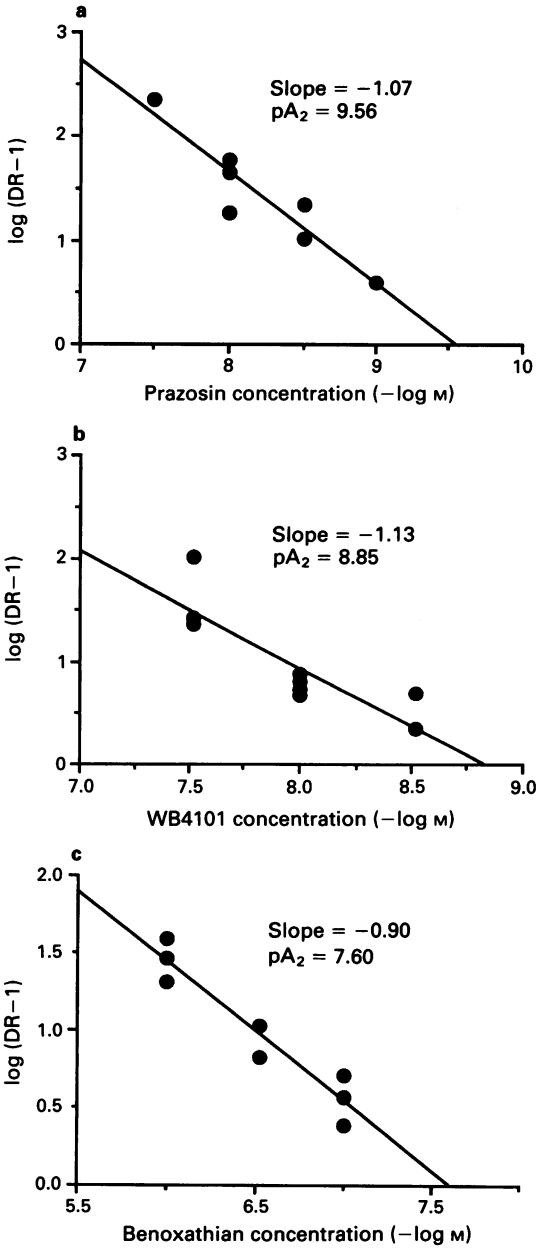

Figure 3 pA_2 values obtained for the competitive antagonists prazosin (a), WB 4101 (b) and benoxathian (c) against contractions to phenylephrine in rat spleen. Each point represents an individual experiment. Full details of pA_2 values are shown in Table 1.

Table 1 Potencies of antagonists against contractions to noradrenaline (NA) in rat vas deferens and aorta, and to phenylephrine in rat spleen

	Prazosin	*WB 4101*	*Benox*	*5-MU*	*CEC*
Vas deferens	9.26	9.54	9.02	8.43	no effect
(α_{1A})	(8.17–11.88)	(8.82–11.70)	(8.35–9.97)	(7.74–9.32)	
Spleen	9.56	8.85	7.60	$6.62 \pm 0.50^{+}$	effective
(α_{1B})	(9.02–10.49)	(8.21–10.13)	(7.27–8.05)		
Aorta	9.45	9.21	8.55	8.12	effective
(non-α_{1A}/α_{1B})	(8.90–10.12)	(8.44–10.16)	(7.86–9.58)	(7.20–10.99)	

Potencies are expressed as pA_2 values (and 95% confidence limits) from the Schild plots shown in Figures 1, 3 and 6, or as a K_B ($\pm$ 95% confidence limits) from the effects of a fixed concentration of antagonist (5-methyl-urapidil in rat spleen). The effects of chloroethylclonidine (CEC, 100 μM) on agonist responses are also listed.
Abbreviations: Benox., benoxathian; 5-MU, 5-methylurapidil

shifts in potency of noradrenaline produced by CEC (10 and 100 μM) were 26.1 ± 11.0 times ($n = 5$) and 464 ± 174 times ($n = 4$), respectively.

Rat spleen

NA and phenylephrine produced isometric contractions with pD_2 values of 5.80 ± 0.21 and 5.37 ± 0.40, and maximum contractions of 0.68 ± 0.10 g ($n = 6$) and 0.18 ± 0.01 g (n = 28). Prazosin, WB 4101, benoxathian and 5-methyl-urapidil produced shifts in the potency of NA and phenylephrine without reducing the maximum response. Schild plots were constructed from the effects of a range of concentrations of each antagonist, and where the slope of these regressions did not differ significantly from negative unity, pA_2 values were calculated (see Figure 3 and Table 1). However, slopes of Schild plots differed from negative unity for all antagonists against NA, so that effects against NA were not quantified. For 5-methyl-urapidil, the slope of the Schild plot against phenylephrine was also significantly different from negative unity, so that potency was expressed as a K_B from the lowest concentration (1 μM) to produce a significant shift in potency of phenylephrine.

The irreversible antagonist CEC (100 μM) significantly reduced the response to phenylephrine (1 mM) (Figure 4).

Whole vas deferens: responses to exogenous agonists

In vehicle-treated whole vas deferens, NA produced contractions consisting of intermittent spikes superimposed on a tonic contraction with a pD_2 of 6.36 ± 0.50 and a maximum contraction (measured as the maximum height of intermittent spikes) of 1.76 ± 0.20 g (mean ± s.e. of mean, $n = 5$). In tis-

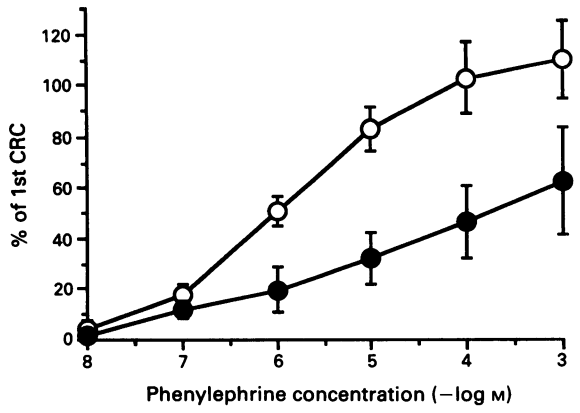

Figure 4 Effects of chloroethylclonidine (CEC, 100 μM) or vehicle on contractions to phenylephrine in rat spleen. Responses in the presence of CEC (●) or vehicle (○) are expressed as a percentage of the maximum response obtained in the first (control) concentration-response curve. Vertical bars represent s.e. of mean from at least 4 experiments.

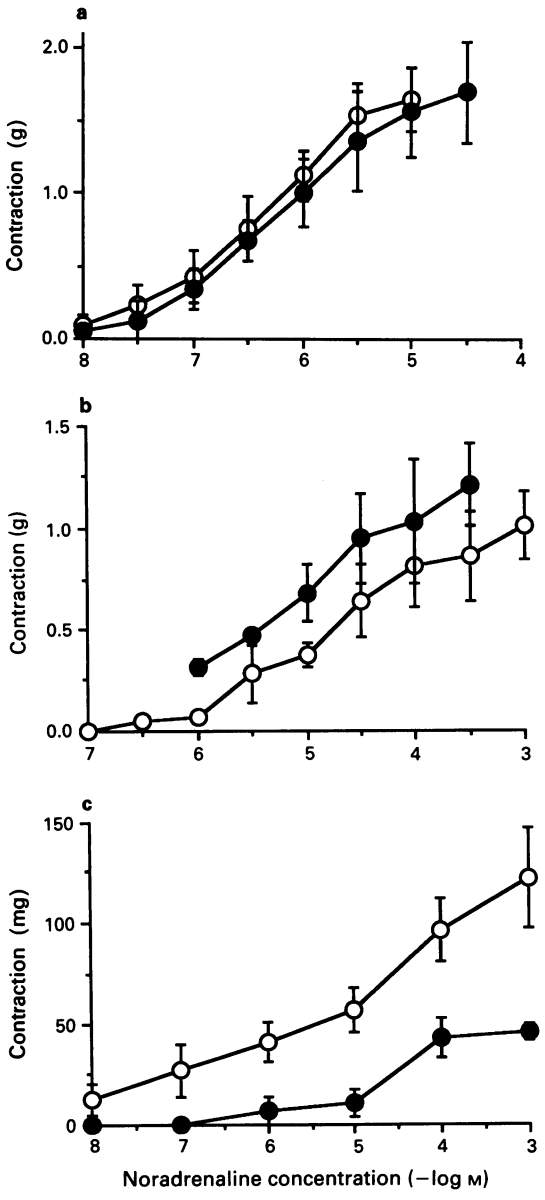

Figure 5 Effects of chloroethylclonidine (CEC) on contractions to noradrenaline (NA) in rat whole vas deferens in the presence of cocaine (a), in the absence of cocaine (b) and in the presence of cocaine and nifedipine (10 μM) (c). Responses are expressed as contraction (g or mg) to NA produced in a single concentration-response curve following exposure to CEC (10 μM) (a), CEC (100 μM) (b,c) (●) or vehicle (○). Vertical bars represent s.e. of mean from at least 4 experiments.

sues pretreated with CEC (10 μM), NA had a pD_2 of 6.38 ± 0.36, and a maximum contraction of 1.62 ± 0.28 g ($n = 5$) (no significant difference from controls) (see Figure 5a). In the absence of cocaine the potency of NA was reduced, with pD_2 values of 4.67 ± 0.94 and 4.57 ± 0.92 and a maximum contraction of 1.01 ± 0.17 g ($n = 3$) and 1.25 ± 0.20 g ($n = 3$) in vehicle-treated and CEC (100 μM)-treated tissues, respectively (no significant difference) (see Figure 5b).

Prazosin, WB 4101, benoxathian and 5-methyl-urapidil produced concentration-dependent shifts in the potency of NA, and behaved competitively in that they did not decrease the maximum contraction to NA, and slopes of Schild plots were not significantly different from negative unity, so that pA_2 values could be calculated (Figure 6).

In the presence of nifedipine (10 μM), NA no longer produced intermittent spikes but produced small tonic contractions with a maximum contraction of 0.12 ± 0.02 g ($n = 5$). In the presence of CEC (100 μM), this contraction was reduced to 0.05 ± 0.01 g ($n = 5$) (significant reduction; $P < 0.05$, Student's t test) (Figure 5c).

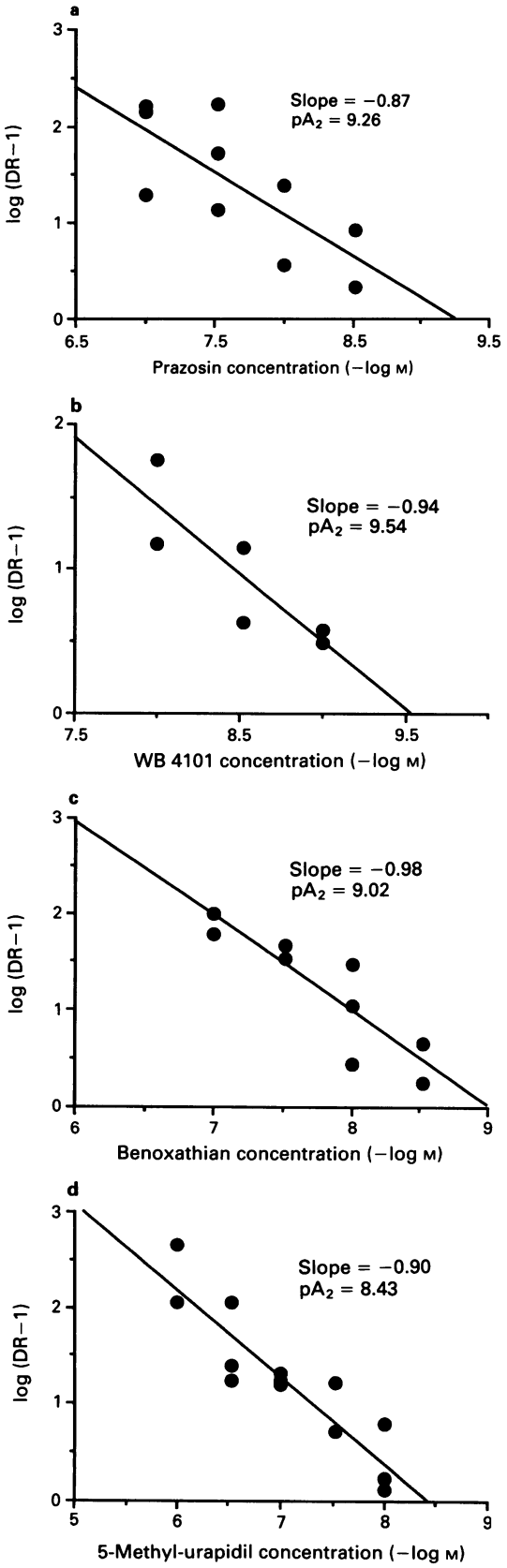

Figure 6 pA_2 values obtained for the competitive antagonists prazosin (a), WB 4101 (b), benoxathian (c) and 5-methyl-urapidil (d) against contractions to noradrenaline in rat vas deferens. Each point represents an individual experiment. Full details of pA_2 values are shown in Table 1.

Prostatic portions of rat vas deferens: responses to exogenous agonists

In prostatic portions of rat vas deferens, NA produced transient contractions with a pD_2 of 5.29 ± 0.11 and a maximum contraction of 1.19 ± 0.18 g ($n = 3$), and this contraction was virtually abolished by nifedipine (10 μM) (reduced to 0.04 ± 0.01 g, $n = 5$). Following nifedipine and CEC (100 μM), the contraction to NA was 0.03 ± 0.01 g ($n = 3$) (no significant difference).

Prostatic portions of rat vas deferens: nerve-mediated responses

In prostatic portions, single pulse electrical stimulation produced a biphasic contraction, consisting mainly of the first non-noradrenergic phase. Amidephrine produced a concentration-dependent increase in the stimulation-evoked contraction, increasing the first phase to 269.2 ± 25.4% of control ($n = 4$) with a pD_2 of 5.82 ± 0.41 in vehicle-treated animals (Figure 7).

In prostatic portions exposed to CEC 100 μM for 30 min, the small second (noradrenergic) phase of the contraction to a single stimulus was abolished and the first (nonadrenergic) phase was reduced to 44.1 ± 6.0% of the response prior to CEC ($n = 4$). Exposure to vehicle for 30 min did not significantly alter the response to a single stimulus (first phase: 102.0 ± 5.4% of response prior to vehicle, $n = 4$). Following exposure to CEC (100 μM), amidephrine produced a concentration-dependent increase in the first phase of the response to a single stimulus, with a maximum increase to 533.1 ± 100.0% of control and a pD_2 of 6.59 ± 1.08 (Figure 7).

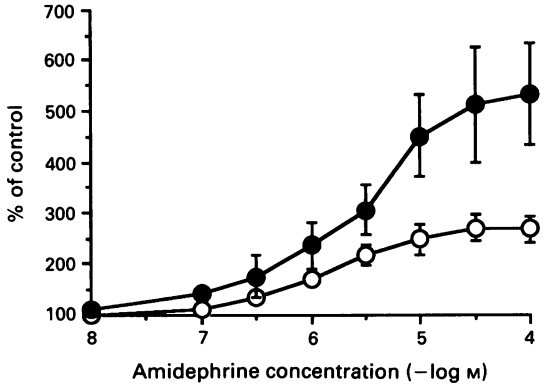

Figure 7 Effects of amidephrine on the isometric contraction produced by single pulse electrical stimulation in prostatic portions of rat vas deferens. Responses in the presence of amidephrine are expressed as a percentage of the response in the absence of amidephrine. Symbols: vehicle-treated (○), chloroethylclonidine (100 μM)-treated (●). Vertical bars represent s.e. of mean from at least 4 experiments.

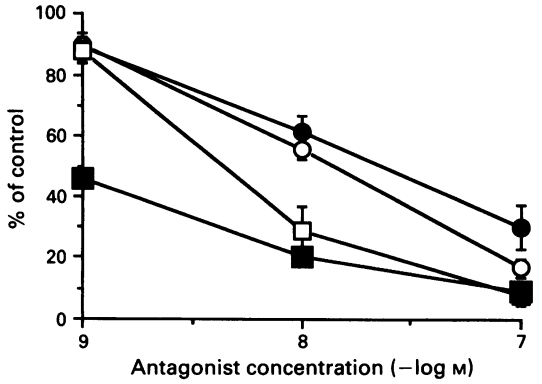

Figure 8 Effects of prazosin (●), WB 4101 (□), benoxathian (○ and 5-methyl-urapidil (■) on isometric contractions produced by single pulse electrical stimulation in epididymal portions of rat vas deferens in the presence of nifedipine (10 μM). Effects of all concentrations of all antagonists shown were significantly different from the effects of vehicle (Student's t test, $P<0.05$). Vertical bars represent s.e.mean from at least 4 experiments.

Although the maximum increase in the evoked response produced by amidephrine was much greater in tissues exposed to CEC, this can be explained entirely by the significant reduction in the evoked contraction produced by CEC. When expressed as a percentage of the initial (prior to exposure to CEC or vehicle) first phase contraction to a single electrical stimulus, amidephrine produced a maximum increase in the evoked contraction to 274.9 ± 31.0% and 226.4 ± 39.9% of control, in vehicle- and CEC-treated tissues, respectively (no significant difference). In experiments in which vehicle replaced amidephrine, there was no significant increase in the twitch response over the same time period.

Epididymal portions of rat vas deferens: nerve-mediated responses

In epididymal portions of rat vas deferens, the initial biphasic twitch to a single electrical stimulus was reduced to a monophasic response by nifedipine (10 μM), which eliminates the first (non-noradrenergic) phase, leaving the second (noradrenergic) phase. Under these conditions, the twitch to a single stimulus was 0.99 ± 0.14 g ($n = 10$), and this was reduced to 4.2 ± 3.7% of control ($n = 5$) by exposure to CEC (10 μM) for 30 min, as compared to 81.8 ± 11.8% of control ($n = 5$) when exposed to vehicle ($P<0.05$).

The competitive antagonists prazosin, benoxathian, WB 4101 and 5-methyl-urapidil produced concentration-dependent inhibitions of the isometric twitch to a single electrical stimulus, with an order of potency 5-methyl-urapidil ≥ WB4101 > benoxathian = prazosin (Figure 8). For all 4 antagonists, nerve-mediated contractions were significantly reduced by a concentration of 1 nM ($P<0.05$ from effects of vehicle).

Discussion

In this study, we have looked at subtypes of α_1-adrenoceptors mediating contractions of the rat aorta, spleen and vas deferens to exogenous agonists (NA, phenylephrine or amidephrine) and contractions of the vas deferens to endogenous NA. These will be discussed separately, beginning with contractions to exogenous agonists.

Responses to exogenous agonists

In whole vas deferens, NA produced concentration-dependent contractions which consisted of intermittent spikes superimposed on a small tonic contraction. Contractions to NA were unaffected by CEC, but potently inhibited by WB 4101, benoxathian and 5-methyl-urapidil. Insensitivity to CEC, which irreversibly inactivates α_{1B}-adrenoceptors, would suggest that the receptors involved in contractions to NA are of the α_{1A}-subtype, and the high potency of WB 4101, benoxathian and 5-methyl-urapidil would tend to confirm this identification. The pA_2 values of WB 4101 (9.54), benoxathian (9.02) and 5-methyl-urapidil (8.48) found in this study compare well with pA_2 values of 9.40, 8.75 and 8.47, respectively, obtained in functional studies of rat vas deferens (Han *et al.*, 1987; Hanft & Gross, 1989), and with ligand binding affinities for α_{1A}-sites in rat hippocampus (9.53, 9.04 and 9.07, respectively: Han *et al.*, 1987; Gross *et al.*, 1988). Since WB 4101, benoxathian and 5-methyl-urapidil have lower affinities for α_{1B}-ligand binding sites (8.57, 7.30 and 7.17, respectively: Han *et al.*, 1987; Gross *et al.*, 1988), our results would confirm the identification of the receptor mediating contractions of rat vas deferens to NA as an α_{1A}-adrenoceptor.

In the presence of the calcium entry blocker nifedipine, the intermittent spikes produced by exogenous NA in whole vas deferens were abolished, and a small tonic contraction remained. Pre-exposure to CEC significantly reduced this contraction, suggesting that the residual contraction is mediated by a non-α_{1A}-adrenoceptor. Mallard *et al.* (1992). have demonstrated that contractions to NA are resistant to CEC in rat vas deferens, but failed to find a component of the response to exogenous NA which was resistant to nifedipine, although Martinotti *et al.* (1991) found a component following calcium entry block which could be blocked by CEC. Various authors have suggested that α_{1B}-adrenoceptors mediate contractions which are resistant to calcium entry blockers, employing calcium stores (Han *et al.*, 1987). Interestingly, in prostatic portions of rat vas deferens, almost no contractile response was left following nifedipine, and what little remained was unaffected by CEC. Hence, the CEC-sensitive component of the response to NA is found mainly in the epididymal portion of the rat vas deferens, where a CEC-sensitive α-adrenoceptor-mediated response to nerve stimulation is present (present results and Mallard *et al.*, 1992), so that this action of higher concentrations of NA may be mediated by receptors in the synaptic region. Since the response to NA in the presence of nifedipine was so small, even in epididymal portions, the interaction with competitive antagonists was not examined, but the CEC-sensitive α-adrenoceptor response to nerve stimulation was examined in more detail (see below).

In rat spleen, it proved impossible to obtain competitive interactions between NA and the antagonists prazosin, WB 4101, and benoxathian. This may be due to the involvement of α_2- as well as α_1-adrenoceptors in this response (Kenakin & Novak, 1988). When phenylephrine was used as a selective α_1-adrenoceptor agonist, contractions were inhibited in a competitive manner by the above antagonists and pA_2 values from Schild plots could be obtained for all antagonists except 5-methyl-urapidil, for which a K_B was calculated. The low potencies of the competitive antagonists benoxathian, WB 4101 and 5-methyl-urapidil, and the effectiveness of CEC all indicated that the receptors involved in noradrenergic contractions of rat spleen are α_{1B}-adrenoceptors, as previously suggested (Han *et al.*, 1987).

The receptor mediating contractions of the rat aorta produced by NA resembled the α_{1A}-adrenoceptor producing contractions of rat vas deferens in that benoxathian, WB 4101 and 5-methyl-urapidil all showed high potency, but resembled the α_{1B}-adrenoceptor of rat spleen in that responses were susceptible to blockade by CEC. Interestingly, CEC did not reduce the maximum response to NA in rat aorta, but produced a parallel shift in the potency of NA. Other authors have reported that CEC causes a parallel shift in the potency of NA in rat aorta (Muramatsu *et al.*, 1991; Oriowo & Ruffolo, 1992). This may suggest either the presence of 2 receptor subtypes, 1 of which is sensitive to CEC, or that

CEC interacts in a complex way with the α_1-adrenoceptor of rat aorta. Piascik *et al.* (1990) found that CEC (100 μM) made the concentration-response curve to phenylephrine in rat aorta biphasic, indicating that CEC inactivated 1 of 2 subtypes of α_1-adrenoceptor. In our studies, the sensitivity to CEC and the high potency of the competitive antagonists suggest that this receptor is a non-α_{1A}-, non α_{1B}-receptor, and may represent a functional α_{1D}-site which is expressed in rat aorta (Perez *et al.*, 1991).

Responses to endogenous noradrenaline: rat vas deferens

In rat whole vas deferens, the electrical stimulation-evoked contraction to a single stimulus consists of a biphasic response, the first phase of which is non-adrenoceptor mediated and predominates in prostatic portions, and the second phase of which is α-adrenoceptor mediated, and predominates in the epididymal portion (see Brown *et al.*, 1983). The second α-noradrenergic phase can be examined in isolation in the epididymal portion in the presence of nifedipine which eliminates the second non-noradrenergic response. Since α_{1A}-adrenoceptors are thought to produce responses via entry of extracellular calcium, this might suggest that the nerve response is mediated by a non-α_{1A}-adrenoceptor. Indeed, CEC abolished the α-noradrenergic contraction, and this has led to the suggestion that the response is mainly α_{1B}-adrenoceptor-mediated (Mallard *et al.*, 1991). However, the high potencies of 5-methyl-urapidil, WB 4101 and benoxathian in inhibiting the twitch response are not consistent with an α_{1B}-site, at which these agents have low potency, so that the receptor mediating nerve stimulation-evoked contractions in the presence of nifedipine may be a non-α_{1A}, non-α_{1B}-site, and may belong to either the α_{1C} or α_{1D}-sites which are CEC-sensitive, but at which 5-methyl-urapidil and WB 4101 show high potency (Perez *et al.*, 1991). Since the α_{1D}-receptor is reported to be expressed in rat vas deferens (Perez *et al.*, 1991), the above receptor may represent a functional α_{1D}-adrenoceptor.

In prostatic portions, the twitch response to a single stimulus is mainly nonadrenergic, but can be increased in a concentration-dependent manner by α_1-adrenoceptor agonists. CEC significantly reduced the first (non-noradrenergic) phase of the contraction but did not affect the ability of the α_1-agonist amidephrine to increase the evoked contraction, confirming that contractions to exogenous α_1-adrenoceptor agonists, be it NA or amidephrine, are mediated predominantly by CEC-insensitive receptors, presumably α_{1A}-adrenoceptors. Furthermore, it is likely that the increase in stimulation-evoked contractions produced by amidephrine is mediated by the same population of receptors as mediate contractions to NA.

The fact that CEC (100 μM) did not affect contractions to exogenous NA but significantly reduced the non-noradrenergic contraction to single pulse electrical stimulation demonstrates that, although CEC may show high selectivity for α_{1B} over α_{1A}-adrenoceptors, it appears to have major effects at other receptors.

It is clear from this study that it is at present difficult to subclassify definitively functional α_1-adrenoceptors with the tools available. However, 3 subtypes can be identified: α_{1A} (resistant to CEC); α_{1B} (sensitive to CEC, at which 5-methyl-urapidil, WB 4101 and benoxathian show low potency), non-α_{1A}, non-α_{1B} (sensitive to CEC but at which the latter antagonists show high potency). Under this classification, contractions of the rat vas deferens to NA are mediated predominantly by α_{1A}-adrenoceptors, but in the presence of nifedipine a second CEC-sensitive component can be identified, particularly in the epididymal portion. The isometric twitch response of the epididymal portion of rat vas deferens in the presence of nifedipine, appears to be mediated by a CEC-sensitive, non-α_{1A}-, non-α_{1B}-adrenoceptor, which may also mediate the CEC-sensitive component of the response to exogenous NA. Isometric contractions of rat spleen to phenylephrine are mediated predominantly by CEC-sensitive α_{1B}-adrenoceptors. Contractions of rat aorta to NA appear to be mediated by CEC sensitive non-α_{1A}, non-α_{1B}-adrenoceptors. Hence, at least 3 subtypes of α_1-adrenoceptor can be identified functionally.

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