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European Journal of Pharmacology 336 (1997) 37–42

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Further investigation of the α-adrenoceptor-mediated actions of chloroethylclonidine in rat aorta

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Received 24 April 1997; revised 14 July 1997; accepted 8 August 1997

Abstract

We have investigated the interaction between chloroethylclonidine and α-adrenoceptors in rat aorta. Chloroethylclonidine has two actions on rat aorta: reduction of the contraction to low concentrations of noradrenaline by α_1-adrenoceptor antagonism and irreversible partial agonism in combination with high concentrations of noradrenaline. The former antagonist action was found to be more marked in vessels from immature rats (1 month). We have examined further the latter agonist actions in adult rats (3 month). In the absence of chloroethylclonidine, exposure to phenoxybenzamine (10 μM for 15 min) virtually abolished contractions to subsequent noradrenaline. However, when tissues were exposed to chloroethylclonidine (100 μM) for 30 min prior to exposure to phenoxybenzamine, a large contraction was produced by subsequent noradrenaline. Receptor protection with noradrenaline or the α_2-adrenoceptor antagonists yohimbine or methoxy-idazoxan (all 10 μM), but not the α_1-adrenoceptor antagonist prazosin (10 μM), significantly reduced the ability of chloroethylclonidine to prevent the actions of phenoxybenzamine against noradrenaline. In ligand binding studies, pre-exposure to chloroethylclonidine (100 μM) for 30 min significantly reduced the maximum binding of [^{3}H]prazosin (B_{max}) to α_{1B}-adrenoceptors in rat spleen membranes to 21.4 $\pm$ 10.2% ($n = 5$) and the maximum binding of [^{3}H]yohimbine (B_{max}) to α_{2D}-adrenoceptors in rat submandibular gland membranes to 34.8 $\pm$ 6.3% ($n = 4$), as compared to pre-exposure to vehicle. These results suggest that chloroethylclonidine interacts irreversibly with α_2-adrenoceptors in rat aorta to make contractions to subsequent noradrenaline resistant to α-adrenoceptor blockade. Chloroethylclonidine appears to act as a silent irreversible agonist (i.e., an agonist which persists following multiple washout but only produces effects in combination with a classical agonist). © 1997 Elsevier Science B.V.

Keywords: Chloroethylclonidine; Prazosin; Aorta; Spleen; Submandibular gland; α_1-Adrenoceptor; α_2-Adrenoceptor; (Rat)

1. Introduction

The alkylating agent chloroethylclonidine has been employed as a tool in the subclassification of α_1-adrenoceptors, since it distinguishes between α_{1A}- and α_{1B}-adrenoceptors (Han et al., 1990). However, chloroethylclonidine binds to varying degrees to all subtypes of α_1-adrenoceptor, and also binds to α_2-adrenoceptors (Michel et al., 1993), so that its usefulness is less certain.

In addition, chloroethylclonidine has been reported to be an irreversible agonist at α_2-adrenoceptors, both prejunctionally and postjunctionally: chloroethylclonidine reduced the release of noradrenaline by irreversible activation of prejunctional α_2-adrenoceptors in rat vas deferens (Bultmann and Starke, 1993); chloroethylclonidine irreversibly contracted the dog saphenous vein with a maximum response approximately 75% of that to phenylephrine (Nunes and Guimaraes, 1993). Chloroethylclonidine also produces a sustained contraction in rat tail artery (Piascik et al., 1992) and a pressor response in the pithed rat (Leclerc et al., 1980), both preparations containing postjunctional α_2-adrenoceptors.

We have previously investigated the interaction between chloroethylclonidine and α-adrenoceptors in the rat aorta and obtained results inconsistent with a single mode of action for chloroethylclonidine (O'Rourke et al., 1995). Chloroethylclonidine had two actions: it inhibited contractile responses to low concentrations of noradrenaline and it made contractions to high concentrations of noradrenaline resistant to subsequent α-blockade. These results were interpreted as follows. Firstly, chloroethylclonidine interacts with low concentrations of noradrenaline in rat aorta as an irreversible α_1-adrenoceptor antagonist. Secondly, all actions of noradrenaline following chloroethylclonidine in rat aorta involve α-adrenoceptors since all the effects of chloroethylclonidine were prevented by receptor protection

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with noradrenaline (O'Rourke et al., 1995). Thirdly, the response to noradrenaline resistant to α-blockade, which only occurred following exposure to chloroethylclonidine, must also involve α-adrenoceptors, but since receptor protection with prazosin (10 μM) was ineffective and receptor protection with yohimbine (10 μM) was inconclusive, this may suggest that chloroethylclonidine may interact as an agonist in a way which is more easily prevented by using an agonist in receptor protection, or that chloroethylclonidine acts at normally silent (i.e., undetectable) α_2-adrenoceptors (O'Rourke et al., 1995). The object of this study was to re-examine the actions of chloroethylclonidine in rat aorta to resolve this issue. In addition, since chloroethylclonidine is reported to produce more marked inhibition of contractions in aorta from immature than adult rats (Gurdal et al., 1995), immature rats were also studied. Some of these results have been reported in abbreviated form (Docherty and O'Rourke, 1997).

2. Methods

Male wistar rats, both immature (1 month: 50–150 g) and young adult (3–4 months: 250–350 g), were obtained from Trinity College Dublin and aorta, spleen and submandibular gland were employed as outlined below. Except where otherwise stated, experiments were carried out on tissues from adult rats.

2.1. 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 and ascorbic acid, 0.28. Additionally, cocaine (3 μM), propranolol (3 μM) and indomethacin (10 μM) were present.

Tissues were contracted with KCl (40 mM), exposed to acetylcholine (10 μM) to test for absence of endothelium-dependent relaxations and washed. The bathing fluid was then changed every 15 min for the next hour. Tissues were then contracted with noradrenaline administered cumulatively in 0.5 log unit increments beginning with 1 nM. Once a maximum response to noradrenaline had been obtained, the tissues were washed and the bathing fluid was changed every 15 min.

In receptor protection experiments, following the first concentration–response curve to noradrenaline, tissues were exposed to vehicle, noradrenaline (10 μM), yohimbine (10 μM), prazosin (10 μM) or methoxy-idazoxan (10 μM) for 15 min before and during the 30 min exposure to chloroethylclonidine (100 μM) or vehicle. After washing for 60 min, the tissues were exposed to phenoxybenzamine (10 μM) for 15 min. After washing for another 60 min, a concentration–response curve to noradrenaline was repeated. In all cases, responses to noradrenaline obtained in the second (test) concentration–response curve were expressed as a percentage of the maximum response obtained in the first (control) concentration–response curve. Vehicle experiments were experiments in which three additions of vehicle replaced protecting agent, chloroethylclonidine and phenoxybenzamine.

2.2. Radioligand binding studies

The preparation of rat submandibular gland membranes was carried out as described in Connaughton and Docherty (1990) and Smith and Docherty (1992) and rat spleen membranes were obtained by the same methods. The resultant pellets were used immediately or stored at −20°C for later use. Pellets were reconstituted in 5 volumes (submandibular) or 10 volumes (spleen) of incubation buffer.

In saturation experiments employing rat spleen, aliquots of membrane suspension were incubated with various concentrations of [^{3}H]prazosin (specific activity 78 Ci/mmol, NEN) at 25°C (0.1–10 nM; incubation buffer: Tris–HCl, 50 mM; EDTA, 5 mM; pH 7.4 at 25°C). In saturation experiments employing rat submandibular gland, aliquots of membrane suspension were incubated with various concentrations of [^{3}H]yohimbine (specific activity 81 Ci/mmol, NEN) at 25°C (0.5–30 nM; incubation buffer: Tris–HCl, 50 mM; EDTA, 5 mM; pH 7.4 at 25°C). Non-specific binding was determined in the presence of phentolamine (10 μM). Specific binding of [^{3}H]prazosin or [^{3}H]yohimbine was 70–90% of total binding. Assays were terminated by washing with ice-cold incubation buffer, followed by rapid vacuum filtration through Whatman GF/C filters, using a Brandel Cell Harvester. Radioactivity retained on filters was determined by liquid scintillation spectroscopy.

Prior to saturation experiments, membrane preparations of rat spleen or submandibular gland were incubated for 30 min at 37°C with chloroethylclonidine (100 μM) or vehicle, followed by centrifugation twice at $14\,000 \times g$ for 12 min. The resulting pellet was used as described above.

2.3. Drugs

Acetylcholine chloride (Sigma, Poole, UK); chloroethylclonidine (Research Biochemicals International, Natick, MA, USA); cocaine hydrochloride (Sigma); corticosterone (Sigma); methoxy-idazoxan (Research Biochemicals International); (−)-noradrenaline bitartrate (Sigma); phenoxybenzamine hydrochloride (Research Biochemicals); prazosin hydrochloride (gift from Pfizer, Sandwich, UK) and DL-propranolol hydrocholoride (Sigma).

Drugs were dissolved in distilled water, except for corticosterone (100% ethanol) and phenoxybenzamine (tartaric acid, 1 mM).

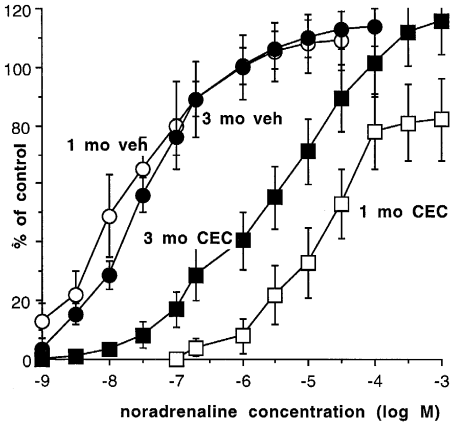

Fig. 1. Effects of chloroethylclonidine (100 μM) or vehicle on contractions to noradrenaline in aorta from immature (1 mo) and adult rats (3 mo). Symbols: vehicle, 1 mo (○); vehicle, 3 mo (●); chloroethylclonidine (100 μM), 1 mo (□); chloroethylclonidine (100 μM), 3 mo (■). Responses to noradrenaline following exposure to chloroethylclonidine 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 8 experiments.

2.4. Statistics

The values are arithmetic mean ± S.E.M. with n the number of experiments. Noradrenaline potency values ($-\log EC_{25}$ or $-\log EC_{50}$) and maximum contractions (% of control) were compared between tissues and were compared with the effects of vehicle using a Student's t-test for unpaired or paired data, where appropriate and by analysis of variance and Dunnett's or Tukey's multiple comparisons test, where appropriate (Graphpad Instat package for MacIntosh). Radioligand binding results were analysed using the Graphpad Prism package for PC.

3. Results

3.1. Rat aorta

In adult rats, noradrenaline produced isometric contractions in the first (control) concentration–response curve with a $-\log EC_{50}$ of 7.45 ± 0.13 ($n = 25$) and a maximum contraction of 0.82 ± 0.09 g ($n = 25$). In vehicle experiments, the $-\log EC_{50}$ of noradrenaline in the second concentration–response curve was 7.47 ± 0.14 ($n = 25$) and the maximum contraction to noradrenaline was 115.0 ± 6.6% of control (see Fig. 1 and Table 1).

In immature rats, noradrenaline produced isometric contractions in the first (control) concentration–response curve with a $-\log EC_{50}$ of 7.87 ± 0.20 ($n = 8$) and a maximum contraction of 0.74 ± 0.08 g ($n = 8$). In vehicle experiments, the $-\log EC_{50}$ of noradrenaline in the second concentration–response curve was 7.90 ± 0.26 ($n = 8$) and the maximum contraction to noradrenaline was 109.4 ± 11.2% of control (see Fig. 1 and Table 1). There were no significant differences between immature and adult rats in any of these parameters.

3.2. Effects of pre-exposure to chloroethylclonidine and phenoxybenzamine

In rat aorta, chloroethylclonidine (100 μM) did not itself produce contractions and chloroethylclonidine pre-exposure failed to reduce the maximum response to noradrenaline but significantly shifted the potency of noradrenaline ($P < 0.001$) (Fig. 1). However, in adult rats, chloroethylclonidine pre-exposure did not produce a clearly parallel shift in the concentration–response curve to noradrenaline, but tended to shift the response to high concentrations of noradrenaline more than the response to low concentrations (Fig. 1). In immature rats, pre-exposure to chloroethylclonidine tended to produce a parallel shift in the noradrenaline concentration–response curve, so that there was no contraction until above noradrenaline 0.1 μM (Fig. 1). The pEC_{50} of noradrenaline was significantly shifted by pre-exposure to chloroethylclonidine in aorta from both adult and immature rats ($P < 0.001$), but there was no significant difference between adult and immature in the pEC_{50} of noradrenaline following chloroethylclonidine (Table 1). However, the pEC_{25} of noradrenaline following chloroethylclonidine was significantly reduced in aorta from immature as compared to adult rats (Table 1). This confirms that chloroethylclonidine causes a more parallel shift of noradrenaline potency in aorta from immature rats.

The effects of pre-exposure to the irreversible antagonist phenoxybenzamine were examined against contractions to noradrenaline. Phenoxybenzamine (10 μM) pre-exposure significantly reduced, and virtually abolished, the contractile response to noradrenaline in both adult and immature rats ($P < 0.001$) (see Fig. 2). However, subsequent to exposure to chloroethylclonidine (100 μM), a component of the response to noradrenaline became resistant to block by phenoxybenzamine (Fig. 2). In adult rats, the concentration–response curve to noradrenaline following chloroethylclonidine was shifted about 1 log unit to the

Table 1
Potency of noradrenaline, expressed as pEC_{25} or pEC_{50} ($-\log$ of the concentration producing 25 or 50% of maximum) and maximum response (% of control maximum) following vehicle or chloroethylclonidine (100 μM) exposure for 30 min in aorta from adult or immature rats

	Immature ($n = 8$)	Adult ($n = 25$)
Vehicle		
Noradrenaline pEC_{50}	7.90 ± 0.26	7.47 ± 0.14
Noradrenaline maximum	109.4 ± 11.2%	115.0 ± 6.6%
Chloroethylclonidine (100 μM)		
Noradrenaline pEC_{50}	5.10 ± 0.30 [a]	5.50 ± 0.19 [a]
Noradrenaline pEC_{25}	5.39 ± 0.31 [b]	6.40 ± 0.22
Noradrenaline maximum	82.1 ± 15.4%	115.7 ± 9.7%

Superscripts denote significance of difference from vehicle (analysis of variance and Tukey multiple comparison test).
[a] $P < 0.001$.
[b] Significantly different from value in adult animals ($P < 0.05$).

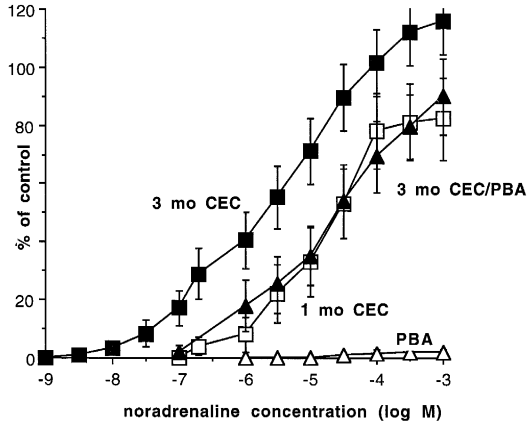

Fig. 2. Effects of chloroethylclonidine (100 μM), phenoxybenzamine (10 μM) and chloroethylclonidine prior to phenoxybenzamine on contractions to noradrenaline in aorta from 1 mo and 3 mo animals. Symbols: phenoxybenzamine (10 μM) (1 mo and 3 mo combined) (△); chloroethylclonidine (100 μM), 1 mo (□); chloroethylclonidine (100 μM), 3 mo (■); phenoxybenzamine (10 μM) following chloroethylclonidine (100 μM), (3 mo) (▲). Responses to noradrenaline following exposure to the various combinations of antagonist and 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.

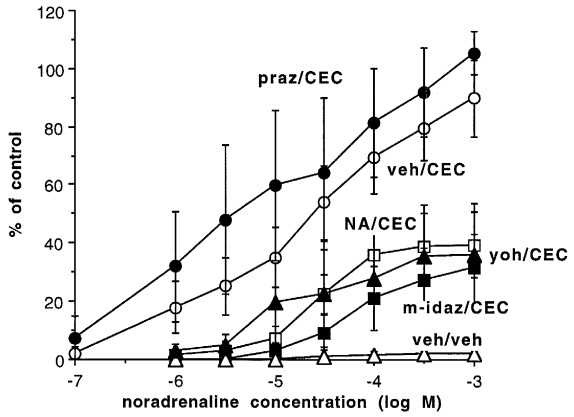

Fig. 3. Effects of chloroethylclonidine and receptor protection prior to chloroethylclonidine on subsequent ability of phenoxybenzamine to abolish contractions to noradrenaline in rat aorta. Symbols: phenoxybenzamine following vehicle (△); phenoxybenzamine following chloroethylclonidine (100 μM) (receptor protection with vehicle) (○); phenoxybenzamine following chloroethylclonidine (100 μM) (receptor protection with prazosin, 10 μM) (●); phenoxybenzamine following chloroethylclonidine (100 μM) (receptor protection with methoxy-idazoxan, 10 μM) (■); phenoxybenzamine following chloroethylclonidine (100 μM) (receptor protection with noradrenaline, 10 μM) (□); phenoxybenzamine following chloroethylclonidine (100 μM) (receptor protection with yohimbine, 10 μM) (▲). Responses to noradrenaline following exposure to the various combinations of antagonist and 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.

right by subsequent exposure to phenoxybenzamine so that the concentration–response curve was virtually superimposed on the concentration–response curve obtained in 1 mo animals following chloroethylclonidine alone (Fig. 2).

3.3. Receptor protection experiments

In preliminary experiments, it was found that exposure to methoxy-idazoxan, noradrenaline, or yohimbine (all 10 μM) for 45 min (vehicle addition after 15 min), followed by 60 min washout, did not affect the ability of subsequent phenoxybenzamine (10 μM) to block contractions to noradrenaline. Prazosin (10 μM) exposure for 45 min followed by 60 min washout did affect the response to phenoxybenzamine, presumably due to incomplete washout: following prazosin and phenoxybenzamine, noradrenaline (100 μM) and above produced contractions so that noradrenaline (1 mM) produced contractions of 21.1 ± 4.5% of control; this effect of noradrenaline was significantly greater than after phenoxybenzamine alone, but was significantly less than the effect of noradrenaline following chloroethylclonidine and phenoxybenzamine (see Fig. 3).

Receptor protection with the α_1-adrenoceptor antagonist prazosin (10 μM) prior to chloroethylclonidine failed to affect the component of the response to noradrenaline resistant to phenoxybenzamine (Fig. 3). Receptor protection with noradrenaline or the α_2-adrenoceptor antagonists yohimbine or methoxy-idazoxan prior to chloroethylclonidine greatly reduced the component of the response to noradrenaline resistant to phenoxybenzamine (Fig. 3). These results suggest that chloroethylclonidine interacts irreversibly with an α_2-adrenoceptor in the rat aorta to make responses to noradrenaline resistant to subsequent α-adrenoceptor blockade by phenoxybenzamine.

3.4. Ligand binding studies

In saturation experiments employing rat spleen membranes (α_{1B}-adrenoceptors), pre-exposure to chloroethylclonidine (100 μM) significantly reduced the maximum number of binding sites (B_{max}) of [^{3}H]prazosin to 21.4 ± 10.2% ($n = 5$) as compared with the effects of vehicle ($P < 0.01$) (see Table 2a), without significantly affecting

Table 2
Effects of pre-exposure to chloroethylclonidine (100 μM) or vehicle for 30 min on affinity (K_D) and maximum binding (B_{max}) of (a) [^{3}H]prazosin at α_{1B}-adrenoceptor ligand binding sites of rat spleen membranes, and (b) [^{3}H]yohimbine at α_{2D}-adrenoceptor ligand binding sites of rat submandibular gland membranes

	Vehicle	Chloroethylclonidine (100 μM)
(a) [^{3}H]prazosin, rat spleen α_{1B}-adrenoceptor		
B_{max} (fmol/mg protein)	184 ± 48	36.0 ± 8.0 [a]
K_D (nM)	0.95 ± 0.44	0.81 ± 0.28
(b) [^{3}H]yohimbine, rat submandibular α_{2D}-adrenoceptor		
B_{max} (fmol/mg protein)	105 ± 19	38.6 ± 22.5 [a]
K_D (nM)	15.2 ± 4.5	12.6 ± 1.7

Values are mean ± S.E.M. from 4 experiments. Superscripts denote values significantly different from these in vehicle experiments.
[a] $P < 0.01$, Student's t-test for paired data.

K_D (Table 2a). In saturation experiments employing rat submandibular gland membranes (α_{2D}-adrenoceptors), pre-exposure to chloroethylclonidine (100 μM) significantly reduced the maximum number of binding sites (B_{max}) of [^{3}H]yohimbine to 34.8 ± 6.3% ($n = 4$) as compared with the effects of vehicle ($P < 0.01$) (see Table 2b), without significantly affecting K_D (Table 2b).

4. Discussion

We have previously investigated the interaction between chloroethylclonidine and α-adrenoceptors in the rat aorta and obtained results inconsistent with a single mode of action for chloroethylclonidine (O'Rourke et al., 1995). Chloroethylclonidine affects the response to low concentrations of noradrenaline in rat aorta as an irreversible α_1-adrenoceptor antagonist, but affects the response to high concentrations of noradrenaline in rat aorta as an irreversible agonist (i.e., an agonist which persists following multiple washout) at α-adrenoceptors. The latter effect of chloroethylclonidine could be prevented by receptor protection with noradrenaline, but not with the α_1-adrenoceptor antagonist prazosin, prior to exposure to chloroethylclonidine. The object of this study was to re-examine the actions of chloroethylclonidine in rat aorta to resolve whether chloroethylclonidine interacts with α-adrenoceptors as an agonist (perhaps by acting at an agonist site) in a way which is more easily prevented by using an agonist (noradrenaline) in receptor protection, or whether chloroethylclonidine acts at normally silent (i.e., undetectable) α_2-adrenoceptors.

Let us first of all consider previous reports of the effects of chloroethylclonidine on rat aorta. In many studies, chloroethylclonidine produced an approximately parallel shift (Oriowo and Bevan, 1990; Tian et al., 1990; Muramatsu et al., 1991; Oriowo and Ruffolo, 1992; Aboud et al., 1993) or a clearly non-parallel shift in the concentration–response curve to α-adrenoceptor agonists (Piascik et al., 1991; O'Rourke et al., 1995), although Han et al. (1990) found a large decrease in the maximum response. Direct contractions to chloroethylclonidine were reported by Muramatsu et al. (1991), but in our studies, clear contractile responses to chloroethylclonidine were observed in very few experiments (O'Rourke et al., 1995).

The contractile response of rat aorta to noradrenaline following chloroethylclonidine pretreatment has been widely reported to be resistant to subsequent α-adrenoceptor antagonism, (Oriowo and Bevan, 1990; Piascik et al., 1991; Oriowo and Ruffolo, 1992; O'Rourke et al., 1995) but sensitive to nifedipine (Piascik et al., 1991; O'Rourke et al., 1995). Even the ability of the irreversible alkylating agent phenoxybenzamine to abolish contractions to phenylephrine or noradrenaline was impaired by chloroethylclonidine pretreatment in rat aorta (Piascik et al., 1991; O'Rourke et al., 1995). In our previous studies, receptor protection against chloroethylclonidine with noradrenaline, but not prazosin, left no component of the response following chloroethylclonidine resistant to the competitive α_1-adrenoceptor antagonist prazosin (O'Rourke et al., 1995). Results obtained with the α_2-adrenoceptor antagonist yohimbine were less clear (see O'Rourke et al., 1995).

Due to this uncertainty as to the actions of the α_2-adrenoceptor antagonist yohimbine, in the present study another approach was taken to the problem of whether chloroethylclonidine acts at an agonist site or an α_2-adrenoceptor site in making contractions to noradrenaline insensitive to subsequent α_1-adrenoceptor antagonists such as prazosin: resistance to the irreversible antagonist phenoxybenzamine was examined. phenoxybenzamine (10 μM) abolished contractions to noradrenaline in rat aorta, but following exposure to chloroethylclonidine, a component of the response to noradrenaline becomes resistant to block by phenoxybenzamine. Receptor protection with noradrenaline or with the α_2-adrenoceptor antagonists yohimbine or methoxy-idazoxan, but not with the α_1-adrenoceptor antagonist prazosin, prior to chloroethylclonidine greatly reduced the component of the response to noradrenaline resistant to phenoxybenzamine (see Fig. 2). These results suggest that chloroethylclonidine interacts with an α_2-adrenoceptor in the rat aorta to make responses to noradrenaline resistant to subsequent α-adrenoceptor blockade. However, this action of chloroethylclonidine at α_2-adrenoceptors is irreversible since subsequent yohimbine (10 μM) did not affect contractions to noradrenaline following exposure to chloroethylclonidine (O'Rourke et al., 1995). Studies of gene expression in rat aorta show the presence of α_{1B}-, α_{1D}- and α_{2A}-adrenoceptors (Ping and Faber, 1993), or α_{1A} and α_{1D} (Rokosh et al., 1994). Hence, the present results suggest that the agonist actions of chloroethylclonidine involve a normally silent (i.e., undetectable) α_{2A}-adrenoceptor (termed α_{2D} in the rat), at which prazosin has low potency (see Smith and Docherty, 1992; Kenny et al., 1994).

In aorta from immature rats, Gurdal et al. (1995) found that noradrenaline was significantly more potent than in aorta from adult rats: responsiveness to a number of vasoconstrictors are reported to decrease in maturation (see Docherty, 1990). In our studies, chloroethylclonidine had greater inhibitory actions against low concentrations of noradrenaline in aorta from immature rats, thus confirming the results of Gurdal et al. (1995). Gurdal et al. (1995) did not examine concentrations of noradrenaline above 10 μM and so did not find any contraction to high concentrations of noradrenaline in their study. However, in the present study, the contractile response to high concentrations of noradrenaline was similar in adult and immature rats. In adult rats, noradrenaline potency following chloroethylclonidine was shifted by phenoxybenzamine treatment to the level of potency found in immature rats following chloroethylclonidine alone. Therefore, it seems that chloroethylclonidine has greater effectiveness as an irreversible blocker of α_1-adrenoceptors in immature rat aorta,

perhaps due to diminished receptor reserve, but has similar agonist actions in combination with high concentrations of noradrenaline. The difference between adult and immature further confirms that the antagonist and agonist actions of chloroethylclonidine are distinct, involving different receptors.

In ligand binding studies of rat spleen, we demonstrated that chloroethylclonidine (100 μM) significantly reduced [^{3}H]prazosin binding to 21% of control, confirming the sensitivity of α_{1B}-adrenoceptors to chloroethylclonidine (see Michel et al., 1993). Since α_2-adrenoceptors may be involved in contractions of rat aorta, given the receptor protection and gene expression information, we also examined the actions of chloroethylclonidine at α_2-adrenoceptor ligand binding sites in rat submandibular gland (α_{2D}-adrenoceptors). The examination of α_{2D}-adrenoceptors in submandibular gland was particularly crucial given that these receptors are reported to be expressed in rat aorta (Ping and Faber, 1993). Chloroethylclonidine (100 μM) was found to bind irreversibly to α_{2D}-adrenoceptors in the concentrations at which it has irreversible silent agonist actions in rat aorta (i.e., irreversible actions which only produce effects in combination with a classical agonist), binding to approximately two-thirds of receptors at that concentration for that time of exposure (30 min). Hence, chloroethylclonidine interacts with α_{2D}-adrenoceptors in the concentrations employed in functional studies.

Finally, we have tried to explain the actions of chloroethylclonidine by using the terms 'silent agonist' and 'irreversible agonist'. These terms are used in a descriptive rather than theoretical sense. We use the term silent agonist since the agonist actions of chloroethylclonidine are usually only seem following addition of noradrenaline: we assume that chloroethylclonidine is bound to the normally undetectable α_2-adrenoceptors but does not activate them sufficiently to produce a contraction. We use the term irreversible agonist to describe chloroethylclonidine simply because its effects persist following washout. Admittedly, irreversible agonism has been demonstrated previously for chloroethylclonidine (see Section 1).

In conclusion, chloroethylclonidine has two major classes of action at α-adrenoceptors (and both can be demonstrated in the rat aorta): irreversible antagonism at α_1-adrenoceptors and irreversible agonism (demonstrable in combination with noradrenaline in rat aorta) at α_2-adrenoceptors.

Acknowledgements

Supported by the Research Committee of the Royal College of Surgeons in Ireland, The Health Research Board (Ireland) and the Irish Heart Foundation. The authors' laboratory is a member of the EU funded Concerted Action EureCa (contract No. BMH1-CT94-1375).

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