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STUDIES ON SOME *para*-SUBSTITUTED CLONIDINE DERIVATIVES THAT EXHIBIT AN α-ADRENOCEPTOR STIMULANT ACTIVITY

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1 α-Adrenoceptor stimulant activity was determined for noradrenaline (NA), clonidine and a series of *para*-substituted derivatives of clonidine on rat aortic strips, a rat brain synaptosome preparation, and anaesthetized pithed rats. The effects on the blood pressure of intraventricular (i.c.v.) injections of *para*-aminoclonidine were also determined in anaesthetized rats.

2 *Para*-substituted derivatives of clonidine (amino-, diethylamino-, ethylamino-, acetamido-, bromoacetamido-, *N*-chloroethyl-*N*-methyl-amino and *N*-β-chloroethyl-*N*-methylaminomethyl-) retain α-adrenoceptor stimulant activity.

3 pD_2 values determined on rat aortic strips were 11.2, 7.67 and 9.05 respectively for *para*-aminoclonidine, clonidine and noradrenaline. The K_i values of these agents, determined on a rat brain synaptosomal preparation with a radioreceptor assay using [^{3}H]-clonidine as ligand, were 1.3, 8.0 and 23 nM respectively for *para*-aminoclonidine, clonidine and NA. When given by i.c.v. injection in rats, *para*-aminoclonidine lowered the blood pressure.

4 *N*-β-chloroethyl-*N*-methylaminomethylclonidine is an alkylating agent with an unusual agonist activity. It elicits contractions of the rat aorta that persist despite repeated washing.

5 α-Adrenoceptor affinities are discussed in relation to their structural features.

Introduction

We have studied the structural and electronic characteristics of clonidine in relation to α-receptors (Wermuth, Schwartz, Leclerc, Garnier & Rouot, 1973; Rouot, Leclerc, Wermuth, Miesch & Schwartz, 1977) and synthesized 22 structural analogues and correlated their peripheral α-adrenoceptor stimulant effects with their steric and electronic parameters (Rouot, Leclerc, Wermuth, Miesch & Schwartz, 1976). Substitution by Cl, CH_3 and OH in the *para* position did not greatly alter the α-adrenoceptor stimulant activity. It was therefore thought that the introduction of an alkylating group in this position, might produce α-adrenoceptor stimulants with prolonged activity. We describe here the pharmacological effects of some of these *para*-substituted analogues.

Methods

Rat aortic strips

Helically cut strips of rat aorta, 1.5 to 2 cm long and 3 to 4 mm wide, were prepared as described by Liebau, Distler & Wolff (1966). Preparations were suspended in 20 ml baths, containing Krebs-Henseleit solution, kept at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. They were set up at a resting tension of 2 g and allowed to stabilize for approximately 2 h before the experiment. Contractions of rat aortic strips were elicited by increasing cumulative amounts of noradrenaline (NA), then, after 3 washings and one hour's rest, by progressive additions of either clonidine or one of the eight clonidine derivatives. The contractions were recorded isometrically on a polygraph (Gilson M5P) by means of a force displacement transducer (Ugo Basile). Phentolamine was used to test for selective antagonism of α-receptor mediated contractions. Dose-response curves for noradrenaline, clonidine and all substituted derivatives except 8, were determined before and after adding phentolamine. Each preparation was tested with only one concentration of phentolamine (10^{-8}, 3×10^{-8} or 3×10^{-7} mol/l) which was added to the bath 30 min before the second assay. For compound 8, because of its long duration of action, dose-response curves were

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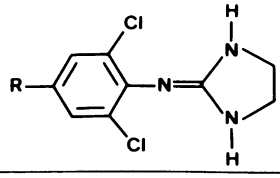
established on eight aortic strips without phentolamine and on ten other strips in the presence of phentolamine. The pD_2 values for the agonists and the pA_2 values for phentolamine were determined by the method of Ariens & Van Rossum (1957). The standard error for pA_2 was calculated according to Miesch, Turlot, Ehrhardt & Schwartz (1977). The intrinsic activity was expressed as the ratio of the maximum response to each compound to the maximum response to noradrenaline (Ariens, 1954).

Binding assays

Studies were performed on a rat brain synaptosomal preparation (De Feudis & Somoza, 1977). Inhibition of [^{3}H]-clonidine binding by competing agents was assessed by the technique of U'Prichard, Greenberg & Snyder (1977). Inhibition was measured at 7 drug concentrations (in duplicate) in the presence of [^{3}H]-clonidine (5 nM). IC_{50} is the concentration of competing agent required for a 50% inhibition of [^{3}H]-clonidine

Table 1 *In vitro* and *in vivo* α-adrenoceptor stimulant activities of *para*-substituted clonidine derivatives, clonidine and noradrenaline



	R	$pD_2 \pm$ s.d.	*Rat aorta* Intrinsic activity	$pA_2 \pm$ s.d.	*Pithed rat* $pD'_2 \pm$ s.d.	*Inhibition of* [^{3}H]-*clonidine binding* $K_i \pm$ s.e. mean
1	O_2N—	4.68 ± 0.08 (5)	0.42	9.31 ± 0.38 (4)	5.26 ± 0.07 (5)	> 500 (6)
2	H_2N—	11.18 ± 0.19 (7)	0.72	8.38 ± 0.15 (4)	8.33 ± 0.03 (6)	1.3 ± 0.3 (6)
3	$(CH_3)_2N$—	6.59 ± 0.15 (4)	0.61	8.71 ± 0.34 (8)	7.22 ± 0.04 (6)	20.5 ± 4 (6)
4	CH_3—CH_2—NH—	7.46 ± 0.56 (7)	0.76	8.48 ± 0.29 (6)	7.25 ± 0.03 (6)	29 ± 5 (6)
5	CH_3—C(=O)—NH—	5.87 ± 0.47 (6)	0.18	8.97 ± 0.13 (4)	6.35 ± 0.05 (5)	130 ± 30 (6)
6	Br—CH_2—C(=O)—NH—	5.06 ± 0.68 (6)	0.50	8.46 ± 0.37 (4)	5.58 ± 0.03 (6)	> 500 (6)
7	Cl—CH_2—CH_2—$N(CH_3)$—	6.42 ± 0.29 (4)	0.70	8.52 ± 0.35 (4)	6.90 ± 0.06 (5)	35 ± 6 (6)
8	Cl—CH_2—CH_2—$N(CH_3)$—CH_2—	6.34 ± 0.34 (8)	0.75	8.53 ± 0.14 (10)	5.26 ± 0.07 (15)	1650 ± 400 (6)
Clonidine H		7.67 ± 0.40 (12)	0.73	8.43 ± 0.17 (4)	7.58 ± 0.04 (8)	8 ± 2 (6)
Noradrenaline		9.05 ± 0.50 (26)	1	8.66 ± 0.20 (8)	8.12 ± 0.06 (8)	23 ± 2 (6)

PD_2 values ± s.d. determined on rat aorta; pA_2 values for phentolamine obtained with each *para*-substituted clonidine derivatives; $pD'_2 \pm$ s.d. in pithed rats and $K_i \pm$ s.e. mean on rat synaptosomal preparations. Figures in parentheses show numbers of experiments.

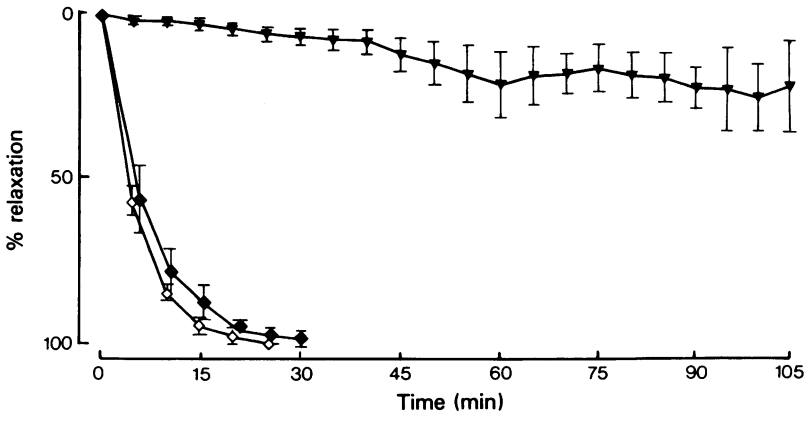


Figure 1 Percentage relaxation of rat aorta, at 5 min intervals after inducing contractions with clonidine (◆), noradrenaline (◇) or compound 8 (▼). The preparations were washed every 5 min. Each point is the mean of eight observations; vertical bars show the s.e. means.

specific binding. IC_{50} values were converted to apparent K_i values (inhibition constant) according to the equation $K_i = IC_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand and K_D, the equilibrium dissociation constant of [^{3}H]-clonidine.

Pressor effect in anaesthetized pithed rats

Rats anaesthetized with sodium pentobarbitone (50 mg/kg) were pithed according to the method of Shipley & Tilden (1947) but were not treated with atropine. Mean blood pressure was measured with a transducer (Statham P23 Db) connected to the carotid artery and recorded on a Gilson M5P polygraph. A jugular vein was cannulated for intravenous injections and dose-response curves were established. Drugs were injected at intervals of not less than 5 min, or when the blood pressure had returned to its pre-dose value. For the most active drugs, the doses ranged from 10^{-9} to 3×10^{-7} mol/kg, and for the less active ones, from 3×10^{-7} to 10^{-4} mol/kg. Doses of compound 8 and clonidine, which increased blood pressure by 30 mmHg, were injected intravenously into pithed rats to compare their durations of action.

Pressor activity *in vivo* is expressed as pD'_2 since the pD_2 defined by Ariens & Van Rossum (1957) applies only at the equilibrium possible in *in vitro* experiments.

Intracerebroventricular (i.c.v.) injections

Rats were anaesthetized and a hole drilled in the skull, 2.5 mm lateral and 0.5 mm posterior to the bregma for direct injection into the lateral brain ventricle. The needle was placed at a depth of 4.5 mm from the top of the skull (Finch, Harvey, Hicks & Owen, 1978). Twenty minutes' rest was allowed before the drug, dissolved in 10 μl of saline, was injected. At the end of each experiment, 10 μl of Evans Blue dye solution was injected and the brain was examined *post mortem* to check the location of the injection into the lateral ventricle. Saline controls were studied similarly. Blood pressure was measured from the carotid artery as described above.

Results

The structures of and the effects produced by several *para*-substituted derivatives of clonidine are summarized in Table 1. *Para*-aminoclonidine, 2, has an outstanding affinity for α-adrenoceptors. On the rat aorta this compound has a pD_2 of 11.18, whereas that of clonidine is 7.67 and that of noradrenaline is 9.05. Compounds 6 and 7 act like clonidine, but are less potent. Although less potent than clonidine ($pD_2 = 6.34$), compound 8 was exceptional in that it produced contractions of the aorta that persisted even after 20 washes (Figure 1).

In the presence of phentolamine, the dose-response curves for the clonidine derivatives were shifted to the right. The pA_2 values of phentolamine were similar whether tested against *para*-aminoclonidine, compound 8, clonidine or noradrenaline.

Brain synaptosomes showed higher affinity for *para*-aminoclonidine (K_i = 1.3 nM) than for clonidine (K_i = 8 nM).

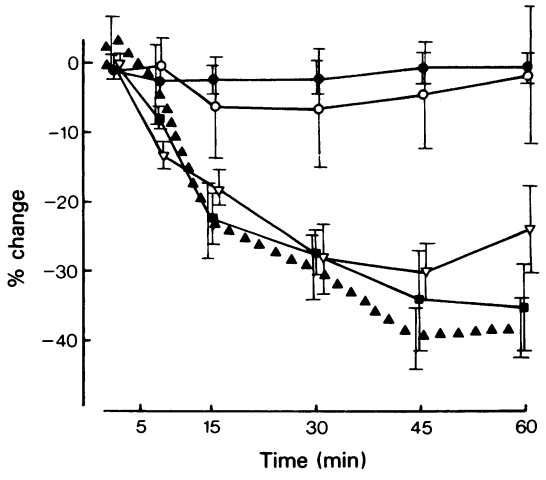


Figure 2 Percentage change in systemic arterial blood pressure after i.c.v. injections in anaesthetized rats of saline in controls (●, $n = 6$) or of *para*-aminoclonidine 2 at doses of 3 μg/kg (○, $n = 6$), 10 μg/kg (▽, $n = 7$), 30 μg/kg (■, $n = 6$), 100 μg/kg (▲, $n = 6$). Each point is the mean of six observations, except for the dose of 10 μg/kg for which 7 observations were made; vertical bars show the s.e. means. Initial blood pressure was 99 mmHg ± 3 ($n = 31$).

High affinity was also encountered in pithed rats. The pD'_2 was for *para*-aminoclonidine 8.33, for clonidine 7.58 and for noradrenaline 8.12. The activities of compounds 6 and 7 were much lower. Compound 8, (pD'_2 5.26) at a dose of 1.5×10^{-6} mol/kg, raised blood pressure by 30 mmHg, and this increase lasted more than 60 min; clonidine at a dose of 1×10^{-8} mol/kg, increased blood pressure to the same extent but its action was over in 5 min.

By i.c.v. injection 10 μg/kg *para*-aminoclonidine showed a marked hypotensive effect in anaesthetized rats (Figure 2). Comparable effects were, produced by clonidine at similar dosages during the 1 h observation period and by compounds 4 and 8 at doses in the range of 0.3 to 1 mg/kg. Compounds 3, 5, 6 and 7 had no effect on blood pressure at i.c.v. doses of up to 3 mg/kg.

Discussion

Para-substitution of the aromatic ring of clonidine influenced the affinity for the α-adrenoceptors but never suppressed it. Affinity for the α-receptors decreased with increasing size of the substituents on the nitrogen atom; the ranking order of potency, $N(Me)_2 > NH\ Et > NH_2$, corresponds with the order of their steric values (Verloop & Tipker, 1977), although size is probably not the only factor involved. Affinity is maximal with the *para*-amino derivative, 2. Its K_i measured on rat brain synaptosome preparations was as high as 1.3 nM. Its unusually powerful stimulant effect on α-adrenoceptors has been demonstrated by intravenous and i.c.v. injection in rats and on rat aortic strips, the pA_2 values for phentolamine being similar whether contractions of the aortic strips were caused by compound 2, clonidine or noradrenaline.

Although it is accepted that clonidine acts presynaptically to inhibit transmitter release (Starke & Altman, 1973), it may act postsynaptically to stimulate α-adrenergic inhibitory neurones in the central nervous system (Van Zwieten, 1973). Since 6-hydroxydopamine does not alter clonidine-binding affinity in brain synaptosome membranes (U'Prichard, Bechtel, Rouot & Snyder, 1979), it would appear that in this preparation clonidine binds to postsynaptic sites. Similar results have been obtained for *para*-aminoclonidine (Rouot & Snyder, 1979).

Compounds 6 and 7 are expected to be weak alkylating agents but they did not produce irreversible or prolonged pharmacological effects. The low alkylating potency of the β-chloroethyl-methylaniline, 7, would be expected as a consequence of the electron-attractive effect of the adjacent dichlorophenyl group which reduces the nitrogen basicity. In the bromoacetamide, 6, the basicity is reduced still more by the carbonyl function adjoining the nitrogen. In both cases, the formation of the aziridinium ion, which is the real reactive species (Triggle, 1976) is prevented. On the other hand, in compound 8, a methylene link interrupts the transmission of the electron-attracting effect of the aromatic nucleus, and the β-halogenoethylamine reactivity is thus maintained. Compound 8 does indeed behave as an irreversible alkylating agent. The duration of its action on rat aorta is similar to that of 2-halogenoethylamine α-adrenoceptor antagonists (Howell & Morgan, 1976), and it is certainly an alpha-adrenoceptor stimulant, since the same pA_2 values for phentolamine were obtained with noradrenaline and compound 8 as agonists. Moreover, its binding characteristics tally with this interpretation.

Para-aminoclonidine, 2, by virtue of its high affinity, and compound 8 with its irreversible action, may become useful agents in investigating pre- and postsynaptic α-receptors. As far as we know, compound 8 is the first example of an α-agonist with an irreversible effect.

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