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Selectivity of Some *Alpha* Adrenoceptor Agonists for Peripheral *Alpha*-1 and *Alpha*-2 Adrenoceptors in the Normotensive Rat[1]

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ABSTRACT

van Meel, J. C. A., A. de Jonge, P. B. M. W. M. Timmermans and P. A. van Zwieten: *Selectivity of some alpha adrenoceptor agonists for peripheral alpha-1 and alpha-2 adrenoceptors in the normotensive rat.* J. Pharmacol. Exp. Ther. **219:** 760–767, 1981.

The selectivity of the *alpha* adrenoceptor agonists (−)-phenylephrine, methoxamine, cirazoline, B-HT 920, B-HT 933 and UK-14,304 with respect to peripheral and central *alpha*-1 and *alpha*-2 adrenoceptors was determined in the normotensive rat. In pithed rats, the pressor responses to (−)-phenylephrine, methoxamine and cirazoline were most sensitive toward antagonism by prazosin, but not by yohimbine. The vasopressor effects of B-HT 920, B-HT 933 and UK-14,304 were strongly inhibited by yohimbine and were not affected by prazosin. In pithed rats, B-HT 920, B-HT 933 and UK-14,304 dose-dependently diminished the increase in heart rate produced by electrical stimulation of the cardioaccelerator sympathetic nerve fibers. (−)-Phenylephrine, methoxamine and cirazoline failed to modify this parameter. B-HT 920, B-HT 933 and UK-14,304 are full agonists of cardiac presynaptic *alpha*-2 adrenoceptors in contrast to clonidine. The results demonstrate that (−)-phenylephrine, methoxamine and cirazoline are selective agonists of *alpha*-1 adrenoceptors, whereas B-HT 920, B-HT 933 and UK-14,304 preferentially stimulate *alpha*-2 adrenoceptors. The *in vitro* affinity of the agonists for [^{3}H]prazosin- and [^{3}H]-clonidine-labeled *alpha*-1 and *alpha*-2 adrenoceptors in rat isolated cerebral membranes generally corresponded with the characterization made by pharmacological means. However, UK-14,304 and cirazoline were found to be relatively potent in displacing [^{3}H]prazosin and [^{3}H]clonidine, respectively. It is concluded that UK-14,304 and B-HT 920, in particular, are potent and most selective, directly acting agonists of *alpha*-2 adrenoceptors. Cirazoline is an effective and selective agonist of *alpha*-1 adrenoceptors. These drugs may be useful tools for the identification and classification of *alpha* adrenoceptors.

The distinct differences in drug selectivity and function between presynaptic *alpha* adrenoceptors at postganglionic sympathetic neurons and postsynaptic *alpha* adrenoceptors at effector cells have become evident. The prefixes *alpha*-1 and *alpha*-2 have been proposed for post- and prejunctionally located *alpha* adrenoceptors, respectively (Langer, 1974). At present, the uniform nomenclature is exclusively based upon the relative activity for agonists and relative affinity for antagonists and is therefore independent of anatomical location (Berthelsen and Pettinger, 1977; Wikberg, 1978; Starke and Langer, 1979).

In general, *alpha* adrenoceptors of the *alpha*-1 type are most effectively activated by (−)-phenylephrine and antagonized by prazosin. The *alpha* adrenoceptor classified as *alpha*-2 is preferentially stimulated by clonidine and tramazoline and inhibited by yohimbine and rauwolscine. Radioligand-binding studies have led to a comparable subclassification (Wood *et al.*, 1979).

Based on the relative affinities of the compounds mentioned above, increasing experimental evidence supports the existence of a mixed population of postsynaptic *alpha*-1 and *alpha* -2 adrenoceptors in vascular smooth muscle *in vivo* of the pithed rat (Drew and Whiting, 1979; Timmermans *et al.*, 1979; Docherty and McGrath, 1980; Kobinger and Pichler 1980; Timmermans and van Zwieten, 1980a,b; Yamaguchi and Kopin, 1980) and pithed dog (Constantine *et al.*, 1980) as well as in the autoperfused hindlimb of rabbits (Madjar *et al.*, 1980) and dogs (Langer *et al.*, 1981). Apart from the commonly known postsynaptic *alpha*-1 adrenoceptor, the postsynaptic *alpha*-2 adrenoceptor site identified in vascular tissue has close similarity to the presynaptic *alpha*-2 adrenoceptor characterized in the rabbit isolated pulmonary artery with respect to the relative affinities for agonists and antagonists (Timmermans *et al.*, 1980b). Both types of *alpha* adrenoceptors induce vasoconstriction upon activation.

By using the relatively simple model of the pithed rat (Timmermans *et al.*, 1980b), it is possible to study both *alpha*-1 and *alpha*-2 adrenoceptors in the vasculature and to determine selectivities of *alpha* adrenoceptor agonists. The present paper reports on six agonists, *viz.* (−)-phenylephrine, methoxamine, cirazoline (LD 3098), B-HT 920, B-HT 933 and UK-14,304. The selection of these compounds was based upon the results of

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[1] Preliminary data were presented at the Joint Meeting of the Scandinavian and German Pharmacological Societies, September 16–18, 1980, Lübeck-Travemünde (van Meel *et al.*, 1980).

preliminary studies. The structures of the last four, rather unknown, agents have been depicted in figure 1. Additionally, their pharmacological profile was examined with respect to their agonistic effect on cardiac presynaptic *alpha*-2 adrenoceptors of the rat. Moreover, their *in vitro* affinity was measured for central *alpha*-1 and *alpha*-2 adrenoceptors labeled by [^{3}H]prazosin and [^{3}H]clonidine, respectively, in rat brain in order to further characterize the compounds.

Methods

Agonistic effect on postsynaptic *alpha*-1 and *alpha*-2 adrenoceptors in the intact circulatory system of the pithed rat. Male normotensive Wistar rats (200–250 g) were pithed by introducing a blunt needle into the spinal canal *via* the orbit under hexobarbitone-sodium anesthesia (150 mg/kg i.p.). Subsequently, the animals were artificially ventilated with room air *via* a tracheal cannula connected to a positive pressure pump (Braun-Melsungen). Rectal temperature was maintained at approximately 37°C. The right jugular vein and common carotid artery were cannulated for the administration of drugs and the recording of arterial pressure. The latter was recorded continuously (Statham P23 Db transducer) and displayed on a Hellige-HE 19 device. Heart rate was obtained from the arterial pulse wave by means of a HSE EKA-puls IC rate meter. Heparin (about 1000 I.U./kg) was injected i.v. During a 15-min period of equilibration, cardiovascular parameters were allowed to stabilize. Agonists (in a volume of 0.5 ml/kg) were injected i.v. 15 min after saline (1 ml/kg) or 15 min after i.v. pretreatment with the *alpha*-1 adrenoceptor antagonist prazosin (0.1 mg/kg) or the *alpha*-2 sympatholytic drug yohimbine (1 mg/kg) or after a combination of the *alpha* adrenoceptor blocking agent and (−)-propranolol (0.5 mg/kg). These experiments were repeated after depletion of catecholamine stores by reserpine (5 mg/kg i.p.) 48 and 24 hr before the start of the study. The increase in diastolic pressure and the effect on heart rate were measured for single doses of the agonists administered i.v. in a random order. Four doses were injected per animal. Full recovery from the pressor effects to preinjection values was ensured between the subsequent doses.

Effects on cardiac presynaptic *alpha*-2 adrenoceptors. After anesthesia with diethyl ether, normotensive male Wistar rats (200–250 g) were pithed as described above. The pithing rod was coated with enamel in such a way as to allow selective electrical stimulation of the preganglionic cardioaccelerator nerves. An indifferent electrode was placed in the dorsum. A femoral vein was cannulated for the administration of drugs. Arterial pressure and heart rate were recorded continuously (see above). Heparin (about 1000 I.U./kg) was injected i.v. Before the start of the experiments, all the animals were given atropine and *d*-tubocurarine i.v. (1 mg/kg of each) and bilateral vagotomy was performed. Continuous stimulation of the sympathetic nerves to the heart was achieved by means of rectangular pulses (0.2 Hz; 2 msec; 50 V) obtained from a HSE stimulator. The position of the pithing rod was adjusted to obtain a 70 to 80 beats/min increase in heart rate, without influencing arterial pressure. The compounds were injected cumulatively in a volume of 0.5 ml/kg. The agonistic potency of the drugs on cardiac presynaptic *alpha*-2 adrenoceptors was quantified as percentage of inhibition of stimulation-induced tachycardia. ED_{50} values were calculated from log dose-response curves.

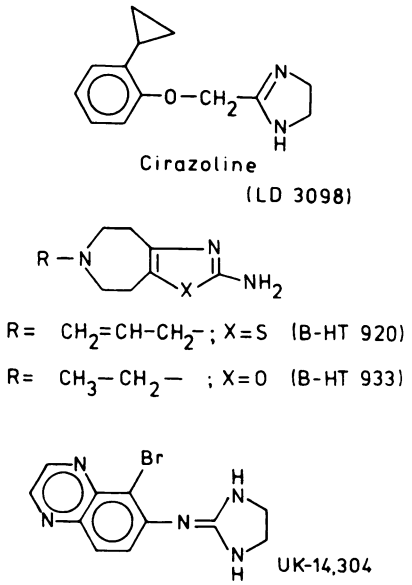

Fig. 1. Structural formulae of cirazoline (LD 3098), B-HT 920, B-HT 933 and UK-14,304.

Affinity for central *alpha*-1 and *alpha*-2 adrenoceptors. Cerebral membranes from male normotensive Wistar rats (200–250 g) were prepared as reported by Greenberg *et al.* (1976) and U'Prichard *et al.* (1977). For this purpose, the animals were decapitated and their brains (minus cerebella) were homogenized in 20 vol (w/v) of ice-cold 50 mM Tris/HCl buffer (pH = 7.7 at 25°C). After centrifugation at 50,000 × *g* for 10 min at 4°C the pellet was rehomogenized in fresh cold buffer and the procedure was repeated. The final tissue pellet was suspended in Tris/HCl buffer. The protein concentration, as determined according to the method of Lowry *et al.* (1951), amounted to 1 mg/ml for the [^{3}H]prazosin and 4 mg/ml for the [^{3}H]clonidine binding assays. Aliquots of 500 μl were incubated at 25°C for 60 min with [^{3}H]clonidine (specific activity, 26.7 Ci/mmol; 0.1–20 nM) or [^{3}H]prazosin (specific activity, 33 Ci/mmol; 0.05–7 nM). The specific binding of [^{3}H]clonidine was determined as the excess over blanks containing 10 μM of (−)-norepinephrine. Accordingly, 2 μM of phentolamine was used to define the specific binding of [^{3}H]prazosin. In the displacement studies, the inhibition of the specific binding of [^{3}H]clonidine (0.4 nM) and [^{3}H]prazosin (0.2 nM) was examined in the presence of various concentrations of the agonists. Incubations (total volume 1 ml) were terminated by rapid vacuum filtration through Whatman GF/B filters.

Filters were washed by three 5-ml portions of ice-cold Tris/HCl buffer, left to solubilize in 10 ml of Instagel for 24 hr and counted for radioactivity at 35 to 40% efficiency.

Drugs and chemicals used. B-HT 920 dihydrochloride and B-HT 933 dihydrochloride (gifts: Thomae, Biberach, FRG); cirazoline hydrochloride (LD 3098) (gift: Synthélabo, Paris, France); clonidine hydrochloride and [^{3}H]clonidine hydrochloride (specific activity 26.7, Ci/mmol; gifts: Boehringer Ingelheim Ltd., Elmsford, NY); heparin (NOVO, Copenhagen, Denmark); hexobarbitone-sodium (Bayer, Leverkusen, FRG); Instagel (Packard-Becker, Groninger, The Netherlands); (±)-methoxamine hydrochloride (Burroughs Wellcome and Company, Research Triangle Park, NC); (−)-norepinephrine, (−)-phenylephrine hydrochloride and yohimbine hydrochloride (Sigma Chemical Company, St. Louis, MO); phentolamine hydrochloride and reserpine (Serpasil; gifts: Ciba-Geigy Corp., Summit, NJ); prazosin hydrochloride, [^{3}H]prazosin hydrochloride (specific activity, 33 Ci/mmol) and UK-14,304 (D)-(+)-tartrate (gifts: Pfizer Inc., New York); (−)-propranolol hydrochloride (gift: Imperial Chemical Industries, Wilmslow, Cheshire, England); and rods with enamel coating were kindly provided by Keramiek en Email Fabriek (Coevorden, The Netherlands). In animal experiments, the drugs were dissolved in saline, except for prazosin which was solubilized in hot distilled water. Doses of drugs quoted are in terms of the salts.

Statistical analysis. Results are expressed as mean ± S.E.M. Analysis of variance and Student's *t* test for paired and unpaired observations were used to determine statistical significance.

Results

Agonistic effect on postsynaptic *alpha*-1 and *alpha*-2 adrenoceptors in the intact circulatory system of the pithed rat. Pithed, normotensive rats had initial mean values of diastolic pressure and heart rate before any drug treatment

of 45.3 ± 0.2 mm Hg and 321 ± 2 beats/min, respectively (n = 203). Log dose-response curves were constructed for the maximal increase in diastolic pressure (millimeters of mercury) brought about by the *alpha* adrenoceptor stimulating drugs (−)-phenylephrine, methoxamine, cirazoline, B-HT 920, B-HT 933 and UK-14,304 after i.v. injections (fig. 2). Cirazoline (ED_{50} = 4.5 × 10^{-9} mol/kg) was about 6 times more potent than (−)-phenylephrine (ED_{50} = 2.6 × 10^{-8} mol/kg) and 38 times more effective than methoxamine (ED_{50} = 1.7 × 10^{-7} mol/kg). Cirazoline, phenylephrine and methoxamine all reached a comparable maximal hypertensive response of approximately 120 mm Hg. In contrast, the maximal pressor effects attained by UK-14,304, B-HT 920 and B-HT 933 amounted to 80 to 90 mm Hg. In addition, the slopes of these log dose-response curves were less steep than those of the former three agonists. UK-14,304 (ED_{50} = 2.8 × 10^{-8} mol/kg) was found 2.5 times more active than B-HT 920 (ED_{50} = 7.0 × 10^{-8} mol/kg) and about 37 times more potent than B-HT 933 (ED_{50} = 1.0 × 10^{-6} mol/kg). (−)-Phenylephrine in the amounts administered and B-HT 933 in the higher doses (11.8 and 39.3 × 10^{-5} mol/kg) caused tachycardia in a dose-dependent manner. The other drugs had no marked effects on cardiac frequency.

After i.v. treatment with prazosin (0.1 mg/kg) the log dose-response curves of (−)-phenylephrine (fig. 3), methoxamine (fig. 4) and cirazoline (fig. 5) were all displaced to the right in a parallel fashion (36-, 11- and 10-fold, respectively). However, yohimbine (1 mg/kg) injected i.v. 15 min before only slightly attenuated the pressor responses to (−)-phenylephrine (displacement: 2.1; fig. 3) and to methoxamine (displacement: 1.7; fig. 4) and failed to significantly reduce the increase in diastolic pressure elicited by cirazoline (fig. 5). (−)-Propranolol (0.5 mg/kg i.v.) caused a potentiation of the pressor effects of the lower doses of (−)-phenylephrine without affecting the maximal response (fig. 3). In pithed rats which had received (−)-propranolol, comparable results were obtained for methoxamine. This

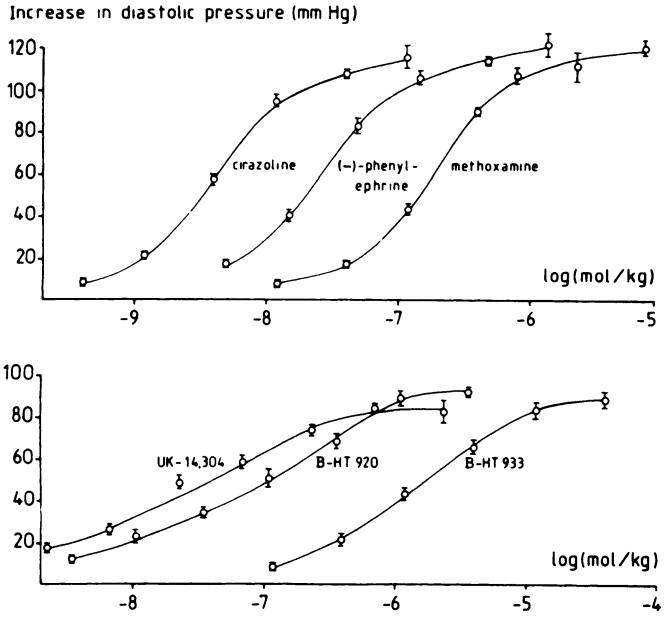

Fig. 2. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of cirazoline, (−)-phenylephrine and methoxamine (upper part) and of UK-14,304, B-HT 920 and B-HT 933 (lower part). The data are given as the mean ± S.E.M. (n = 5–7).

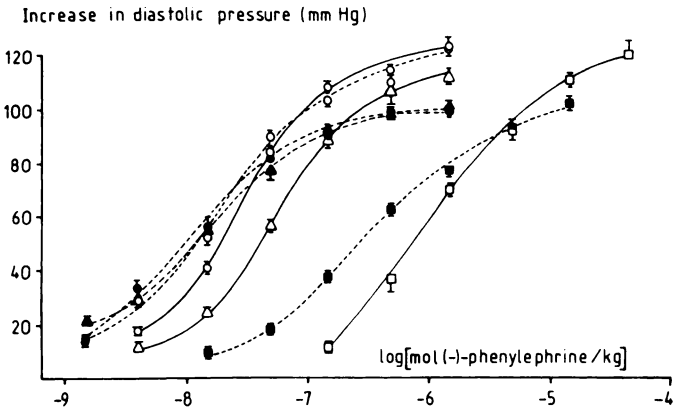

Fig. 3. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of (−)-phenylephrine. O——O, 15 min after saline; △——△, 15 min after yohimbine (1 mg/kg); □——□, 15 min after prazosin (0.1 mg/kg); O- - -O, 15 min after saline in (−)-propranolol (0.5 mg/kg)-treated pithed rats; ●- - -●, 15 min after saline in (−)-propranolol (0.5 mg/kg)- and reserpine-treated pithed rats; ▲- - -▲, 15 min after yohimbine (1 mg/kg) in (−)-propranolol (0.5 mg/kg)- and reserpine-treated pithed rats; ■- - -■, 15 min after prazosin (0.1 mg/kg) in (−)-propranolol (0.5 mg/kg)- and reserpine-treated pithed rats. Symbols represent mean values ± S.E.M. (n = 5–7).

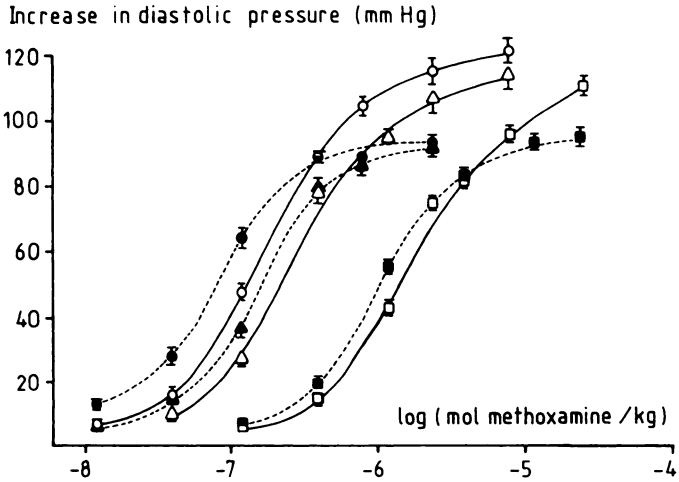

Fig. 4. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of methoxamine. For explanation of symbols see the legend to figure 3. Means ± S.E.M. (n = 5–7).

dose of (−)-propranolol did not influence the log dose-response curve of cirazoline. The tachycardia produced by (−)-phenylephrine was reduced after (−)-propranolol. The depletion of the catecholamine stores by reserpine depressed the maximal plateau of activity of (−)-phenylephrine and methoxamine from about 120 mm Hg (see Fig. 2) to approximately 90 to 100 mm Hg, but had no effect on the maximal increase in diastolic pressure reached by cirazoline. Pretreatment with reserpine reduced, but not eliminated, the positive chronotropic action of (−)-phenylephrine. A combination of reserpine and (−)-propranolol showed the combined effects of the individual treatments, *viz.* the pressor responses to (−)-phenylephrine and methoxamine were potentiated in the lower dose range and the maximally reachable increase in diastolic pressure was reduced, when compared with nonpretreated pithed, normotensive rats

(figs. 3 and 4). The combined treatment with reserpine and (−)-propranolol caused no substantial alteration of the pressor effects of cirazoline (fig. 5). After the combination of reserpine and (−)-propranolol, the displacement of the log dose-response curve to (−)-phenylephrine, methoxamine and cirazoline by prazosin (0.1 mg/kg) amounted to 28-, 13- and 16-fold, respectively. Under these conditions, no shift was noticed for the dose-response curve of (−)-phenylephrine after yohimbine (1 mg/kg; fig. 3). The same combined treatment of reserpine and (−)-propranolol resulted in similar slight displacements of the log dose-response characteristics of methoxamine (fig. 4) and cirazoline (fig. 5) after yohimbine (1 mg/kg), compared to those measured after this antagonist in nonpretreated pithed rats.

In untreated pithed, normotensive rats the previous administration of yohimbine (1 mg/kg) caused parallel shifts to the right of the log dose-response curves of B-HT 920 (fig. 6), B-HT 933 (fig. 7) and UK-14,304 (fig. 8), resulting in 20-, 16- and 5.2-fold displacements, respectively. Prazosin (0.1 mg/kg) did not significantly modify the pressor responses to either of these three agonists (figs. 6–8). (−)-Propranolol (0.5 mg/kg) did not significantly influence the log dose-pressor effect curves of B-HT 920, B-HT 933 and UK-14,304. In the presence of (−)-propranolol, the reserpine-treated animals showed a small but insignificant ($P > .05$) increase in the sensitivity toward the pressor responses to B-HT 920, B-HT 933 and UK-14,304. The curves had slightly but significantly ($P < .05$) depressed maximal responses, when compared to nonpretreated control pithed rats. In catecholamine-depleted and (−)-propranolol-administered animals, the tachycardia induced by the higher doses of B-HT 933 (see above) was totally abolished. The combined pretreatment with (−)-propranolol and reserpine slightly diminished the inhibitory effect of yohimbine (1 mg/kg) on the vasopressor responses to B-HT 920 (displacement: 14; see Fig. 6) and B-HT 933 (displacement: 8; see fig. 7). No influence was found on the yohimbine-shifted rises in diastolic pressure of UK-14,304 (displacement: 5.5; see fig. 8). (−)-Propranolol and reserpine also had no effect on the log dose-response curves obtained in the presence of prazosin (0.1 mg/kg).

Effects on cardiac presynaptic *alpha*-2 adrenoceptors. After pithing and equilibration, mean heart rate amount to 314 ± 3 beats/min ($n = 42$). Electrical stimulation of the preganglionic sympathetic nerves to the heart increased cardiac frequency by 78 ± 1 beats/min ($n = 42$). When the tachycardia was fully developed (about 5 min), the effects of the *alpha* adrenoceptor agonists (−)-phenylephrine, methoxamine, cirazoline, B-HT 920, B-HT 933 and UK-14,304 were tested against the cardioaccelerator response to electrical stimulation by injecting them i.v. in a cumulative manner. Dose-response curves

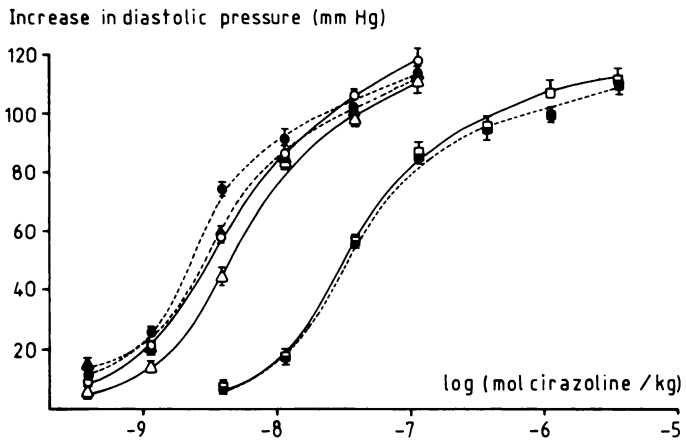

Fig. 5. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of cirazoline. For explanation of symbols see the legend to figure 3. Means ± S.E.M. ($n = 5–7$).

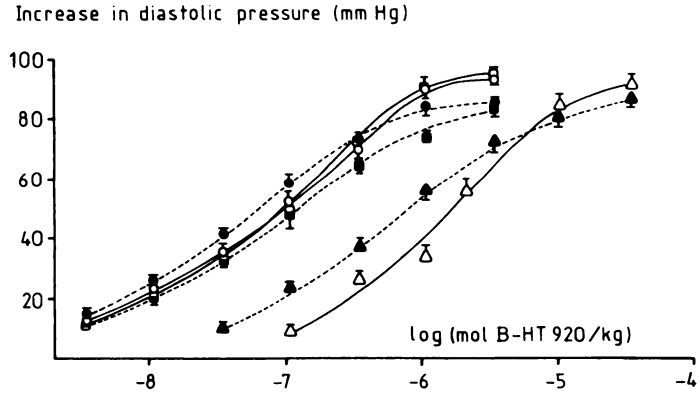

Fig. 6. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of B-HT 920. For explanation of symbols see the legend to figure 3. Means ± S.E.M. ($n = 5–7$).

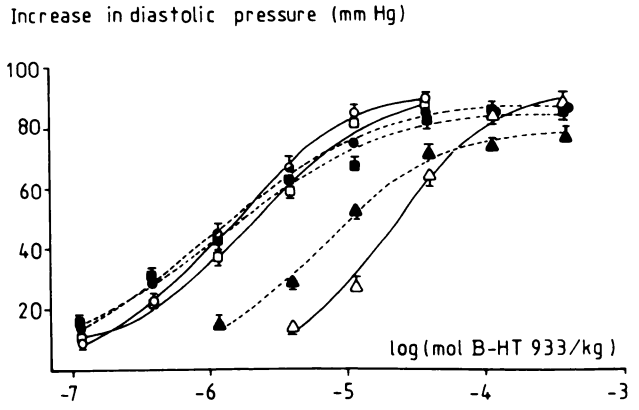

Fig. 7. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of B-HT 933. For explanation of symbols see the legend to figure 3. Means ± S.E.M. ($n = 5–7$).

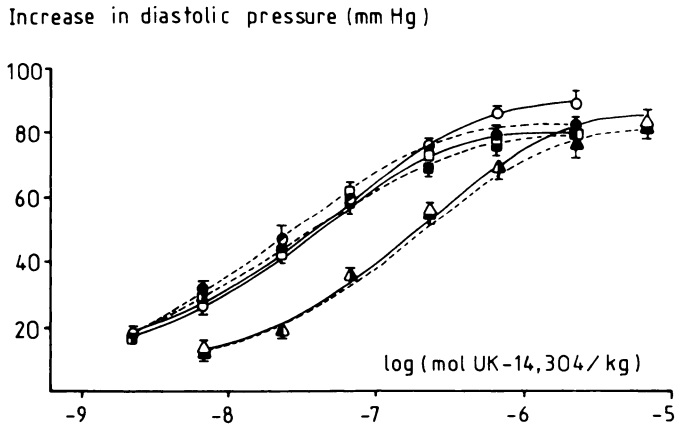

Fig. 8. Log dose-response curves with respect to the increase in diastolic pressure of pithed, normotensive rats induced by i.v. injections of UK-14,304. For explanation of symbols see the legend to figure 3. Means ± S.E.M. ($n = 5–7$).

were constructed by plotting the log dose against percentage of inhibition (fig. 9). As illustrated in this figure, cirazoline and methoxamine did not reduce the electrically induced increase in heart rate in the doses tested. In lower doses, (−)-phenylephrine had no inhibitory effect and in higher amounts it caused slight tachycardia. UK-14,304, B-HT 920 and B-HT 933 had a marked cardioinhibitory effect, like clonidine, which was included for comparison. The former three drugs completely abolished stimulation-induced tachycardia in contrast to clonidine which acted as a partial agonist in this model. UK-14,304 behaved as the most potent agonist of these cardiac presynaptic *alpha*-2 adrenoceptors ($ED_{50} = 3.4 \times 10^{-9}$ mol/kg). It was about 3 times more active than B-HT 920 ($ED_{50} = 9.9 \times 10^{-9}$ mol/kg) and 2 times more effective than clonidine ($Ed_{50} = 6.0 \times 10^{-9}$ mol/kg). B-HT 933 ($ED_{50} = 1.5 \times 10^{-7}$ mol/kg) was found approximately 40 times less potent than UK-14,304. The inhibitory effects of the compounds on the heart rate response to electrical stimulation were completely reversed by phentolamine (1 mg/kg) or yohimbine (1 mg/kg) injected i.v. at the end of each experiment.

Affinity for central *alpha*-1 and *alpha*-2 adrenoceptors. The specific binding of [^{3}H]clonidine and of [^{3}H]prazosin to membranes isolated from rat brain was saturable and of high affinity. Scatchard analysis of the binding data showed single populations of binding sites for both radioligands and afforded apparent dissociation constants (K_D) of 3.4 ± 0.5 nM for [^{3}H]-clonidine and 0.24 ± 0.06 nM (n = 5) for [^{3}H]prazosin. The maximal number of specific binding sites (B_{max}) was 197 ± 20 fmol of [^{3}H]clonidine and 88 ± 9 fmol of [^{3}H]prazosin per mg of protein (n = 5). Both radioligands were most effectively displaced by the corresponding nonradioactive reference compounds resulting in sigmoid displacements curves (figs. 10 and 11). The following decreasing order of potency in competing for [^{3}H]clonidine binding (0.4 nM) was determined: clonidine ≥ UK-14,304 > B-HT 920 > cirazoline > B-HT 933 > (−)-phenylephrine > methoxamine > prazosin. The concentration of the drugs inhibiting the specific [^{3}H]clonidine binding by 50% (IC_{50}) is listed in table 1. A completely different order of potency was observed with respect to the affinity for the specific binding sites occupied by [^{3}H]prazosin (0.2 nM): prazosin ≫ cirazoline > clonidine > UK-14,304 > (−)-phenylephrine > methoxamine > B-HT 920 ≥ B-HT 933. B-HT 920 and B-HT 933 in particular lacked affinity for [^{3}H]prazosin binding sites. The IC_{50} values of the drugs are reported in table 1.

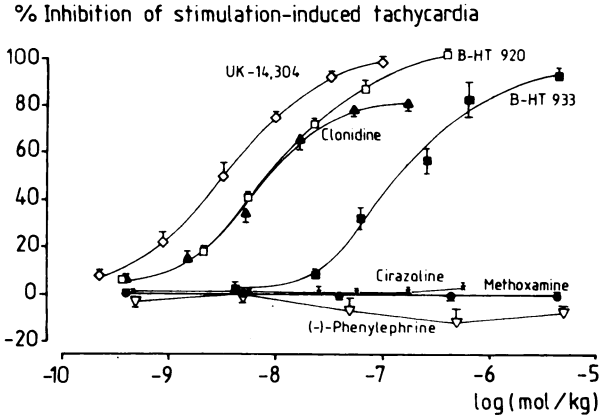

Fig. 9. Log dose response characteristics with respect to the inhibitory effect of UK-14,304, B-HT 920, clonidine, B-HT 933, cirazoline, methoxamine and (−)-phenylephrine on the stimulation-induced increase in heart rate of pithed, normotensive rats after i.v. administration. Continuous electrical stimulation (0.2 Hz, 2 msec, 50V) of the preganglionic, sympathetic nerves to the heart was performed *via* the pithing rod. Results are given as mean values ± S.E.M. (n = 5–6).

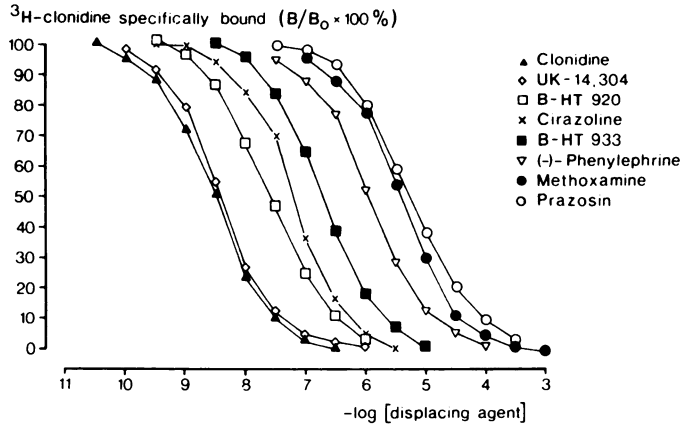

Fig. 10. Displacement of [^{3}H]clonidine (0.4 nM) from its specific binding sites in isolated rat cerebral membranes (M) by increasing concentrations of unlabeled clonidine, UK-14,304, B-HT 920, cirazoline, B-HT 933, (−)-phenylephrine, methoxamine and prazosin. Means of four separate determinations were performed in duplicate. B, fraction radioligand specifically bound in the presence of competing displacer; B_0, in the absence of competing displacer.

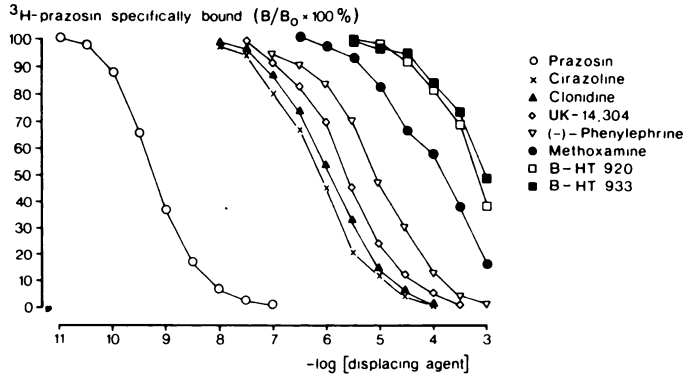

Fig. 11. Displacement of [^{3}H]prazosin (0.2 nM) from its specific binding sites in isolated rats cerebral membranes (M) by increasing concentrations of unlabeled prazosin, cirazoline, clonidine, UK-14,304, (−)-phenylephrine, methoxamine, B-HT 920 and B-HT 933. Means of four separate determinations were performed in duplicate. B, fraction radioligand specifically bound in the presence of competing displacer; B_0, in the absence of competing displacer.

Discussion

It was the aim of the present investigation to evaluate the selectivity of a number of *alpha* adrenoceptor agonists for *alpha*-1 and *alpha*-2 adrenoceptors. Substantial experimental evidence shows that both types are located postsynaptically in vascular smooth muscle *in vivo* (see "Introduction"). Both populations upon their activation elicit an increase in arterial pressure. The relatively simple *in vivo* model of the pithed rat was used to study the inhibition of the selective *alpha* adrenoceptor antagonists prazosin and yohimbine on the vasoconstrictor responses of the agonists in order to characterize the class of *alpha* adrenoceptor involved. Prazosin was chosen as the most selective antagonist of *alpha*-1 adrenoceptors (Cambridge *et al.*, 1977; Doxey *et al.*, 1977). A dose of 0.1 mg/kg does not influence blood pressure responses mediated *via* vascular *al-*

TABLE 1

Inhibition of [^{3}H] clonidine and [^{3}H]prazosin specific binding in isolated rat cerebral membranes

The displacement of the specific binding of [^{3}H]clonidine (incubation concentration, 0.4 nM) and [^{3}H]prazosin (incubation concentration, 0.2 nM) was determined with nine concentrations of competing drugs assayed in duplicate. The concentration (nanomoles) decreasing the specific binding of the ligands by 50% (IC_{50}) was calculated by log probit analysis. The mean value of four separate determinations is given. Also see figures 10 and 11.

	IC_{50} (nM)	
	[^{3}H]Clonidine	[^{3}H]Prazosin
Prazosin	5,000	0.6
Cirazoline	59	760
Clonidine	3.1	1,200
UK-14,304	3.6	2,420
(−)-Phenylephrine	1,080	8,200
Methoxamine	3,600	155,000
B-HT 920	25	500,000
B-HT 933	185	940,000

pha-2 adrenoceptors (Timmermans and van Zwieten, 1980a; Docherty and McGrath, 1980). Yohimbine is one of the most selective blocking agents of *alpha*-2 adrenoceptors (Starke *et al.*, 1975a; Borowski *et al.*, 1977; Weitzell *et al.*, 1979). A dose of 1 mg/kg leaves the pressor effects of *alpha*-1 adrenoceptor activation virtually unaffected (Timmermans and van Zwieten, 1980a; Docherty and McGrath, 1980).

The vasopressor responses to (−)-phenylephrine, methoxamine and cirazoline were effectively impaired by prazosin and were relatively resistant toward yohimbine, indicating that these three agonists predominantly stimulate the vascular *alpha*-1 adrenoceptor subpopulation in causing vasoconstriction. The preferential *alpha*-1 agonistic activity of (−)-phenylephrine and methoxamine toward vascular *alpha*-1 adrenoceptors has previously been demonstrated by several authors (see Starke *et al.*, 1975b and references quoted in "Introduction"). The present experiments identify cirazoline as a potent and selective agonist of *alpha*-1 adrenoceptors.

The use of (−)-phenylephrine for the identification of *alpha*-1 adrenoceptors is hampered by the fact that the drug can simultaneously act as a stimulant of *beta* adrenoceptors and that it possesses an additional indirect sympathomimetic action (Stanton *et al.*, 1965; Luchelli-Fortis and Langer, 1974; Lefèvre *et al.*, 1977; Ledda *et al.*, 1980). The tachycardia in pithed rats and the partial but not complete reduction of this action after either reserpine or (−)-propranolol treatment are in agreement with these properties of (−)-phenylephrine mentioned above. It has been reported that (−)-phenylephrine also exhibits a minor agonistic activity at postsynaptic *alpha*-2 adrenoceptors (Flavahan and McGrath, 1981). The small but significant inhibition of the pressor effects to (−)-phenylephrine by yohimbine would seem consistent with this suggestion. However, the lack of antagonism of (−)-phenylephrine by yohimbine after catecholamine depletion indicates that this response of (−)-phenylephrine may be the result of an indirect sympathomimetic action. The released norepinephrine (and epinephrine) are nonselective agonists of *alpha*-1 and *alpha*-2 adrenoceptors (Drew and Whiting, 1979; Flavahan and McGrath, 1980), and thus are partly susceptible for antagonism by yohimbine.

Methoxamine is not taken up in neuronal compartments and is devoid of *beta* adrenoceptor stimulating activity (Trendelenburg *et al.*, 1970). Therefore, the drug would be particularly suitable as a tool for the demonstration of *alpha*-1 adrenergic effects. However, a complicating factor may be that it is probably not a full agonist of *alpha*-1 adrenoceptors (Schümann and Endo, 1976; Bevan *et al.*, 1977; Bradshaw *et al.*, 1980).

Cirazoline (LD 3098) is an imidazoline derivative with potent postsynaptic *alpha* adrenoceptor stimulating properties both under *in vitro* and *in vivo* conditions (Lefèvre *et al.*, 1975, 1976, 1979). This study shows the marked selectivity of cirazoline to act as an agonist of vascular *alpha*-1 adrenoceptors. In accordance with earlier findings (Lefèvre *et al.*, 1977), this compound has no detectable indirect sympathomimetic effects and does not stimulate *beta* adrenoceptors.

Reserpine treatment produced a general depression of the maximally attainable vasoconstrictor responses to (−)-phenylephrine and methoxamine. This phenomenon cannot be explained yet, but it is interesting to note that the maximal pressor effects of cirazoline were not influenced by a pretreatment with reserpine.

The increase in diastolic pressure of pithed rats brought about by B-HT 920, B-HT 933 and UK-14,304 were attenuated by yohimbine, whereas prazosin was virtually inactive in this respect. Consequently, these substances can be classified as selective agonists of vasopressor *alpha*-2 adrenoceptors situated in the vascular wall. The occurrence of this subpopulation of postsynaptic *alpha*-2 adrenoceptors has been demonstrated by various authors (see "Introduction"). The results obtained with B-HT 920 and B-HT 933 agree with similar findings on the preferential *alpha*-2 agonistic activity of these two representatives out of a new class of compounds (Pichler *et al.*, 1980; Kobinger and Pichler, 1980; Timmermans and van Zwieten, 1980a,b; Timmermans *et al.*, 1980b).

The imidazolidine derivative UK-14,304 is the most potent *alpha*-2 adrenoceptor agonist studied. Data on its pharmacological properties are limited (Timmermans *et al.*, 1980a). UK-14,304 seems to have promising properties for being an additional tool in the characterization of *alpha*-2 adrenoceptors. B-HT 920, B-HT 933 and UK-14,304 are directly acting *alpha*-2 agonists. Reserpine treatment hardly affected the vasopressor activity of these drugs apart from a slight depression of the maximal plateau of activity. In higher doses, B-HT 933 caused tachycardia which is possibly due to its mild ganglion stimulating potency (J. C. A. van Meel, unpublished observations).

The cardioaccelerator response of the rat heart to sympathetic nerve stimulation can be inhibited by *alpha* adrenoceptor agonists, such as clonidine, which acts at prejunctional *alpha* adrenoceptors on the nerve terminals to diminish the release of neurotransmitter norepinephrine (Armstrong and Boura, 1973; Drew, 1976; Doxey, 1977; Robson *et al.*, 1978; Docherty and McGrath, 1979a). Prevention or reversal of this clonidine-induced bradycardia can be accomplished by antagonists of *alpha*-2 adrenoceptors, *e.g.* yohimbine and piperoxan (Drew, 1976; Doxey, 1977; Robson *et al.*, 1978; Docherty and McGrath, 1979b). In contrast to (−)-phenylephrine, methoxamine and cirazoline, B-HT 920, B-HT 933 and UK-14,304 dose-dependently reversed the positive chronotropic response to stimulation of the sympathetic outflow in the pithed rat. The results confirm the classification of these agonists as selective *alpha*-1 or *alpha*-2 adrenoceptor stimulants made above. The presynaptic diminution of the increase in heart rate produced by electrical stimulation of the cardioaccelerator sympathetic nerve fibers by B-HT 933 and B-HT 920 has also been reported by Kobinger and Pichler (1980) and Pichler *et al.* (1980). UK-14,304, B-HT 920 and B-HT 933 are full agonists on cardiac presynaptic *alpha*-2 adrenoceptors, in contrast to clonidine

which inhibited the tachycardic response by about 80%. The partial agonistic action of clonidine on presynaptic *alpha*-2 adrenoceptors (rabbit pulmonary artery) has also been demonstrated by Medgett *et al.* (1978).

This study gives no indication for the presence of presynaptic *alpha*-1 adrenoceptors in the rat heart. The existence of a minor population of cardiac presynaptic *alpha*-1 adrenoceptors has been proposed by Kobinger and Pichler (1980) using methoxamine as the agonist. However, in the present investigation, methoxamine appeared to be ineffective up to 1 mg/kg. Even cirazoline which was found about 38 times more potent than methoxamine as an *alpha*-1 adrenoceptor stimulant was without effect on the electrically induced tachycardia. This result is compatible with that of Roach *et al.* (1978). It should be added, however, that a pronounced effect on the presynaptic *alpha* adrenoceptors in the perfused cat spleen has been reported for cirazoline (Dubocovich *et al.*, 1980). At present, the discrepancy between these findings is difficult to explain.

Radioligand binding studies have supported the hypothesis of distinct *alpha*-1 and *alpha*-2 adrenoceptor sites. The classification which is based upon binding experiments (Wood *et al.*, 1979) generally agrees with the characterization obtained by pharmacological means. The selective occupation of *alpha*-1 adrenoceptor-like binding sites in brain and peripheral tissues can be achieved by the radiolabeled *alpha*-1 adrenoceptor antagonist [^{3}H]WB-4101 (Greenberg *et al.*, 1976; U'Prichard *et al.*, 1977) and in particular by [^{3}H]prazosin (Greengrass and Bremner, 1979; Lyon and Randall, 1980; Miach *et al.*, 1980; Timmermans *et al.*, 1981). [^{3}H]Clonidine has proven a useful radioligand for the labeling of *alpha*-2 adrenoceptors (Greenberg *et al.*, 1976; U'Prichard *et al.*, 1977; Glossmann and Presek, 1979). The binding experiments showed the greater affinity of cirazoline over (−)-phenylephrine and methoxamine for *alpha*-1 adrenoceptors and the lack of affinity of B-HT 920 and B-HT 933 for these receptive sites. Moreover, UK-14,304 is more potent than B-HT 920 and the latter is more effective than B-HT 933 in competing for [^{3}H]clonidine-occupied *alpha*-2 adrenoceptors, whereas (−)-phenylephrine and methoxamine behave as weak displacers. These data are in accordance with the *in vivo* pharmacological characterization of the agonists (see above). However, all agonists had lower affinity to the [^{3}H]-prazosin (*alpha*-1) site than to the [^{3}H]clonidine (*alpha*-2) site. This would suggest that every compound exhibited selectivity to *alpha*-2 adrenoceptors which is in apparent contradiction to the outcome of the pharmacological studies (see above). A similar inconsistency between binding data and functional selectivity of some *alpha* adrenoceptor agonists has been reported by Starke and Docherty (1980). As discussed by these authors, the use of Tris buffer in *alpha* receptor binding studies without the monovalent cations, such as sodium, will give rise to a systematic overestimation of agonist affinity for *alpha*-2 adrenoceptors leading to a systematic underestimation of their *alpha*-1/*alpha*-2 selectivity ratio. Furthermore, it should be realized that receptor binding studies merely give a measure of the affinity of the drug for the particular binding site and do not provide adequate information concerning agonistic (intrinsic) activity. Therefore, moderate affinities of agonists as measured in binding experiments can be translated into high potencies as quantified in pharmacological studies due to the high efficacies of these drugs. The relatively pronounced *in vitro* affinity of UK-14,304 and cirazoline for *alpha*-1 and *alpha*-2 adrenoceptors, respectively, indicate that the former may also behave as an antagonist of *alpha*-1 adrenoceptors and that the latter may possess an additional measurable blocking activity of *alpha*-2 adrenoceptors. However, at present no pharmacological data are available to support this.

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