MOLECULAR PHARMACOLOGY, 20:295–301

Identical Antagonist Selectivity of Central and Peripheral *Alpha*$_1$-Adrenoceptors

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Received March 13, 1981; Accepted May 21, 1981

SUMMARY

TIMMERMANS, P. B. M. W. M., F. KARAMAT ALI, H. Y. KWA, A. M. C. SCHOOP, F. P. SLOTHORST-GRISDIJK, AND P. A. VAN ZWIETEN. Identical antagonist selectivity of central and peripheral *alpha*$_1$-adrenoceptors. *Mol. Pharmacol.* **20**:295–301 (1981).

The present study was undertaken to test the relationship between binding affinity for *alpha*$_1$-adrenoceptors and functional antagonism of drug-induced effects mediated by this type of *alpha*$_1$-receptor sites for *alpha*-sympatholytic drugs. The radioligand [^{3}H]prazosin was used to identify *alpha*$_1$-adrenoceptors in rat brain membranes. The specific binding of [^{3}H]prazosin was rapid, reversible, saturable, and of high affinity (K_D = 0.21 nM) and involved a single class of binding sites (B_{max} = 95 fmoles/mg of protein). A variety of *alpha*-adrenoceptor blocking drugs inhibited the specific binding of [^{3}H]prazosin (0.2 nM) according to sigmoid displacement curves from which the corresponding log IC_{50} values were calculated. The binding sites of [^{3}H]prazosin in rat cerebral membranes possessed the characteristics of *alpha*$_1$-adrenoceptors. Antagonists such as rauwolscine, tolazoline, yohimbine, and piperoxan, known as selective blocking drugs of *alpha*$_2$-adrenoceptors, were found to be weak competitors. However, 2-[(2′,6′-dimethoxyphenoxyethyl)-aminomethyl]-1,4-benzodioxane (WB-4101) and unlabeled prazosin as well as two of its derivatives, 2-[4-(ethoxyethyloxy)-piperidine-1-yl]-4-amino-6,7-dimethoxyquinazoline (UK-18,596) and 2-[4-(2-(1,4-benzodioxoyl))-piperazine-1-yl]-4-amino-6,7-dimethoxyquinazoline (UK-33,274), which have been classified as antagonists preferably occupying *alpha*$_1$-adrenoceptors, strongly interfered with this binding. The same *alpha*-adrenolytic drugs were studied with respect to their antagonism of (−)-phenylephrine-induced increases in diastolic pressure mediated via vascular, postsynaptic *alpha*$_1$-adrenoceptors in pithed, normotensive rats. This antagonism was quantified with the aid of pA_2 values. For the *alpha*-adrenoceptor antagonists studied (n = 21), the pA_2 *in vivo* correlated well with the log $1/IC_{50}$ (r^2 = 0.86). Similarly, a close relationship was calculated between the binding data and reported pA_2 values with respect to the antagonism of *alpha*$_1$-adrenoceptor-mediated constrictor effects of the rabbit pulmonary artery *in vitro* (r^2 = 0.90). The results demonstrate that the binding affinity *in vitro* of antagonists for *alpha*$_1$-adrenoceptors corresponds with their functional antagonism of *alpha*$_1$-adrenoceptors *in vivo* and *in vitro*. Moreover, these findings point to a similarity among peripheral and central *alpha*$_1$-adrenoceptors.

INTRODUCTION

Postsynaptic *alpha*-adrenoceptors are known to mediate the response of the target cells, whereas presynaptic *alpha*-adrenoceptors modulate neurotransmitter release (for reviews and monographics, see refs. 1–4). Originally, the subclassification of *alpha*-adrenoceptors into *alpha*$_1$ for postsynaptic receptor sites and *alpha*$_2$ for presynaptic receptor sites has been proposed (5). However, it has been recognized that this terminology should be used independently of the anatomical position and function of the *alpha*-adrenoceptors (6–8), since *alpha*-adrenoceptors similar to the presynaptic ones have also been identified outside noradrenergic synapses and even at postsynaptic locations (for review see ref. 9). As a consequence thereof, the prefixes *alpha*$_1$ and *alpha*$_2$ are exclusively based upon the relative activities and affinities of agonists and antagonists, respectively.

A comparable subclassification of *alpha*-adrenoceptors can be made in the central nervous system. Radioligand binding studies have identified distinct and non-interconverting binding sites in brain homogenates possessing the characteristics of *alpha*$_1$- and *alpha*$_2$-adrenoceptors. The two binding sites of [^{3}H]dihydroergocryptine (10, 11) represent *alpha*$_1$- and *alpha*$_2$-adrenoceptors (12, 13).

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Among a variety of radioligands, tritiated clonidine and *p*-aminoclonidine have frequently been used for the direct labeling of central *alpha*$_2$-adrenoceptor sites (14–18). The tritiated antagonists WB-4101 and prazosin have been employed as selective radioligands of *alpha*$_1$-adrenoceptors (14, 15, 19–21).

This study reports on a quantitative comparison of the affinities *in vitro* of numerous *alpha*-adrenoceptor antagonists for central *alpha*$_1$-adrenoceptors in rat brain identified with [^{3}H]prazosin and the antagonistic potency of these *alpha*-sympatholytic drugs toward peripheral *alpha*$_1$-adrenoceptors mediating vasoconstrictor responses *in vitro* and *in vivo*. The present experiments were performed to test the relationship between affinity *in vitro* and functional antagonism *in vitro* and *in vivo* and to investigate the similarity between peripheral and central *alpha*$_1$-adrenoceptor populations.

MATERIALS AND METHODS

Materials. The following materials were obtained from the indicated sources: Azapetine phosphate (Hoffmann-La Roche, Nutley, N. J.); 7-chloropiperoxane hydrochloride and 6-methylpiperoxane hydrochloride (synthesized by Dr. J. de Boer, University of Amsterdam); clozapine (Wander); corynanthine hydrochloride and rauwolscine hydrochloride (Roth, Karlsruhe, Federal Republic of Germany); dihydroergotamine methanesulfonate (Sandoz, Basel, Switzerland); WB-4101[1] (Ward Blenkinsop, Wembley, Middlesex, United Kingdom); droperidol, haloperidol, and piperoxane hydrochloride (Janssen Pharmaceutica, Beerse, Belgium); heparin (Novo); hexobarbitone-sodium (Bayer, Leverkusen, Federal Republic of Germany); indoramine hydrochloride (Wyeth Laboratories, Philadelphia, Pa.); labetalol hydrochloride (Glaxo, Ware, Hertfordshire, United Kingdom); mianserin hydrochloride (Organon Oss, The Netherlands); phentolamine hydrochloride and tolazoline hydrochloride (Ciba-Geigy, Summit, N. J.); (−)-phenylephrine hydrochloride and yohimbine hydrochloride (Sigma Chemical Company, St. Louis, Mo.); prazosin hydrochloride, [^{3}H]prazosin hydrochloride (specific activity 33 Ci/mmole), UK-18,596, and UK-33,274 (Pfizer, Sandwich, Kent, United Kingdom); and thymoxamine hydrochloride (Opilon, Warner, Easleigh, Hampshire, United Kingdom). For animal experiments the drugs were dissolved in saline (0.9% aqueous sodium chloride solution). In competition studies *in vitro* the drugs were dissolved in distilled water and dilutions were made in Tris-HCl buffer. Clozapine was taken up in 0.02 M tartaric acid. Prazosin and UK-33,274 were solubilized in distilled water containing 5% (w/v) glucose and 5% (w/v) glycerol. Dihydroergotamine methanesulfonate was used as a commercial solution (Dihydergot). The vehicles neither affected the pressor effects of (−)-phenylephrine nor influenced the affinity for [^{3}H]prazosin binding sites. All of the doses of the drugs quoted are expressed in terms of the form indicated above.

Preparation of rat brain membranes. Male, normotensive Wistar rats weighing 200–250 g were killed by decapitation. Their brains (minus cerebella) were isolated and homogenized in 20 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°) in motor-driven glass/Teflon homogenizers. The homogenate was centrifuged at 50,000 × *g* for 10 min at 4°. The pellet was rehomogenized in fresh cold Tris-HCl buffer and the suspension was centrifuged again (see above). The final material was resuspended in Tris-HCl buffer at a concentration of 1 mg of protein per milliliter for routine use in the binding assays. The protein concentration was determined by the method of Lowry *et al.* (22), using bovine serum albumin as standard and 50 mM Tris-HCl buffer as blank.

[^{3}H]Prazosin binding assay. Standard [^{3}H]prazosin binding assays were performed by incubating 500 μl of rat brain membrane suspension (see above) at 25° for about 60 min with [^{3}H]prazosin (specific activity 33 Ci/mmole); 0.05–7 nM) with shaking in a total volume of 1 ml of incubation buffer. In displacement experiments, the inhibition of the specific binding of [^{3}H]prazosin (0.2 nM) was determined in the presence of various concentrations of competing drugs. Incubations were terminated by rapid vacuum filtration through Whatman GF/B filters. Filters were rapidly washed with three 5-ml portions of ice-cold Tris-HCl buffer, left to solubilize in 10 ml of Instagel (Packard-Becker, Groningen, The Netherlands) for 24 hr, and counted at an efficiency of about 40%. Nonspecific binding of [^{3}H]prazosin is defined as binding which is not displaced by a 2 μM concentration of the *alpha*-sympatholytic drug phentolamine. These blanks were subtracted from the total binding to obtain the specific binding of [^{3}H]prazosin.

As a measure of the affinity of drugs for the *alpha*$_1$-adrenoceptor sites identified by [^{3}H]prazosin, the concentration (molar) inhibiting the specific binding of [^{3}H]prazosin (0.2 nM) by 50% (IC_{50}) was calculated by log probit analysis.

Antagonism of alpha$_1$*-adrenoceptor-mediated vasopressor effects in vivo.* Male, normotensive Wistar rats weighing 200–250 g were anesthetized with hexobarbitone-sodium, administered i.p. (150 mg/kg). The animals were placed on thermostat-equipped tables to keep body temperature at approximately 37°. The tracheae were cannulated and the animals were ventilated artificially by means of a respiration pump with positive pressure. A blunt, stainless steel needle was introduced into the spinal canal via the orbit and pushed forward in caudal direction. Pithed rats are most suitable for quantifying vasopressor effects *in vivo*. The central nervous system being destroyed in these animals, peripheral responses are not affected by counteracting reflexogenic and/or central mechanisms. Left carotid arterial pressure was measured continuously (Statham P23 Db transducer) and displayed on a Hellige HE 19 recorder. The right jugular vein was cannulated for the i.v. administration of drugs. The pithed rats received heparin (about 1000 IU/kg, i.v.). An equilibration period of about 15 min elapsed before the commencement of the experiments. Vascular postsynaptic *alpha*$_1$-adrenoceptors were stimulated with the aid of the selective agonist (−)-phenylephrine injected i.v. in various amounts in single doses (0.5 ml/kg) 15 min after i.v. treatment with saline (1 ml/kg) or the particular *alpha*-adrenoceptor antagonist (1 ml/kg). The

[1] The abbreviations used are: WB-4101, 2-[(2′,6′-dimethoxyphenoxyethyl)-aminomethyl]-1,4-benzodioxane; UK-18,596, 2-[4-(ethoxyethyloxy)-piperidine-1-yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride; UK-33,274, 2-[4-(2-(1,4-benzodioxoyl))-piperazine-1-yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride.

increase in diastolic pressure was measured, and log dose-response curves were constructed. Displacements of the log dose-pressor effect curves caused by the antagonists were calculated at the level of half-maximal activity. The pA_2 values were obtained from the equation: pA_2 = log (dose ratio − 1)-log (antagonist concentration). Independent of the pretreatment (saline or antagonist), no more than three or four separate injections of (−)-phenylephrine were made per pithed animal in random order. Recovery of the pressor effects to preinjection control values was ensured between administration of the subsequent doses.

Antagonism of alpha$_1$-adrenoceptor-mediated vasoconstrictor effects in vitro. The antagonism of various *alpha*-sympatholytic drugs toward (−)-noradrenaline- or (−)-phenylephrine-induced *alpha*$_1$-adrenoceptor-mediated constrictor effects of the rabbit isolated pulmonary artery has been evaluated by Borowski *et al.* (23) and Weitzell *et al.* (24). The pA_2 values reported in Table 1 are taken from these studies or were kindly provided by Professor K. Starke (Freiburg, Federal Republic of Germany).

Correlations. By means of linear regression analysis, pharmacological and binding data were correlated via the method of least squares. The correlation coefficient (r), the standard deviation (SD), and the significance of the regression (F) are given. The figures between parentheses represent 95% confidence intervals.

RESULTS

[^{3}H]Prazosin binding to rat brain membranes. Part of the total binding of [^{3}H]prazosin to rat isolated cerebral membranes was not inhibited by excess (2 μM) phentolamine (nonspecific binding). Total binding depended on the concentration of [^{3}H]prazosin. Nonspecific binding increased linearly up to 7 nM, the highest concentration examined. At 0.2 nM [^{3}H]prazosin, used in routine binding assays, the nonspecific binding represented about 6% of the total binding. The specific binding of the ligand (0.05–7 nM) to rat brain membranes was a saturable process according to a simple hyperbolic function, with a plateau at 1–2 nM (Fig. 1). Scatchard analysis of six such saturation experiments indicated one distinct class of binding sites yielding an K_D of 0.21 ± 0.05 nM (mean ± standard error of the mean) and a maximal number of binding sites of 95 ± 10 fmoles/mg of protein (Fig. 1).

The association rate of the [^{3}H]prazosin binding (0.2 nM) is rapid, with greater than 50% of the specific binding achieved within approximately 5 min and equilibrium reached within 15–20 min at 25° (association rate constant: 0.24 nM^{-1} min^{-1}). Specific binding was stable for up to 1 hr at 25°. Dissociation of [^{3}H]prazosin (0.2 nM) from its specific binding sites in rat brain membranes was assessed by adding an excess (2 μM) of phentolamine to an equilibrated mixture. The binding was readily reversible with a half-time of approximately 10 min (dissociation rate constant: 0.072 min^{-1}).

Affinities of alpha-adrenoceptor antagonists for [^{3}H] prazosin specific binding sites. The displacement of [^{3}H] prazosin (0.2 nM) from its specific binding sites in rat cerebral membranes was determined for 21 *alpha*-adrenoceptor antagonists, including unlabeled prazosin. Figure 2 shows the displacement curves of some of these drugs. All competing compounds ultimately caused a 100% inhibition of the binding of 0.2 nM of [^{3}H]prazosin. Their competition characteristics were found to be virtually parallel with Hill coefficients not significantly different from unity. Table 1 lists the concentration (molar) of the agents required to reduce this specific binding of [^{3}H]-prazosin by 50% (IC_{50}), calculated by log probit analysis. Substantial differences in affinity for the [^{3}H] prazosin binding sites were observed among the *alpha*-antagonists studied (about 4 log units). Unlabeled prazosin was most potent in this respect, displacing this binding with an IC_{50} value of 0.58 nM. The compounds UK-18,596 and UK-33,274, which are structurally related to prazosin, were also potent inhibitors as well as was WB-4101. The order of potency was as follows: droperidol = indoramine > dihydroergotamine > thymoxamine > phentolamine > haloperidol. The diastereoisomers yohimbine, rauwolscine, and corynanthine differed in their potency to compete for the [^{3}H]prazosin binding sites. Corynanthine was found considerably more effective than yohimbine, which in turn was more potent than rauwolscine. This latter drug behaved as a weak displacer. Clozapine, azapetine, and labetalol possessed comparable affinities as measured for corynanthine. The substances mianserin, piperoxane, and tolazoline proved moderate inhibitors of [^{3}H]prazosin binding. The 6-methyl- and 7-chloro-substituted analogues of piperox-

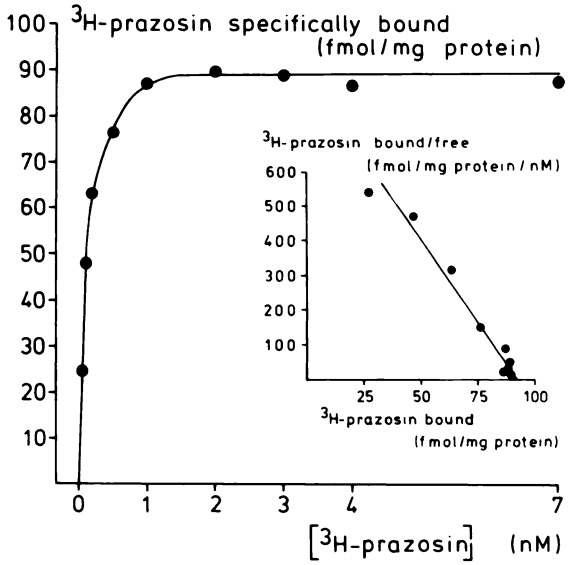

FIG. 1. *Specific binding of [^{3}H]prazosin to rat brain membranes as a function of increasing concentration of the ligand*

Membrane suspensions (0.5 mg of protein per milliliter; total volume 1 ml) were incubated for 60 min at 25° with various concentrations of [^{3}H]prazosin as described under Materials and Methods. Nonspecific binding was that occurring in the presence of 2 μM phentolamine. Specific binding was defined as the difference between total and nonspecific binding. Each value is the mean of six experiments performed in duplicate.

Inset. Scatchard plot of [^{3}H]prazosin binding to rat isolated brain membranes. The *line* indicated was determined by linear regression analysis. The number of binding sites per milligram of protein was calculated from the intercept of the plot with the abscissa. The equilibrium dissociation constant at 25° resulted from the slope of the line. Values are means of six determinations performed in duplicate.

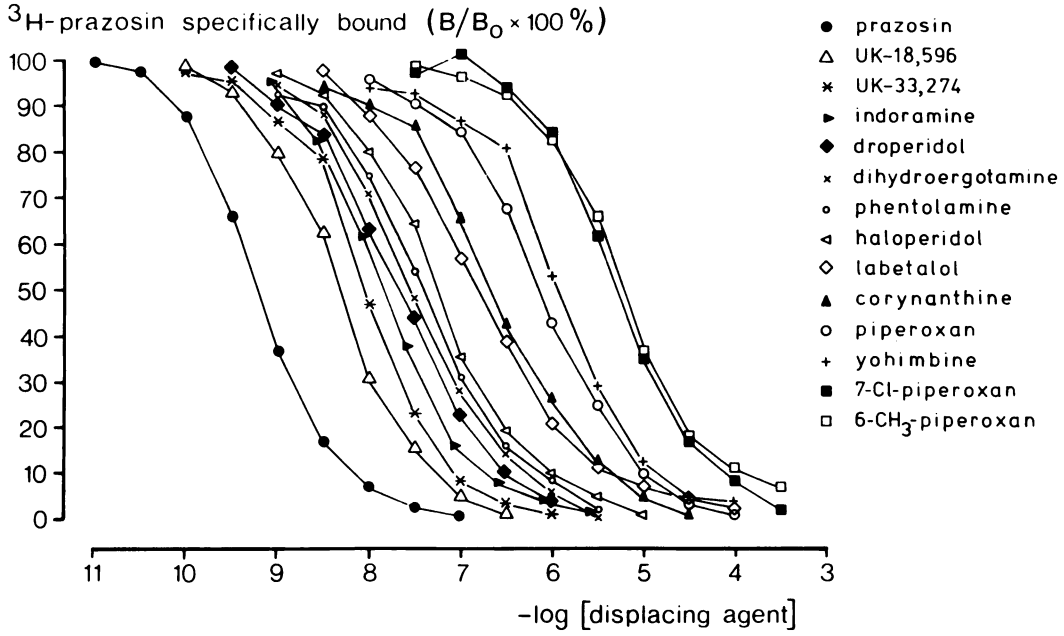

FIG. 2. *Inhibition of specific [^{3}H]prazosin binding to rat cerebral membranes by various alpha-adrenoceptor antagonists*
Membrane suspensions were incubated with 0.2 nM [^{3}H]prazosin in the presence or absence of nine increasing concentrations (molar) of competing nonlabeled drug under standard assay conditions (see Materials and Methods). Nonspecific binding was defined as that part of the total binding which was not displaced by excess (2 μM) phentolamine. *Points* are given as means of four individual experiments performed in duplicate. *B*, Fraction of radioligand specifically bound in the presence of competing displacer; B_0, in the absence of competing displacer.

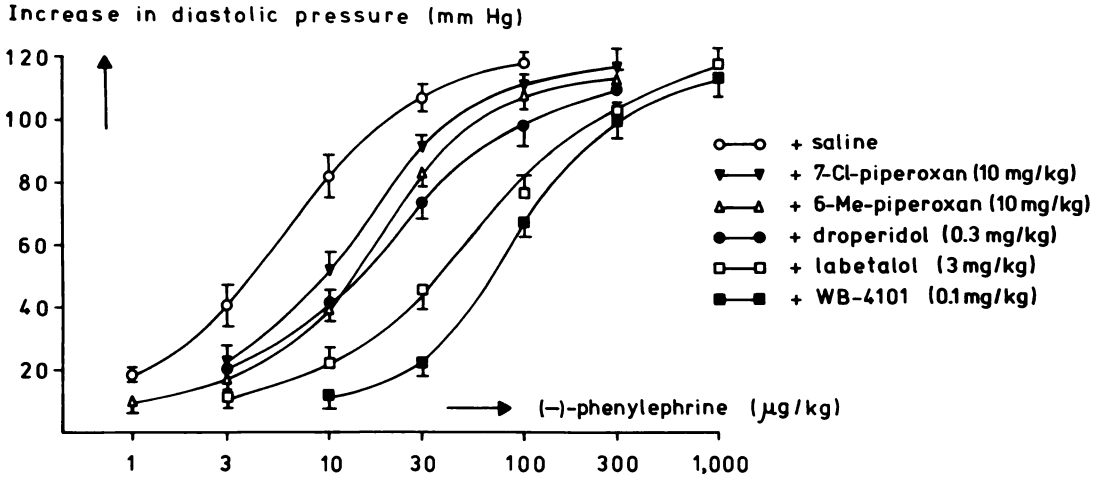

FIG. 3. *Log dose-pressor response curves of (−)-phenylephrine in pithed, normotensive rats*
Various doses (micrograms per kilogram) of (−)-phenylephrine were injected i.v. into pithed, normotensive rats 15 min after saline treatment (control) or 15 min after i.v. application of *alpha*-adrenoceptor antagonists indicated. The increase in diastolic pressure was measured. Symbols represent mean values ± standard error of the mean (n = 5–7).

ane were about 10 times less active than the parent compound. The displacement of [^{3}H]prazosin from its specific binding sites occurred in high concentrations only.

Antagonism of (−)-phenylephrine-induced vasopressor effects in pithed rats. After appropriate equilibration, the mean diastolic pressure of pithed, normotensive rats amounted to 43.2 ± 0.2 mm Hg (mean ± standard error of the mean; n = 631). The log dose-response curve of i.v. (−)-phenylephrine with respect to the increase in diastolic pressure after saline pretreatment is shown in Fig. 3. A maximal increase in diastolic pressure of about 120 mm Hg was reached by this *alpha*$_1$-adrenoceptor agonist. Previous i.v. treatment of these pithed animals with various doses of the antagonists resulted in parallel shifts to the right of this log dose-vasopressor response curve of (−)-phenylephrine. Some representative curves are depicted in Fig. 3. For all antagonists these parallel shifts occurred without a depression of the maximal pressor effect of (−)-phenylephrine. Table 1 reports the pA$_2$ values of the *alpha*-adrenoceptor-blocking drugs with respect to this antagonism *in vivo*. As can be deduced from Table 1, prazosin, its two congeners UK-18,596 and UK-33,274, and WB-4101 were by far the most effective antagonists of (−)-phenylephrine-induced increases in diastolic pressure in pithed rats. The rank order of de-

TABLE 1

Antagonism of various alpha-adrenoceptor blocking drugs toward alpha$_1$-adrenoceptor-mediated vasopressor effects in vivo ($pA_{2\ in\ vivo}$) and vasoconstrictor responses in vitro ($pA_{2\ in\ vitro}$) and inhibition of [^{3}H]prazosin binding in rat isolated brain membranes

Antagonism *in vivo* of (−)-phenylephrine-induced increases in diastolic pressure of pithed, normotensive rats was assessed as described under Materials and Methods. $pA_{2\ in\ vitro}$ refers to the antagonism of the constrictor effect of the isolated pulmonary artery of the rabbit (data from refs. 23 and 24). Inhibition of the specific binding of [^{3}H] prazosin (0.2 nM) was determined with nine concentrations of competing drugs in duplicate. The concentration (molar) decreasing the specific binding of the tritiated ligand by 50% (IC_{50}) was calculated by log probit analysis. The mean value of four separate determinations is given. Log $1/IC_{50}$ was used in the correlations.

Alpha-adrenoceptor antagonist	$pA_{2\ in\ vivo}$	$pA_{2\ in\ vitro}$	IC_{50}	log $1/IC_{50}$
Prazosin	8.33	8.70	0.58×10^{-9}	9.24
UK-18,596	7.84	—	4.8×10^{-9}	8.32
UK-33,274	7.77	—	8.8×10^{-9}	8.06
WB-4101	7.75	—	7.0×10^{-9}	8.15
Phentolamine	6.95	7.49	3.9×10^{-8}	7.41
Dihydroergotamine	6.68	7.55	2.7×10^{-8}	7.57
Droperidol	6.50	—	1.65×10^{-8}	7.78
Indoramine	6.36	—	1.7×10^{-8}	7.77
Clozapine	6.29	7.43	1.25×10^{-7}	6.91
Corynanthine	6.25	6.60	2.2×10^{-7}	6.66
Thymoxamine	6.15	—	3.1×10^{-8}	7.51
Azapetine	6.09	6.96	2.05×10^{-7}	6.69
Labetalol	5.99	—	1.6×10^{-7}	6.80
Haloperidol	5.96	—	5.1×10^{-8}	7.29
Yohimbine	5.72	6.40	1.3×10^{-6}	5.89
Piperoxane	5.71	6.06	7.2×10^{-7}	6.14
Tolazoline	5.66	5.41	2.1×10^{-6}	5.68
Mianserin	5.41	6.60	3.8×10^{-7}	6.42
Rauwolscine	5.12	5.89	6.3×10^{-6}	5.20
6-Methyl-piperoxane	4.75	—	6.0×10^{-6}	5.22
7-Chloro-piperoxane	4.52	—	5.2×10^{-6}	5.28

creasing blocking activity *in vivo* is further listed in Table 1. This order of potency generally corresponds with the affinity of the drugs for [^{3}H]prazosin-binding sites measured *in vitro*.

Correlations. Linear regression equations were calculated to test the quantitative relationship between affinity *in vitro* of *alpha*-adrenoceptor antagonists for central *alpha*$_1$-adrenoceptors identified by [^{3}H]prazosin and functional antagonism of these blocking drugs toward vasoconstrictor effects mediated by peripheral *alpha*$_1$-adrenoceptors *in vivo* (pA_2 *in vivo*) and *in vitro* (pA_2 *in vitro*). The following equations were generated:

$$pA_{2\ in\ vivo} = 0.840(\pm 0.17) \log 1/IC_{50} + 0.437 \quad (1)$$
$$n = 21;\ r = 0.926;\ SD = 0.393;\ F = 113.77$$

$$pA_{2\ in\ vitro} = 0.800(\pm 0.21) \log 1/IC_{50} + 1.456 \quad (2)$$
$$n = 11;\ r = 0.949;\ SD = 0.310;\ F = 80.87$$

Equations 1 (Fig. 4) and 2 (Fig. 5) identify close linear correlations between the potencies of the drugs as antagonists of functional *alpha*$_1$-adrenoceptors in vascular smooth muscle *in vivo* and *in vitro* and their *in vitro* affinities for central *alpha*$_1$-adrenoceptors ([^{3}H]prazosin-binding sites). The binding values *in vitro* account for

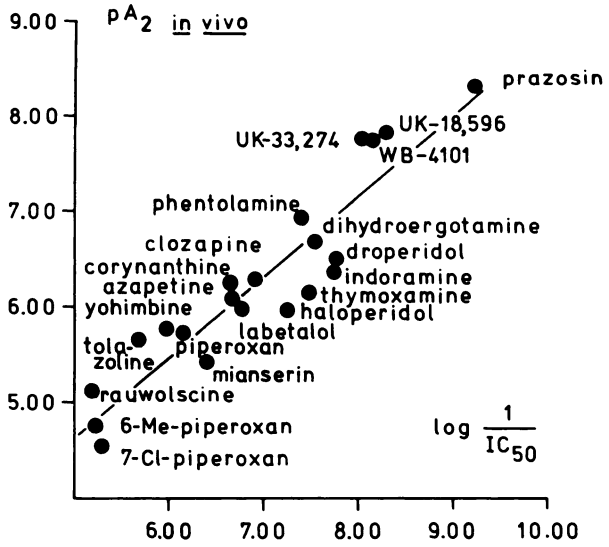

FIG. 4. *Relationship between the antagonistic potency of various alpha-adrenoceptor blocking drugs toward alpha$_1$-adrenoceptor-mediated vasopressor effects in vivo (pA_2 in vivo) and inhibition of [^{3}H] prazosin binding in rat brain membranes (log $1/IC_{50}$)*

Values for pA_2 *in vivo* refer to the antagonism of the drugs with respect to (−)-phenylephrine-induced increases in diastolic pressure of pithed, normotensive rats. Log $1/IC_{50}$ values resulted from displacement experiments. For details see Materials and Methods. The correlation is expressed by Eq. 1 (see text).

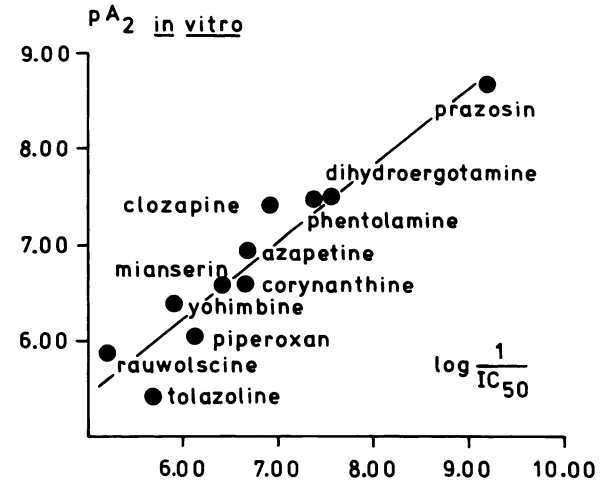

FIG. 5. *Relationship between the antagonistic efficacy of various alpha-adrenoceptor-blocking drugs with respect to alpha$_1$-adrenoceptor-induced constrictor responses in vitro (pA_2 in vitro) and inhibition of [^{3}H]prazosin binding in rat brain membranes (log $1/IC_{50}$)*

The pA_2 *in vitro* values were obtained with the rabbit isolated pulmonary artery (data from refs. 23 and 24). Log $1/IC_{50}$ values were calculated from displacement data. For details see Materials and Methods. The correlation is given by Eq. 2 (see text).

86% (r^2) (Eq. 1) and 90% (Eq. 2) of the variance in the pharmacological data.

DISCUSSION

The use of the radioligand [^{3}H]prazosin in this study has allowed the identification of binding sites in rat brain membranes which possess the characteristics of *alpha*$_1$-adrenoceptors. The process of [^{3}H]prazosin binding was

found rapid and reversible. The binding occurred with high affinity to a finite and limited number of a single class of specific sites. The *alpha*$_1$-adrenergic character of [^{3}H]prazosin-specific binding sites in rat cerebral membranes is indicated by the rank order of potencies of *alpha*-adrenoceptor antagonists competing for this binding. Prazosin itself, its derivates UK-18,596 and UK-33,274, and WB-4101, classified as selective antagonists of *alpha*$_1$-adrenoceptors by pharmacological means (25–27), were most potent displacers of [^{3}H]prazosin binding, whereas rauwolscine, tolazoline, yohimbine, and piperoxane, identified as more or less specific blocking drugs of *alpha*$_2$-adrenoceptors (23, 24), behaved as relatively weak competitors. [^{3}H]Prazosin has been used by other workers to label central *alpha*$_1$-adrenoceptors in brain tissue (19–21). The results presented in this study are in good agreement with those reported.

At least two distinct classes of postsynaptic *alpha*-adrenoceptors can be identified in vascular smooth muscle. The two types resemble *alpha*$_1$- and *alpha*$_2$-adrenoceptors, and both are involved in drug-induced vasoconstriction *in vivo* (9). Among a great number of nonselective agonists, (−)-phenylephrine has proven a selective stimulant of the *alpha*$_1$-adrenoceptor population in the intact circulatory system of the pithed rat (ref. 9 and refs. quoted therein). It may be remarked that the known indirect sympathomimetic as well as the *beta*-agonistic activity of (−)-phenylephrine may have hampered an accurate determination of the pA_2 values *in vivo* reported in this study. However, in reserpine-treated, *beta*-blocked pithed rats, the antagonism toward (−)-phenylephrine-induced hypertensive responses is not dramatically changed (28). This indicates that the influence of these additional properties of (−)-phenylephrine on the pA_2 values determined *in vivo* in pithed rats is not particularly great. In addition, the blocking potencies of the drugs generally correspond with their antagonistic activity at other *alpha*$_1$-adrenoceptor sites determined with the aid of different pharmacological methods (9, 23–25).

A close linear relationship was demonstrated to exist between the antagonistic efficacy *in vivo* of the blocking drugs at peripheral *alpha*$_1$-adrenoceptors triggered by (−)-phenylephrine and their binding affinity *in vitro* for central *alpha*$_1$-adrenoceptors. A relationship of comparable statistical quality was generated for the data *in vitro* referring to the antagonism of *alpha*$_1$-adrenoceptor-mediated constrictor effects of the rabbit isolated pulmonary artery *in vitro* (23, 24). This finding strongly suggests that peripheral, vascular *alpha*$_1$-adrenoceptors stimulated *in vivo* as well as *in vitro* and central *alpha*$_1$-adrenoceptors labeled by [^{3}H]prazosin *in vitro* possess similar specificities toward their antagonists.

Meaningful quantitative relationships between pharmacological potencies of *alpha*-adrenoceptor agonists and antagonists induced by *alpha*$_1$-adrenoceptors in the rat vas deferens, rabbit heart, and rabbit spleen and affinities for [^{3}H]WB-4101 binding sites have been reported (29). In addition, the blocking potencies of various benzodioxane *alpha*-adrenoceptor antagonists in the rat vas deferens correlated with the inhibition data for [^{3}H]WB-4101 binding (26). However, in this latter study some pronounced discrepancies between absolute pharmacological activity and affinity for [^{3}H]WB-4101-binding sites were noticed. The present study shows no obvious anomalies between functional antagonism and affinity with respect to *alpha*$_1$-adrenoceptors within the series of *alpha*-adrenoceptor antagonists tested. It may be added that multiple central binding sites have recently been reported for the radioligand [^{3}H]WB-4101 (30). Among the compounds examined thus far, [^{3}H]prazosin is presently the most selective ligand for identifying *alpha*$_1$-adrenoceptors.

ACKNOWLEDGMENTS

The generous gifts of drugs by Ciba-Geigy, Glaxo, Hoffmann-La Roche, Janssen Pharmaceutica, Organon, Sandoz, Ward Blenkinsop, Wander, Warner, and Wyeth are gratefully acknowledged. The authors are most obliged to Dr. M. J. Davey (Pfizer, Sandwich, Kent, United Kingdom) for providing tritiated and unlabeled prazosin, UK-18,596, and UK-33,274, and to Dr. J. de Boer (University of Amsterdam) for the synthesis of 6-methyl- and 7-chloropiperoxane.

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