Biochemical Pharmacology, Vol. 28, pp. 2409–2411.

0006-2952/79/0801-2409 $02.00/0

Interaction of prazosin with alpha-adrenergic receptors—*In vitro* binding and *in vivo* antagonism

(*Received* 20 *February* 1978; *accepted* 15 *January* 1979).

Prazosin hydrochloride (MINIPRESS) has been shown to reduce blood pressure in both experimental animals [1, 2] and in man [3]. The mechanism(s) of its antihypertensive action reportedly involve peripheral vasodilation resulting from both direct vascular smooth muscle relaxation (possibly through inhibition of phosphodiesterase activity) and interference with peripheral sympathetic function [4, 5]. There is an accumulating body of evidence suggesting that prazosin possesses alpha-adrenergic blocking properties [6–11]. In a preliminary study, we reported that prazosin interacts with peripheral alpha-adrenergic receptor sites [12]. More recent biochemical studies suggest that the drug binds to central alpha adrenergic receptors [13, 14]. In this communication, we provide direct biochemical evidence that prazosin competes with [^{3}H-]dihydroergocryptine for specific alpha-adrenergic binding sites in rabbit uterine membrane preparations. In addition, we have extended these *in vitro* findings to evaluate *in vivo* alpha-adrenergic blocking potency of the drug.

Alpha-adrenergic receptor assays. All procedures were conducted at 5°, according to the method of Williams *et al.* [15]. Frozen type II mature rabbit uteri were purchased from Pel-Freeze Biologicals, Rogers, AR. After thawing, fat was removed and endometrial cells were scraped free with a scalpel. Six uteri were sliced and finely minced with scissors in a solution containing 0.25 M sucrose 5 mM Tris–HCl (pH 7.4), and 1 mM $MgCl_2$. The minced tissue was homogenized 4 times for 5 sec intervals using a Tekmar model SDT tissuemizer at maximum setting. After filtration through cheese cloth, the homogenate was centrifuged at 400 *g* for 10 min, and the supernatant fraction was collected with a Pasteur pipette. The supernatant liquid was then centrifuged for 10 min at 39,000 *g*. The resulting pellet was homogenized using a Teflon–glass tissue homogenizer in 50 mM Tris–HCl (pH 7.4) and 10 mM $MgCl_2$. The resuspended pellet was centrifuged for 10 min at 39,000 *g* , and the pellet washed twice more in the same buffer. The final pellet was homogenized in 20 ml of the same buffer.

In the binding assay, the radioligand used was dihydroergocryptine-[9, 10 ^{3}H-(N)], purchased from New England Nuclear, Boston, MA with a specific activity of 24 Ci/mmole. Non-labeled drugs were dissolved or suspended in 10% dimethylsulfoxide (DMSO) in water. Ascorbic acid (0.1%) was added when catecholamines were studied. The incubation consisted of 25 μl of [^{3}H-]dihydroergocryptine (~ 40,000 cpm; ~ 2 pmoles) 50 μl of the test drug and 100 μl of the uterine membrane. The incubation was conducted for 15 min at 25° in 12 × 75 mm plastic test tubes (Falcon 2052). The binding reaction was terminated by the addition of 5 ml of room temperature 50 mM Tris–HCl (pH 7.5) and 10 mM $MgCl_2$. The membranes were then washed with five additional 5-ml aliquots of the same buffer.

Specific binding of [^{3}H]dihydroergocryptine to alpha-adrenergic binding sites was calculated by subtracting the radioactivity bound to uterine membranes in the presence of 1.5×10^{-4} M phentolamine from that bound in the absence of phentolamine. Standard non-labeled alpha-adrenergic agonist and antagonists were consistent with previous work which [^{3}H]dihydroergocryptine binding to this level. Specific binding averaged 53 ± 2 per cent. Binding of the ligand to the filter disc was approximately 20 per cent. Specific binding to uterine receptor sites was saturable; Scatchard analysis of saturation experiments revealed a K_d for [^{3}H]dihydroergocryptine of 5.3 nM and 0.1 pmole/mg of protein binding sites. Titration experiments indicated that specific binding was linear within the range of the membrane protein concentration (2–4 mg protein/ml) used in the assays. The relative affinities of selected standard adrenergic agonists and antagonists were consistent with previous work which characterized alpha-adrenergic receptors (Table 1).

Phosphodiesterase inhibition assays. Phosphodiesterase inhibition assays were conducted in duplicate by methods described previously [16]. The enzyme sources were rat brain and cynomolgas monkey thoracic and abdominal aorta, and the substrate was adenosine 3′, 5′-cyclic monophosphate, ammonium salt [^{3}H(C)] (sp. act. approximately 40 Ci/mmole), purchased from New England Nuclear.

Alpha-adrenergic blocking potency in vivo. Nine adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (35 mg/kg, i.v.). The dogs were bilaterally vagotomized and a femoral artery and vein were cannulated to monitor arterial blood pressure (Statham P23AA) and to administer drugs respectively. Recordings were made on a Beckman Dynograph. Alpha-adrenergic blocking potency was determined against phenylephrine-induced increases in diastolic blood pressure. Dose ratios (DR_{10}, dose of antagonist to cause an agonist dose ratio of 10) were calculated according to the method of Arunlakshana and Schild [17] by determining complete dose–response curves of phenylephrine before and after cumulative doses of prazosin or phentolamine. Dose–response curves were estimated by regression lines and one agonist dose ratio was extrapolated for each antagonist dose at the 50 per cent agonist response level. Data were then analyzed by a Schild plot [17]. Only one drug (prazosin or phentolamine) was tested in each dog. Data are expressed as DR_{10} and 95 per cent confidence limits.

Binding to alpha-adrenergic binding sites. The competition of prazosin and phentolamine with [^{3}H]dihydroergocryptine for specific alpha-adrenergic binding sites on rabbit uterine membranes is compared in Fig. 1. While phentolamine exhibited a sigmoidal binding-inhibition curve characteristic of standard alpha-adrenergic agonists and antagonists reported previously [15], prazosin had a biphasic inhibition curve, with an apparent IC_{50} for all binding sites of 1 μM. However, prazosin bound with high affinity to one population of sites titrated with [^{3}H]dihydroergocryptine, which it appeared to

Table 1. Competition of adrenergic agents for binding of [^{3}H]dihydroergocryptine to specific binding sites in rabbit uterine membranes

Compounds	IC_{50} (μM)
Phentolamine hydrochloride	0.04
Indoramine hydrochloride	1.5
Tolazoline hydrochloride	2.0
Propranolol hydrochloride	100.0
l-Epinephrine bitartrate	2.0
l-Norepinephrine bitartrate	3.5
l-Isoproterenol bitartrate	>150.0

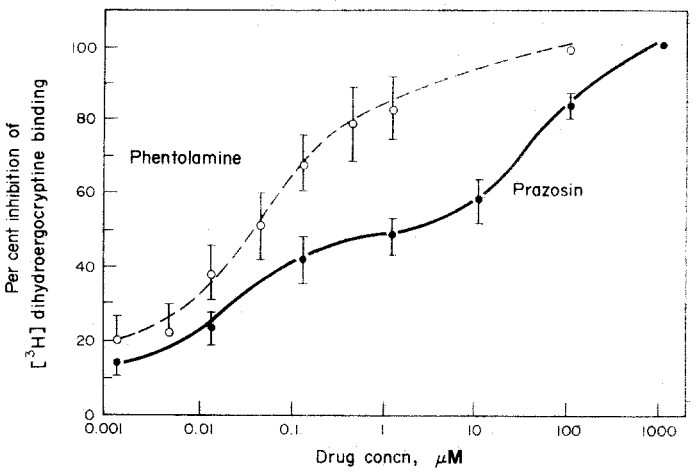

Fig. 1. Inhibition of binding of [^{3}H]dihydroergocryptine to specific alpha-adrenergic sites on rabbit uterine membranes by phentolamine (open circles; $\bar{x}$ + S.E.M. of eight determinations) and prazosin (closed circles; $\bar{x}$ + S.E.M. of twelve determinations).

saturate at a concentration of approximately 1 μM. Higher concentrations of the drug bound to additional sites. Complete displacement of the ligand was accomplished only at a concentration of 1000 μM. Assuming that the higher affinity population of binding sites labeled with [^{3}H]dihydroergocryptine was saturated by prazosin at approximately 1 μM, the half-displacement concentration of prazosin for those sites can be estimated at 0.015 μM. The IC_{50} for phentolamine was 0.04 μM.

Inhibition of phosphodiesterase activity. Prazosin was found to inhibit phosphodiesterase from rat brain and monkey aorta. Double reciprocal plots revealed prazosin to be a competitive inhibitor of the enzyme from brain, while the compound was a mixed inhibitor of the enzyme from aorta. Prazosin was intermediate in brain phosphodiesterase inhibitory potency (IC_{50} = 45 μM) between the potent inhibitor SQ20009 (IC_{50} = 3.7 μM) and theophylline (IC_{50} = 240 μM). Its relative potency against aorta phosphodiestrase was essentially the same.

In vivo *alpha-adrenergic antagonism.* Phenylephrine dose–response curves show a dose-related shift to the right with increasing doses of phentolamine or prazosin (Figs. 2 and 3 respectively). The DR_{10} of phentolamine (1.47 mg/kg) is in agreement with its DR_{10} previously reported in a similar experimental design (1.2 mg/kg) by Brittain and Levy [18]. Prazosin (0.37 mg/kg) was found in our studies to be four times more potent as a blocker of alpha-adrenergic receptors than phentolamine.

Our data indicate that prazosin interacts with both the active site of the enzyme phosphodiesterase and with peripheral alpha-adrenergic receptors. Prazosin competes with [^{3}H]dihydroergocryptine for specific alpha-adrenergic binding sites on rabbit uterine membranes. Prazosin exhibited a biphasic binding inhibition curve, which suggests that the compound binds to two different populations with different relative binding affinities for the drug. In this regard, U'Prichard *et al.* [13] demonstrated recently that prazosin has significantly greater affinity for alpha-receptors labeled [^{3}H]WB4101 (an alpha-antagonist) than for [^{3}H]clonidine (an agonist). Because [^{3}H]dihydroergocryptine is a partial agonist, it labels both populations of receptors [13]. It is reasonable to hypothesize that the competition of prazosin

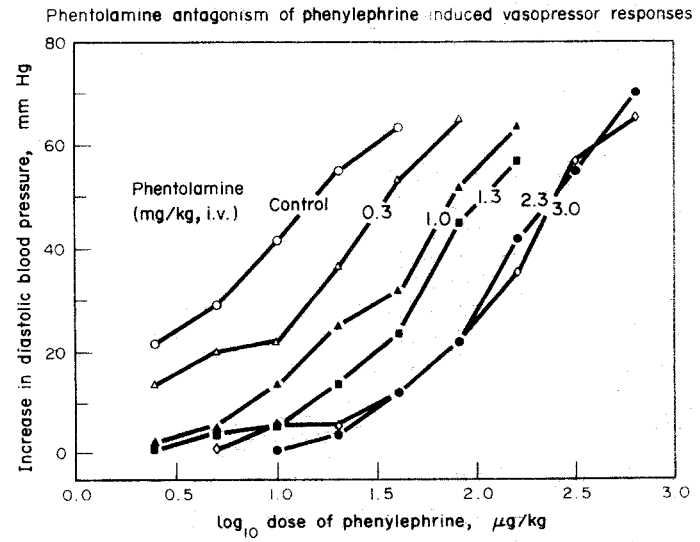

Fig. 2. Phenylephrine dose–response curves in vagotomized dogs before (control) and after cumulative doses of phentolamine. The DR_{10} (mg/kg, i.v.) and 95 per cent confidence intervals were calculated to be 1.47 (1.28, 1.73).

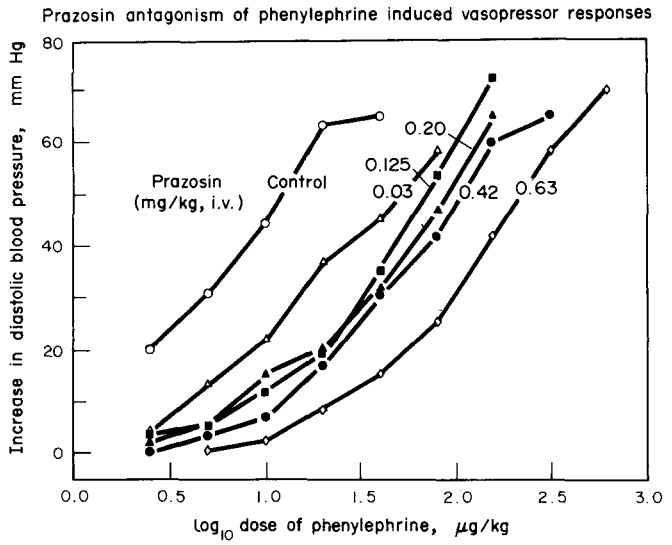

Fig. 3. Phenylephrine dose–response curves in vagotomized dogs before (control) and after cumulative doses of prazosin. The DR_{10} (mg/kg, i.v.) and 95 per cent confidence intervals were calculated to be 0.37 (0.36, 0.80).

with [^{3}H] dihydroergocryptine at the high affinity population of binding sites represents the greater affinity of the drug for selective antagonist-like sites.

Consistent with the *in vitro* binding data demonstrating the existence of at least two populations of alpha-adrenergic receptors are the pharmacological data of Berthelsen and Pettinger [19]. They hypothesized two distinct post-synaptic alpha-adrenergic receptors, one with properties associated with classical post-synaptic receptors (α_1), the other with properties similar to pre-synaptic autoreceptors (α_2). There is, in fact, evidence that alpha-adrenergic binding sites labeled with alpha antagonists may correspond to post-synaptic (α_1) receptors, while sites labeled with alpha-agonists correspond to pre-synaptic (α_2) receptors [20]. If prazosin binds with higher affinity to antagonist-like sites, we would speculate that it acts preferentially at α_1-receptors.

In *our vivo* alpha-blocking potency comparison, we found prazosin to be four times more potent than phentolamine. A number of studies previously conducted *in vivo* and *in vitro* [7, 8, 13] consistently indicate that prazosin is a more potent antagonist (2.5–13 times, depending on the experimental preparation) of alpha-adrenergic receptors than is phentolamine.

Data derived from our studies, although confirming that inhibition of phosphodiesterase is a biochemical effect of prazosin, provide direct evidence that it interacts with alpha-receptors and that blockade of these receptors is a major pharmacological property of the drug.

Divisions of Biochemical Research and Pharmacology, Ortho Pharmaceutical Corp., Raritan, NJ 08869, *U.S.A.*

FORREST C. GREENSLADE
CYNTHIA K. SCOTT
MARK CHASIN
SHERRIE M. MADISON
ALFONSO J. TOBIA

REFERENCES

1. M. J. Davey and R. Massingham, *Curr. med. Res. Opin.* **4**, 47 (1976).
2. J. W. Constantine, W. K. McShane, A. Scriabine and Hans-Jürgen Hess, *Postgrad. Med.* **58**, 18 (1975).
3. G. S. Stokes and M. A. Weber, *Br. med. J.* **2**, 298 (1974).
4. R. Scivoletto, A. J. O. Toledo, A. C. Gomes DaSilva and D. Nigro, *Archs. int. pharmacodyn. Thér* **223**, 333 (1976).
5. H. J. Hess, *Postgrad. Med.* **58**, 9 (1975).
6. R. M. Graham, H. F. Oates, L. M. Stoker, and G. S. Stokes, *J. Pharmac. exp. Ther.* **201**, 747 (1977).
7. P. Bolli, A. J. Wood, E. L. Phelan, D. R. Lee and F. O. Simpson, *Clin. Sci. Med.* **48**, 1775 (1975).
8. M. Commarato, A. E. Langley, D. H. Dugan, E. C. Lattime, R. D. Smith, D. K. Tessman and H. R. Kaplan, *Clin. exp. Hyper.* **1**, (2), 191 (1978).
9. I. Cavero and F. Lefevre, *Clin. exp. Pharmac. Physiol.* **3**, (suppl.), 61 (1976).
10. H. F. Oates, R. M. Graham, L. M. Stoker and G. S. Stokes, *Archs int. Pharmacodyn Thér* **224**, 239 (1976).
11. J. Constantine, R. A. Weeks, and W. K. McShane, *Eur. J. Pharmac.* **50**, 51 (1978).
12. C. K. Scott, K. M. Krider, K. L. Newquist, M. Chasin and F. Greenslade, *Fedn. Proc. Fedn Am. Socs exp. Biol.* **37**, 351 (1978).
13. D. C. U'Prichard, M. E. Charness, D. Robertson and S. Snyder, *Eur. J. Pharmac.* **50**, 87 (1978).
14. P. J. Miach, J. Dausse and P. Meyer, *Nature, Lond.* **274**, 492 (1978).
15. L. T. Williams, D. Mullikin and R. J. Lefkowitz, *J. biol. Chem.* **251**, 6915 (1976).
16. M. Chasin and D. N. Harris, in *Methods in Cyclic Nucleotide Research* (Ed. M. Chasin), p. 169. Marcel Dekker, New York (1972).
17. O. Arunlakshana and H. O. Schild, *Br. J. Pharmac. Chemother.* **14**, 48 (1959).
18. R. T. Brittain and G. P. Levy, *Br. J. clin. Pharmac.* **3**, (4) (suppl.), 681 (1976).
19. S. Berthelsen and W. A. Pettinger, *Life Sci.* **21**, 595 (1978).
20. S. J. Peroutka, D. A. Greenberg, D. C. U'Prichard and S. H. Snyder, *Molec. Pharmac.* **14**, 403 (1978).