Biochemical Pharmacology, Vol. 28, pp. 1233–1237.

0006-2952/79/0401-1233 $02.00/0

ALPHA-ADRENERGIC BLOCKING ACTIVITY OF PRAZOSIN

EFFECT ON CATECHOLAMINE LEVELS AND CATECHOLAMINE SYNTHETIC ENZYMES

FLOYD L. ATKINS and GIAN L. NICOLOSI*

Department of Medicine, University of Kansas Medical Center, 39th and Rainbow Blvd, Kansas City, KS 66103, U.S.A.†

(*Received* 5 *June* 1978; *accepted* 1 *September* 1978)

Abstract—Prazosin, a new antihypertensive agent, was demonstrated to be an effective and long lasting α-blocker. In comparison with phenoxybenzamine, prazosin caused a similar hypotensive response but failed to induce tachycardia. Both drugs depleted the norepinephrine content and stimulated an increase in tyrosine hydroxylase activity of the adrenal gland. Also, the norepinephrine content of the heart was decreased by chronic administration of each drug. Cardiac dopamine-beta-hydroxylase activity, although decreased by phenoxybenzamine, was unchanged by prazosin. The effect of prazosin on heart rate and cardiac dopamine-beta-hydroxylase differs from the traditional α-blockers and could reflect the affinity of the drug for the post-synaptic alpha-receptor.

Prazosin is a new antihypertensive agent which has been demonstrated to effectively lower blood pressure in many mammalian species [1–3]. Although its hypotensive action has been ascribed mainly to direct relaxation of vascular smooth muscle [4], prazosin has potent alpha-adrenergic blocking properties and recent investigations have suggested that interference with peripheral sympathetic function contributes to the vasodilatation [5]. Accordingly, we compared the effects of prazosin on heart rate and blood pressure in normotensive rats with those of phenoxybenzamine, a known alpha-blocking agent. To determine if prazosin affects catecholamine metabolism, we also measured norepinephrine (NE) levels in the heart and adrenals of these animals as well as plasma and cardiac dopamine-beta-hydroxylase (DBH) and adrenal tyrosine hydroxylase activities.

MATERIALS AND METHODS

Normotensive, male Sprague–Dawley rats weighing 200–250 g were used. Prazosin (10 mg/kg) or phenoxybenzamine (25 mg/kg) as the chloride salts, dissolved either in ethanol or as a suspension in 5% dextrose in water, was injected intraperitonealy twice daily for 3–4 days. The animals were studied 2 hr after the last dose.

Animals were anesthetized with sodium amobarbital (15 mg/100 g). The left femoral artery was catheterized and connected to a pressure transducer for recording blood pressure on a Gilson polygraph. Electrocardiograms were used to monitor heart rate. After monitoring for 5 min, a 1-ml sample of arterial blood was quickly withdrawn for plasma DBH and plasma renin determinations. The animal was then killed by cervical dislocation and the heart and adrenal glands were removed rapidly, rinsed, blotted dry and frozen for catecholamine and enzyme determinations.

In some experiments, the internal jugular vein was also cannulated and blood pressure responses to injected norepinephrine (0.45 μg/kg) were determined before and after intravenous injections of either prazosin (1 mg/kg) or phentolamine (0.4 mg/kg).

Some animals were denervated chemically by the administration of a single dose of 6-hydroxydopamine (80 mg/kg, i.p.) and studied 48 hr after this dose. Hearts from animals treated in this way contained 0.099 ± 0.06 μg/g of NE, while control hearts contained 0.802 ± 0.03 μg/g of NE.

Assay of norepinephrine. Hearts were homogenized with a Willems Polytron homogenizer (Brinkman Instruments, Westbury, NY) in 4 vol. of distilled water, and aliquots were removed for enzyme analysis. The remainder was placed in 4 ml of 15% trichloroacetic acid (TCA) and spun at 12,100 *g* in a Sorvall refrigerated centrifuge for 15 min. The supernatant fluid was adjusted to pH 8.4 with NaOH and passed over an alumina column (aluminum oxide, BDH Chemicals, Poole, England). After washing with 50 cc water, norepinephrine was eluted from the alumina in 0.2 N perchloric acid. A portion of the eluate was assayed for norepinephrine by the addition of potassium ferricyanide and alkaline ethylene diamine solution and read within 30 min in a Farrand spectrofluorometer at an activation wave length of 408 nm and emission at 508 nm [6].

Adrenals were homogenized in 19 vol. of distilled water and 100 μl was added to 4 ml of 15% TCA, which was then taken through the same procedure outlined above.

Tyrosine hydroxylase assay. Tyrosine hydroxylase was assayed by a micromodification of the method of Nagatsu *et al.* [7], measuring tritium release from labeled tyrosine. The reaction mixture consisted of 0.1 ml of adrenal homogenate, 4×10^4 cpm of [3-5-^{3}H]-L-tyrosine (40–60 Ci/m-mole), 4.0 nmoles of cold L-tyrosine, 40 nmoles NSD-1055, 200 nmoles ferrous

* Present address: Cardiologia, Ospedale Civile, Pordenone, Italy.

† Reprint requests should be sent to Dr. F. L. Atkins at this address.

ammonium sulfate, 40 nmoles mercaptoethanol, 400 nmoles $DMPH_4$ (2-amino-4 hydroxy-6-7 dimethyl-5,6,7,8-tetrahydropteridine hydrochloride) and 40 m-moles of acetate buffer, pH 6.0.

The reaction mixture was incubated in a Thunberg tube at 37.5° for 10 min in a water bath and was then frozen in the bottom of the tube; the tubes were evacuated to a pressure of 10 μ of Mercury with a vacuum pump. The Thunberg tubes were closed and the top head was placed in dry ice; the bottom was left at room temperature. The tritiated water was allowed to sublime into the head of the Thunberg tube and a 100-μl aliquot was removed and assayed for radioactivity in a liquid scintillation spectrometer (Searle Mark III); the counting efficiency was 23 per cent for ^3H.

Serum and tissue DBH. One ml of whole blood was removed from the arterial cannula in a heparinized syringe. Plasma was separated by centrifugation at 300 *g* for 5 min and stored at 75° until assayed. The heart was homogenized in 4 vol. of distilled water and assayed on the same day. The radioenzymatic assay of Wilcox and Beaven [8] was used for measurement of DBH in both serum and tissues. A 100-μl aliquot of plasma or tissue was mixed with 100 μl of a reagent solution which contained ascorbate, 8 μmoles; dibasic sodium fumarate, 11.5 μmoles; catalase, 1500 units; *N*-ethylmaleimide, 6 μmoles; [7–^{3}H]dopamine, 10 pmoles (8×10^4 dis./min); and sodium acetate buffer, pH 5.2, 100 μmoles. Monoamine oxidase inhibitors were not included in the incubation media since we demonstrated that they did not affect enzyme activity. The incubation was carried out in the bottom of a Thunberg tube which was flushed with approximately 0.5 liter of oxygen and closed. The tubes were incubated at 37° for 1 hr, with constant shaking, and the reaction was stopped by placing the tubes in ice water and adding 200 μl of 0.8 N perchloric acid containing dopamine (250 μg/ml. The tritiated water was sublimed and counted as described in the tyrosine hydroxylase assay. Statistical analysis was carried out by Student's *t*-test for comparison between groups. Plasma renin was measured by Dr. Alan Poisner using a radioimmunoassay [9].

Materials. Prazosin was a gift from Pfizer Laboratories (Groton, CT). Phenoxybenzamine was a gift from Smith Kline & French Laboratories (Philadelphia, PA). Phentolamine HCl was a gift from Ciba Pharmaceuticals (Summit, NJ). Sodium amorbarbital (Amytal Sodium) was purchased from the Eli Lilly Co. (Indianapolis, ID). Labeled compounds, ([7–^{3}H]dopamine and [3–5-^{3}H]-tyrosine) were obtained from the New England Nuclear Corp. (Boston, MA); 6-OH dopamine HBr was purchased from the Regis Chemical Co., (Morton Grove, IL).

RESULTS

The effects of prazosin or phenoxybenzamine on systolic blood pressure and heart rate are shown in Fig. 1. Prazosin and phenoxybenzamine both caused a comparable and significant decrease in systolic blood pressure. However, while in the phenoxybenzamine group there was a significant increase in heart rate, the heart rates of the prazosin-treated animals were unchanged from the control values.

In acute experiments we measured the blood pressure response to a 0.45 μg/kg i.v. bolus of norepinephrine before and after 1 mg/kg of i.v. prazosin (Fig. 2). Prazosin produced an immediate drop of about 20 mm Hg in systolic blood pressure and markedly inhibited the subsequent response to intravenous norepinephrine. in animals treated for 3 days with prazosin (10 mg/kg, i.p.), the antagonism to an injected bolus of norepinephrine lasted for at least 18 hr after the last dose. Because others have reported the need for an intact nervous system for prazosin to be effective, we studied the effect of the drug in chemically sympathectomized animals (Table 1). The pressor response to a bolus of norepinephrine was exaggerated significantly in the denervated group, indicating a denervation hypersensitivity. However, despite physiological and biochemical evidence of denervation, the hypotensive response to prazosin occurred.

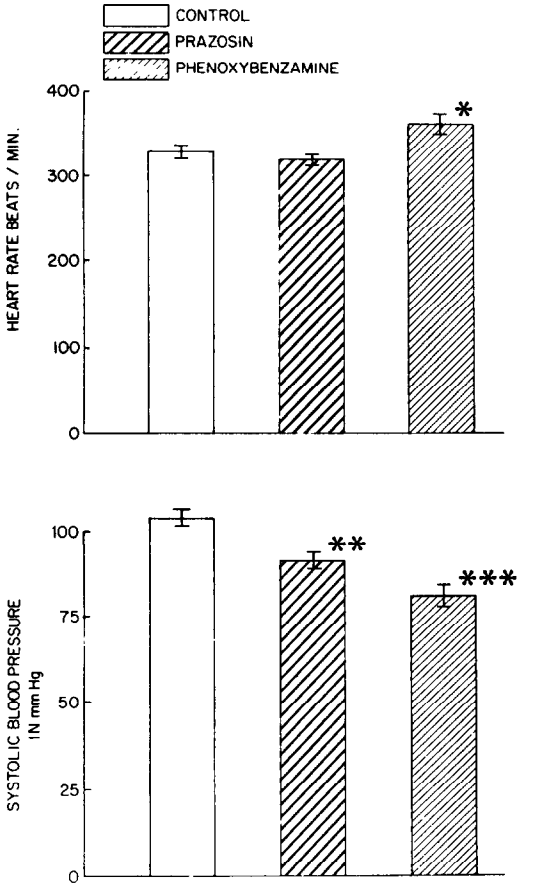

Fig. 1. Effects of prazosin and phenoxybenzamine on rat blood pressure and heart rate. The bars and brackets show means ± S. E. M. Asterisks denote that the values are significantly different from control: *P < 0.05; **P < 0.01; and ***P < 0.001.

In experiments where a rapidly acting alpha-blocking agent, phentolamine, was given in a dose of 0.4 mg/kg, the addition of intravenous prazosin caused no further drop in blood pressure. Conversely, no further effect was noted when prazosin was given first, followed by phentolamine.

Both prazosin and phenoxybenzamine, when administered chronically, depleted the adrenal content of

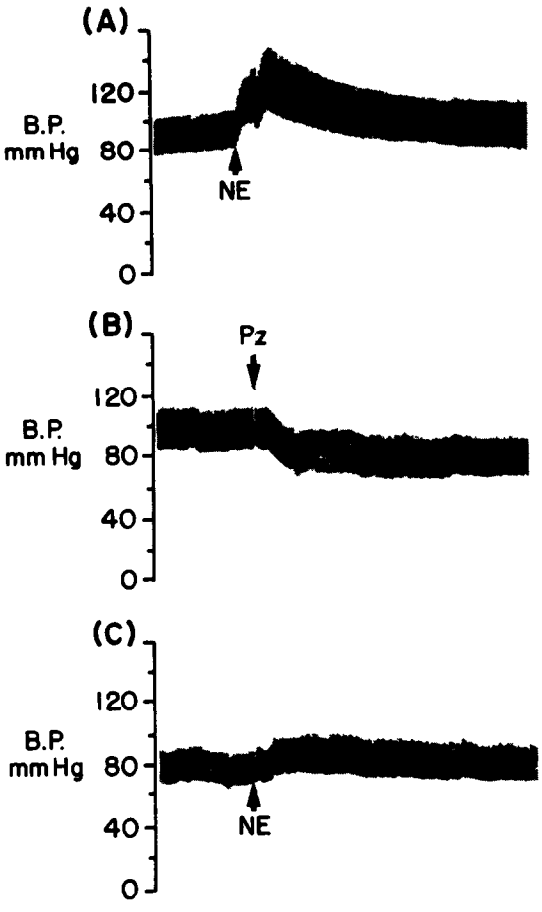

Fig. 2. Typical response of blood pressure to an i.v. bolus of 0.45 μg/kg of NE (A); 1 mg/kg, i.v., prazosin (B); and 0.45 μg/kg of NE after 1 mg/kg of prazosin (C).

norepinephrine markedly. This depletion stimulated a reflex increase of 30–60 per cent in the activity of adrenal tyrosine hydroxylase (Table 2). Cardiac stores of norepinephrine were also depleted by both of these drugs, in the doses administered.Phenoxybenzamine also caused a significant decrease in DBH activity in the heart, while that in the prazosin-treated animals was unchanged from controls (Table 3). Plasma DBH was also unchanged by chronic treatment with either prazosin or phenoxybenzamine (Table 4).

Plasma renin activity was found to be increased in the animals treated chronically with phenoxybenzamine, but was unchanged from controls in the group receiving prazosin chronically (Table 5).

DISCUSSION

Initial studies of prazosin attributed its hypotensive action primarily to direct relaxation of arteriolar vascular smooth muscle [10]. More recent investigations have confirmed the α-receptor blocking properties of prazosin and suggested a sympatholytic mode of action [2, 11]. The present study also confirms the α-blocking properties of prazosin since it inhibits the pressor response to norepinephrine. Destruction of the sympathetic nervous system with 6-OH dopamine did not interfere with its hypotensive action, probably because the denervation was not complete; other studies utilizing ganglionic blockade have failed to demonstrate a direct relaxant effect of the drug [12]. The lack of additional response with serial administration of phentolamine or prazosin in any order suggests that these agents are competing for the same α-receptor sites, and recent *in vitro* binding studies support the affinity of prazosin for the α-receptor [13]. As demonstrated in this study, prazosin differs from the α-adrenergic blocking agent phenoxybenzamine in that its chronic administration does not cause tachycardia. In addition, we demonstrate that chronic prazosin administration does not evoke the increase in plasma renin activity seen after phenoxybenzamine. This lack of renin increase with prazosin has been reported by others after the acute administration of the drug [12, 14]. These differences in response between prazosin and phenoxybenzamine are examples of dissimilarities in the action of these alpha-blocking drugs. Accordingly the functional α-receptor blockade by prazosin has been demonstrated to be unlike that caused by conventional blocking agents. Prazosin appears to have

Table 1.*

BLOOD PRESSURE RESPONSE TO ADMINISTRATION OF NOREPINEPHRINE AND PRAZOSIN

TREATMENT	n	NOREPINEPHRINE	PRAZOSIN	NOREPINEPHRINE AFTER PRAZOSIN
CONTROLS	6	+43.4 ± 3.2	-20.3 ± 7.4	+23 ± 3.4**
DENERVATED	7	+76.4 ± 8.6*	-16.2 ± 4.7	+22 ± 2.3***

* Values are means ± S. E. M. Key for symbols: + = increase; − = decrease.A single asterisk (*) beside a value indicates a $P < 0.005$ when compared to the nondenervated group; a double asterisk (**) indicates $P < 0.005$, when compared to the response before prazosin in the control group; and a triple asterisk (***) indicates $P < 0.001$, when compared to the response before prazosin in the denervated group.

Table 2.*

EFFECT OF TREATMENT WITH PRAZOSIN OR PHENOXYBENZAMINE ON ADRENAL NOREPINEPHRINE LEVELS AND ADRENAL TYROSINE HYDROXYLASE ACTIVITY

TREATMENT	NOREPINEPHRINE (μg/gland)	TYROSINE HYDROXYLASE ACTIVITY (nmoles/hr/gland)
CONTROL	8.41 ± 0.65 (20)	6.02 ± 0.31 (21)
PRAZOSIN	4.61 ± 0.51 •• (19)	10.08 ± 0.76 •• (20)
PHENOXYBENZAMINE	3.78 ± 0.63 •• (19)	8.26 ± 0.60 • (21)

* Values are means ± S. E. M. The number of animals is given in parentheses. Asterisks beside values denote significant difference from control: *P < 0.005; and **P <0.001.

Table 3.*

CARDIAC NOREPINEPHRINE LEVELS AND CARDIAC DOPAMINE-B-HYDROXYLASE ACTIVITY AFTER THE ADMINISTRATION OF PRAZOSIN OR PHENOXYBENZAMINE

TREATMENT	NOREPINEPHRINE (μg/g)	DOPAMINE-B-HYDROXYLASE ACTIVITY (pmoles/hr/g)
CONTROL	0.654 ± 0.041 (15)	0.959 ± 0.063 (15)
PRAZOSIN	0.379 ± 0.024** (15)	0.851 ± 0.033 (13)
PHENOXYBENZAMINE	0.253 ± 0.020** (15)	0.694 ± 0.035 • (14)

* Values are means ± S. E. M. The number of animals is given in parentheses. Asterisks denote significant difference from control: *P < 0.005; and **P < 0.001.

Table 4.*

PLASMA DOPAMINE-B-HYDROXYLASE ACTIVITY AFTER PRAZOSIN OR PHENOXYBENZAMINE ADMINISTRATION

TREATMENT	n	PLASMA DOPAMINE-B-HYDROXYLASE ACTIVITY pmole/100 μl/hr
CONTROL	(10)	0.579 ± 0.046
PRAZOSIN	(8)	0.567 ± 0.053 (NS)
PHENOXYBENZAMINE	(8)	0.559 ± 0.049 (NS)

* Values are means ± S. E. M. NS = not significant from control.

Table 5.*

PLASMA RENIN ACTIVITY AFTER THE ADMINISTRATION OF PRAZOSIN OR PHENOXYBENZAMINE

TREATMENT	n	PLASMA RENIN ACTIVITY (ng/hr/ml)
CONTROL	10	70.5 ± 13.1
PRAZOSIN	10	84.1 ± 12.7
PHENOXYBENZAMINE	11	209 ± 25.2*

* Values are means ± S. E. M. A single asterisk beside a value denotes a significant difference from control, $P < 0.05$.

an affinity for post-synaptic α-receptors, while phenoxybenzamine blocks both pre- and post-synaptic α-receptors [15, 16]. Our *in vivo* findings that DBH is depleted significantly by phenoxybenzamine are in agreement with the data of Reid and Kopin [17] who noted similar reductions in cardiac DBH without changes in the serum enzyme. The lack of DBH depletion by prazosin may possibly reflect its decreased affinity for the pre-synaptic alpha-receptor. De Potter *et al.* [18] were able to demonstrate, with low frequency nerve stimulation of the isolated spleen, that there was an overflow of DBH, as well as of norepinephrine, in the presence of phenoxybenzamine. Others have shown that prazosin can block the overflow of norepinephrine [15].

The effects of these two drugs on adrenal norepinephrine and tyrosine hydroxylase were the same and typical of those reported previously for α-blockade [19], being mediated probably through increased cholinergic input to the adrenal gland [20]. It appears that these changes are due primarily to the effects of the drugs on alpha-receptors and are not just reflex changes due to hypotension. Hydralazine derivatives, which also produce hypotension, but by direct vasodilatation, have been shown to have little or no effect on adrenal catecholamine content [21].

It is evident that prazosin produces an effective and long-lasting α-blockade. Its effects on catecholamine metabolism parallel in some respects those of phenoxybenzamine. Its effects on heart rate, cardiac dopamine-beta-hydroxylase and plasma renin activity are different and probably reflect the lack of affinity of prazosin for the pre-synaptic alpha-receptor.

Acknowledgements—This study was supported in part by a grant from the National Institutes of Health HL21973-01. We wish to thank Dr. Alan Poisner for performing the plasma renin assays.

REFERENCES

1. A. J. Wood, E. L. Phelan and F. O. Simpson, *Clin. exp. Pharmac. Physiol.* **2**, 297 (1975).
2. R. Scivoletto, A. J. O. Toledo, A. C. Gomes DaSilva and D. Nigro, *Archs int. Pharmacodyn. Thér.* **223**, 333 (1976).
3. P. Kincaid-Smith, I. M. MacDonald, A. Hua, M. C. Laver and P. Fang, *Med. J. Aust.* **1**, 327 (1975).
4. J. W. Constantine, W. K. McShane, A. Scribine and H. J. Hess, in *Hypertension: Mechanisms and Management* (Eds G. Onesti, K. E. Kim and J. H. Moyer), p. 429. Grune & Stratton, New York (1973).
5. R. M. Graham, H. F. Oats, L. M. Stoker and G. S. Stokes, *J. Pharmac. exp. Ther.* **201**, 747 (1977).
6. M. C. Boadle-Biber, J. Hughes and R. H. Roth, *Br. J. Pharmac.* **40**, 702 (1970).
7. T. Nagatsu, M. Levitt and S. Udenfriend, *Analyt. Biochem.* **9**, 122 (1964).
8. G. Wilcox and M. A. Beaven, *Analyt. Biochem.* **75**, 484 (1976).
9. D. L. Eaton and A. M. Poisner, *Proc. Soc. exp. Biol. Med.* **154**, 6 (1977).
10. J. W. Constantine, in *Prazosin—Evaluation of a New Antihypertensive Agent* (Ed. D. Cotton), Excerpta Medica, Amsterdam (1974).
11. I. Cavero, F. Lefevre and A. Poach, *Fedn. Proc.* **36**, 955 (1977).
12. H. Oates, R. Graham, L. Stoker and G. Stokes, *Archs int. Pharmacodyn. Thér.* **224**, 239 (1976).
13. C. K. Scott, K. M. Krider, K. L. Newquist, M. Chasin and F. C. Greenslade, *Fedn Proc.* **37**, 351 (1978).
14. R. Massingham and M. Hayden, *Eur. J. Pharmac.* **30**, 121 (1975).
15. D. Cambridge, M. Davey and R. Massingham, *Br. J. Pharmac.* **59**, 514P (1977).
16. S. Langer, *Biochem. Pharmac.* **23**, 1793 (1974).
17. J. Reid and I. Kopin, *J. Pharmac. exp. Ther.* **193**, 748 (1975).
18. W. De Potter, I. Chubb, A. Put and A. Schaepdryver, *Archs int. Pharmacodyn. Thér.* **193**, 191 (1971).
19. W. Dairman and S. Udenfriend, *Molec. Pharmac.* **6**, 350 (1970).
20. R. Patrick and N. Kirshner, *Molec. Pharmac.* **7**, 87 (1970).
21. O. Linet, P. Van Zwieten and G. Hertting, *Eur. J. Pharmac.* **6**, 121 (1969).