J. Pharm. Pharmacol. 1986, 38: 113–117
Received August 14, 1985

α-Adrenoceptor blocking activity of fenoldopam (SK&F 82526), a selective DA_1 agonist

SHIGERU NAKAMURA, JAI D. KOHLI, AND SOL I. RAJFER*

Committee on Clinical Pharmacology, Departments of Pharmacological and Physiological Sciences and Medicine, Section of Cardiology, The University of Chicago, 947 East 58th Street, Chicago, Illinois 60637, USA

The α-adrenoceptor blocking activity of fenoldopam (SK&F 82526), a selective dopamine vascular receptor (DA_1) agonist, was evaluated in isolated segmental preparations of rabbit aorta, dog mesenteric and rabbit splenic arteries. Fenoldopam, in concentrations ranging from 10^{-6} to 10^{-4} M, produced parallel, dextral shifts of concentration–contractile response curves to noradrenaline. Slopes of the Schild regression lines were not significantly different from unity in the three vessels. pA_2 values for fenoldopam in the rabbit aorta, dog mesenteric and rabbit splenic arteries were 5·48 ± 0·08, 5·78 ± 0·05, and 5·20 ± 0·05, respectively. In experiments where the drug was added to the bathing medium before exposing the vascular segments to the irreversible α-adrenoceptor antagonist, phenoxybenzamine, fenoldopam provided nearly complete protection against α-adrenoceptor blockade. These results demonstrate that fenoldopam possesses α-adrenoceptor antagonist activity and competes with α-phenoxybenzamine, for occupancy at the same receptor site.

In studies characterizing the vascular dopamine (DA_1) receptor, pretreatment of the preparations with phenoxybenzamine was necessary to exclude vasoconstrictor activity of DA and putative DA_1 agonists (Goldberg & Toda 1975; Hilditch & Drew 1981; Brodde 1982). With the discovery of fenoldopam (SK&F 82526; 6-chloro-7,8-dihydroxy-1-(*p*-hydroxyphenyl)-2,3,4,5-tetrahydro-[1*H*]-3-benzazepine) as a selective DA_1 agonist (Hahn et al 1982), it became possible to study DA_1-mediated vascular relaxation without the use of an α-adrenoceptor blocking agent because the drug does not possess α-adrenoceptor agonist activity (Berkowitz & Ohlstein 1984). Thus, Ohlstein et al (1984) used noradrenaline (NA) as a contracting agent, in addition to the previously used contracting agents, KCl and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), to evaluate DA_1-mediated relaxation of isolated arterial vessels. They reported that fenoldopam was much weaker in relaxing preparations contracted with KCl or $PGF_{2\alpha}$ than it was in relaxing NA-contracted arterial segments. In all previous studies, however, $PGF_{2\alpha}$ was found to be the most suitable contracting agent for studying DA_1-induced relaxation (for references, see Brodde 1982). We hypothesized that the preferential relaxing activity of fenoldopam on NA-contracted preparations may be due to α-adrenoceptor blocking activity and undertook the present study to determine whether this agent possessed such activity.

* Correspondence.

METHODS

Two vascular preparations that have been used most frequently for studying vascular DA receptors (dog mesenteric and rabbit splenic arteries; for references, see Brodde 1982) and one preparation that has been classically used for studying α-adrenoceptors (rabbit aorta: Furchgott & Bhadrakom 1953) were included in this investigation.

Adult mongrel dogs of either sex, 20–25 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) and the mesenteric arteries excised. In addition, New Zealand White rabbits, 2–3 kg, and of either sex, were killed by an overdose of sodium pentobarbitone (60 mg kg^{-1} i.v.) and the splenic artery and thoracic aorta excised rapidly. The isolated vessels were cleaned of adherent connective tissue and cut into segments of approximately 4 mm in length. The rings were mounted in 20 ml organ baths filled with Krebs-Henseleit bicarbonate solution of the following composition (mM): NaCl 118·2; KCl 4·6; KH_2PO_4 1·2; $MgSO_4$ 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 24·8; dextrose 10·0. The solution was maintained at 37 ± 0·5 °C and aerated with a mixture of 95% O_2 and 5% CO_2. The bathing medium contained the β-adrenoceptor blocking agent, propranolol hydrochloride (3×10^{-6} M), the neuronal uptake inhibitor, cocaine hydrochloride (10^{-5} M), and the extraneuronal uptake inhibitor, hydrocortisone 21-hemisuccinate (3×10^{-5} M). Rings were fixed between hooks under a resting tension of 8 g for dog mesenteric artery and rabbit aorta, and 1 g for

rabbit splenic artery. Tension was measured by Grass FT03 force-displacement transducers and responses were recorded on a Grass model 7D polygraph. The preparations were allowed to equilibrate for a minimum of 45 min before testing; the bathing medium was replaced every 10 min during equilibration.

After the preparations had been tested with 30 mM KCl and 3×10^{-5} NA, cumulative concentration–response curves of contraction were generated by stepwise addition of 10^{-8} to 3×10^{-4} M NA. Successive concentration–response curves generated by NA in the absence of fenoldopam were reproducible in all three preparations with less than 2% variation. Fenoldopam (10^{-6} to 3×10^{-4} M) was added to the bathing medium 10 min before the concentration–response curve for NA was repeated. (Preliminary experiments demonstrated similar antagonism of the contractile responses to NA when the tissue preparations were exposed to fenoldopam for 10, 30, and 60 min before the second concentration–response curve for NA was generated.) With this approach, two or three incremental concentrations of drug could be studied in a given preparation with appropriate intervals (60–90 min) between successive concentration–response curves. Following the same procedure, effects of selected high concentrations of fenoldopam were also tested on contractions induced by $PGF_{2\alpha}$ (10^{-7} to 3×10^{-5} M) and KCl (10 to 90 mM) in the dog mesenteric artery and rabbit aorta. Additional experiments were conducted with SCH 23390 (10^{-7} M), a selective DA_1 antagonist (Goldberg et al 1984), added to the bathing medium 10 min before the study of the antagonism of NA by fenoldopam in the dog mesenteric artery.

To support further the notion that the drug was interacting with α-adrenoceptors, its ability to protect them against irreversible blockade by phenoxybenzamine was studied in dog mesenteric artery and rabbit aorta. Each experiment consisted of four preparations from the same vessel. One preparation was exposed to agonist (NA) alone and tested every 15 min for the stability of its contractile response to NA, 10^{-6} M, up to 90 min. The second and the third preparations were exposed to phenoxybenzamine and fenoldopam, respectively, for 10 min, washed, and then tested for their response to NA as in the first strip. The fourth segment was exposed first to fenoldopam for 10 min and then without washing, phenoxybenzamine was added to the bath for another 10 min. After both antagonists had been washed out, the preparation was then tested for its response to NA, 10^{-6} M, every 15 min up to 90 min. Serial exposure to NA commenced in each vessel at identical times after an initial contractile response to 10^{-6} M NA was obtained. Contractile responses are expressed as a percentage of the initial contraction elicited by 10^{-6}M NA.

Calculations and statistical analysis

Dose ratios (ratios of the EC50 values of NA before and after the addition of fenoldopam, where EC50 is the concentration of NA producing 50% of the maximal response) were calculated for each experiment from the concentration–response curves generated before and after the addition of different concentrations of fenoldopam. Schild plots were generated from these values and the line of best fit was determined by linear regression analysis. pA_2 values for fenoldopam were calculated from the equation: pA_2 = log (dose ratio-1)-log (antagonist concentration) (MacKay 1978). All results are expressed as mean ± standard error of the mean (s.e.m.). Where applicable, statistical analysis was accomplished using Student's *t*-test.

Drugs

Drugs used were: (−)-noradrenaline bitartrate and hydrocortisone 21-hemisuccinate (Sigma Chemical Co., St. Louis, MO); prostaglandin $F_{2\alpha}$ (Upjohn Co., Kalamazoo, MI); cocaine hydrochloride (Merck Chemical, Rahway, NJ); propranolol hydrochloride (Ayerst Laboratories, Inc., New York, NY); fenoldopam (SK&F 82526) and phenoxybenzamine hydrochloride (Smith Kline Laboratories, Philadelphia, PA); and SCH 23390 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol) (Schering Corp., Bloomfield, NJ).

RESULTS

In the first series of experiments, the effects of 10^{-6} to 3×10^{-4} M fenoldopam on the concentration–contractile response curves of NA were studied in canine mesenteric artery, rabbit thoracic aorta, and rabbit splenic artery. In each vessel the drug, in a concentration-dependent manner, shifted the NA concentration–response curves to the right. The shifts were parallel and without suppression of the maximal effect of NA, except for a slight depression in the maximal response at the highest concentration (3×10^{-4} M) of fenoldopam. As an example, concentration–response curves in the absence and in the presence of different concentrations of the drug for the dog mesenteric artery are shown in Fig. 1.

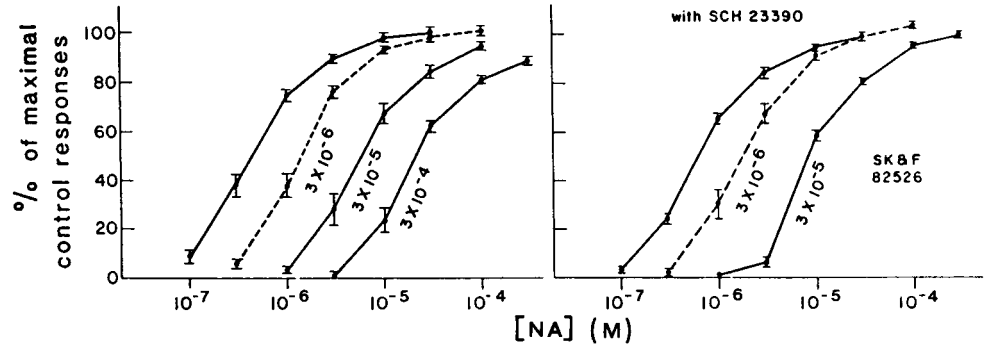

FIG. 1. Concentration–response curves of contraction induced by noradrenaline in dog mesenteric arterial rings in the absence and in the presence of different concentrations (as shown) of fenoldopam. In the right panel, 10^{-7} M SCH 23390 was added 10 min before adding fenoldopam. Vertical bars denote s.e.m.

Schild plots were generated using concentrations of the drug between 10^{-6} and 10^{-4} M, and the resulting slopes for the three vascular preparations did not differ significantly from unity (Table 1). The pA_2 values are also in Table 1.

Table 1. pA_2 values for fenoldopam as an α-adrenoceptor antagonist against noradrenaline.

Preparation	pA_2 values	Schild slope (95% confidence limits)
Dog mesenteric artery (n = 28)	5·78 ± 0·05	0·98 ± 0·08 (0·82–1·14)
Rabbit aorta (n = 22)	5·48 ± 0·08	1·25 ± 0·13 (0·99–1·51)
Rabbit splenic artery (n = 15)	5·20 ± 0·05	1·19 ± 0·12 (0·93–1·44)

Values are expressed as mean ± s.e.m.

The effects of fenoldopam on the contractions induced by KCl (10 to 90 mM) and $PGF_{2\alpha}$ (10^{-7} to 3×10^{-5} M) were also studied. In the dog mesenteric arterial rings, 3×10^{-5} M fenoldopam drug had no effect on contractions produced by $PGF_{2\alpha}$ (n = 9) or KCl (n = 5). Similarly, 3×10^{-5} M fenoldopam had no significant effect on the concentration–response curve for KCl (10 to 90 mM) in the rabbit thoracic aorta (n = 4).

To exclude the possibility that the DA_1 receptor-mediated relaxing effect of fenoldopam may have contributed to the decreased contractile activity of NA, the DA_1 action of the drug was eliminated by pre-exposure of dog mesenteric artery to 10^{-7} M SCH 23390, a selective DA_1 antagonist (Goldberg et al 1984), for 10 min before addition of fenoldopam and repeating concentration–response curves for NA. As shown in Fig. 1 (right panel), the rightward shift of concentration–response curves for NA after exposure to SCH 23390 and fenoldopam was not different from that caused by fenoldopam alone. The pA_2 value of 5·7 ± 0·10 (Schild slope = 1·03 ± 0·21, n = 6) obtained with the drug in the presence of SCH 23390 is similar to that obtained in the absence of SCH 23390 (Table 1).

In the second series of experiments the ability of fenoldopam to protect α-adrenoceptors against irreversible blockade by phenoxybenzamine was studied. Concentrations and duration of exposure to the different drugs used were selected following preliminary experiments. In the absence of any antagonist 10^{-6} M NA elicited reproducible responses in dog mesenteric artery throughout the 90 min experimental period (Fig. 2). Exposure to 10^{-8} M phenoxybenzamine for 10 min produced marked inhibition of the response to NA (10^{-6} M) throughout the 90 min. Antagonism produced by 10

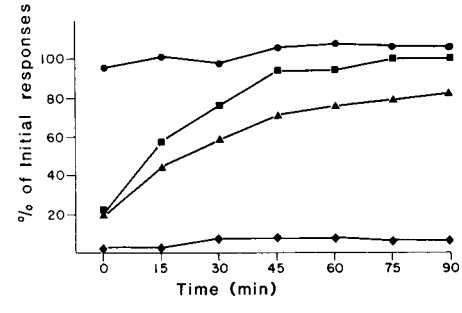

FIG. 2. Contractile responses to 10^{-6} M noradrenaline in four individual segments of dog mesenteric artery, recorded every 15 min and expressed as a percentage of the initial response to 10^{-6} M noradrenaline. In the first segment (●) no antagonist was added; in the second segment (■) 3×10^{-5} M fenoldopam was added for 10 min and washed out; in the third segment (▲) 10^{-8} M phenoxybenzamine was added for 10 min following initial exposure to 3×10^{-5} M fenoldopam for 10 min, and then both drugs were washed out. In the fourth strip (◆), 10^{-8} M phenoxybenzamine was added for 10 min and washed out. (For further explanation, see text.)

min exposure to 3×10^{-5} M fenoldopam disappeared 60–90 min after washing out the antagonist. When fenoldopam was added before phenoxybenzamine, the contractile response to NA attained 77·4 ± 6·9% of its control value at 90 min (Figs 2 & 3). The mean responses (n = 6) recorded at 90 min after washing out the antagonists are shown in Fig. 3.

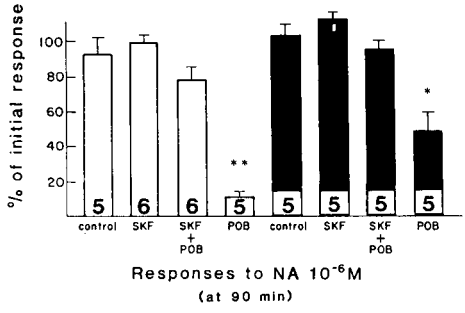

FIG. 3. Contractile responses to 10^{-6} M noradrenaline in isolated segments of dog mesenteric artery (open bars) and rabbit aorta (solid bars) expressed as a percentage of the initial response to 10^{-6} M noradrenaline. Responses were measured 90 min after the addition and subsequent removal 10 min later of fenoldopam (SKF) and phenoxybenzamine (POB), as well as after the sequential addition of fenoldopam (10 min) and POB (10 min, together with fenoldopam) and their subsequent removal (SKF + POB). Vertical bars denote s.e.m. $^{*}P < 0{\cdot}01$ for the difference in responses after POB and SKF + POB. $^{**}P < 0{\cdot}001$ for the difference in responses after POB and SKF + POB. The number of experiments is shown in each column.

Similar experiments were also conducted with segments of rabbit thoracic aorta using slightly different concentrations of antagonists (fenoldopam, 10^{-4} M; phenoxybenzamine, 3×10^{-8} M) as determined in preliminary experiments. Results are shown in Fig. 3. Exposure of the rabbit aorta to fenoldopam before phenoxybenzamine resulted in almost total elimination of the antagonism by phenoxybenzamine against the contractile responses to 10^{-6} M NA.

DISCUSSION

The results of this investigation demonstrate that fenoldopam is a competitive α-adrenoceptor antagonist with apparent dissociation constants that ranged from $1{\cdot}6 \times 10^{-6}$ to $6{\cdot}3 \times 10^{-6}$ M among the three vascular preparations. Its ability to protect α-adrenoceptors against irreversible block by phenoxybenzamine provides additional evidence that the same receptors are occupied by the two antagonists. These observations are supported by the report of Foley & Sarau (1984) who documented high affinity binding of fenoldopam to α_2-adrenoceptors and weaker affinity for α_1-adrenoceptors. Although we did not assess the relative α_1- and α_2-adrenoceptor blocking activity of the drug, our results are consistent with the report of Foley & Sarau (1984).

The inhibitory effect of α-adrenoceptor antagonists against contractions induced by NA (a non-selective α-adrenoceptor agonist) in rabbit thoracic aorta has been evaluated by other investigators. The pA_2 values obtained with the selective α_2-adrenoceptor antagonists, yohimbine (6.04, Ruffolo et al 1982) and rauwolscine (5·75, Timmermans et al 1984), are comparable to the value observed with fenoldopam in the present study. A similar pA_2 value (5·72) was seen with rauwolscine against another non-selective α-adrenoceptor agonist, α-methylnoradrenaline, in the rabbit aorta (Docherty & Starke 1981); prazosin, a selective α_1-adrenoceptor antagonist, was more potent (pA_2 = 8·4) than rauwolscine as an antagonist of the contractions elicited by α-methylnoradrenaline. In the canine mesenteric artery, previous studies have also demonstrated weaker antagonism of NA-induced contractions by yohimbine than by prazosin (Agrawal et al 1984; Sakakibara et al 1982). However, the pA_2 values obtained with yohimbine (7·2 to 7·6) and prazosin (8·4 to 8·7) were considerably higher than the pA_2 value determined for fenoldopam. Comparable data are not available for the rabbit splenic artery. These observations tend to suggest that the drug is a weak α-adrenoceptor antagonist that may possess greater affinity for the α_2-adrenoceptor subtype.

The observation that fenoldopam possesses α-adrenoceptor antagonist activity has several consequences. First, it invalidates the use of NA as a contracting agent for measuring DA_1-mediated relaxing activity of fenoldopam or for that matter, any DA_1 agonist that may have α-adrenoceptor agonist or antagonist activity. It may be noted that the EC50 of the drug as a DA_1 agonist (10^{-6} M; Ohlstein et al 1984) is only 2- to 3-fold lower than its pA_2 value as an α-adrenoceptor antagonist. Second, while our observation explains the preferential relaxing activity of fenoldopam on NA-contracted arterial strips vis-à-vis other contracting agents, it questions the identity of DA_1 receptors studied by in-vitro methods. Fenoldopam has been reported to be 3- to 10-fold more potent than DA as a DA_1 agonist when evaluated in-vivo (Hahn et al 1982; Weinstock et al 1983). According to the in-vitro results of Ohlstein et al (1984), however, when $PGF_{2\alpha}$ is used as the contracting agent, fenoldopam would appear to be much weaker (up to 100-fold)

than DA (Hilditch & Drew 1981; Brodde 1982). Additional studies are necessary to resolve this discrepancy.

In summary, our results demonstrate that fenoldopam, a selective DA_1 receptor agonist, possesses α-adrenoceptor antagonist activity.

Acknowledgements

This work was supported by NIH grants GM-22220 and HL-00872. Dr. Rajfer is the recipient of a Clinical Investigator Award (HL-00872) from the National Institutes of Health.

REFERENCES

Agrawal, D. K., Triggle, C. R., Daniel, E. E. (1984) J. Pharmacol. Exp. Ther. 229: 831–838

Berkowitz, B. A., Ohlstein, E. H. (1984) J. Cardiovasc. Pharmacol. 6: S559–S563

Brodde, O. E. (1982) Life Sci. 31: 289–306

Docherty, J. R., Starke, K. (1981) J. Cardiovasc. Pharmacol. 3: 854–866

Foley, J. J., Sarau, H. M. (1984) Fed. Proc. 43: 555, 1580

Furchgott, R. F., Bhadrakom, S. (1953) J. Pharmacol. Exp. Ther. 108: 129–143

Goldberg, L. I., Glock, D., Kohli, J. D., Barnett, A. (1984) Hypertension 6: I-25–I-30

Goldberg, L. I., Toda, N. (1975) Circ. Res. 36 and 37 Suppl. I-97–I-102

Hahn, R. A., Wardell, Jr., J. R., Sarau, H. M., Ridley, P. T. (1982) J. Pharmacol. Exp. Ther. 223: 305–313

Hilditch, A., Drew, G. M. (1981) Eur. J. Pharmacol. 72: 287–296

MacKay, D. (1978) J. Pharm. Pharmacol. 30: 312–313

Ohlstein, E. H., Zabko-Potapovich, B., Berkowitz, B. A. (1984) J. Pharmacol. Exp. Ther. 229: 433–439

Ruffolo, Jr., R. R., Waddell, J. E., Yaden, E. L. (1982) Ibid. 221: 309–314

Sakakibara, Y., Fujiwara, M., Muramatsu, I. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319: 1–7

Timmermans, P. B. W. M., Qian, J. Q., Ruffolo, Jr., R. R., van Zwieten, P. A. (1984) J. Pharmacol. Exp. Ther. 228: 739–748

Weinstock, J., Wilson, J. W., Ladd, D. L., Brenner, M., Ackerman, D. M., Blumberg, A. L., Hahn, R. A., Hieble, J. P., Sarau, H. M., Wiebelhaus, V. D. (1983) in: Kaiser, C., Kebabian, J. W. (eds) Dopamine Receptors, American Chemical Society, Washington, DC, pp 157–169