Analysis of the agonist activity of fenoldopam (SKF 82526) at the vascular 5-HT₂ receptor

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1 The 5-HT₂ receptor agonist activity of fenoldopam (SKF 82526) was characterized in the rabbit isolated aorta preparation.

2 Fenoldopam was an agonist at the vascular 5-HT₂ receptor with lower affinity and efficacy than the naturally occurring agonist 5-hydroxytryptamine (5-HT). Fenoldopam had an affinity (pK_A) of 5.84 ± 0.04 and efficacy (τ) of 0.57 ± 0.04 , whereas 5-HT had a pK_A of 6.65 ± 0.12 and τ of 2.66 ± 0.41 .

3 The constrictor effects of fenoldopam and 5-HT were competitively antagonized by the 5-HT₂ antagonist, ketanserin, with pK_B values of 8.81 ± 0.11 and 8.83 ± 0.10 respectively.

4 Prior incubation with fenoldopam produced a concentration-related rightward shift of a subsequent 5-HT concentration-response curve. This inhibition was specific for 5-HT since constrictor responses to angiotensin II were unaffected.

5 This study indicates that the D₁ receptor agonist, fenoldopam, acts as an agonist at the vascular 5-HT₂ receptor, but with an affinity and efficacy less than that of the naturally occurring agonist, 5-HT.

Keywords: Rabbit aorta; 5-HT₂ receptor; fenoldopam

Introduction

Fenoldopam (SKF 82526) is a 3-benzazepine D₁ receptor agonist currently in clinical trials as a novel vasodilator for the treatment of hypertension, congestive heart failure and renal failure. Whilst it is relatively selective as an agonist at the D₁ receptor (Hahn *et al.*, 1982), fenoldopam also acts as an antagonist at α_1 - and α_2 -adrenoceptors (Ohlstein *et al.*, 1985). More recently, fenoldopam has also been reported to act as an agonist at the 5-HT₂ receptor expressed in canine tracheal smooth muscle (Gretler *et al.*, 1992). In order to analyse fully the activity of fenoldopam at the vascular 5-HT₂ receptor, we examined its properties in the endothelium-denuded rabbit aortic ring preparation. This preparation was chosen because its response to 5-hydroxytryptamine (5-HT) is consistent and well characterized and, in contrast to other preparations such as rat caudal artery, the 5-HT₂ antagonist, ketanserin, behaves as a simple competitive antagonist (Leff & Martin, 1986).

A preliminary report of these results has been presented to the British Pharmacological Society (Christie & Smith, 1991).

Methods

Tissue preparation

New-Zealand White/Half-lop rabbits (1.9–3.5 kg) were killed by an overdose of pentobarbitone (100 mg kg⁻¹) injected into a marginal ear vein. The thoracic aorta was removed, cleaned of excess fat and connective tissue, everted and the intimal surface rubbed with a moistened cotton bud to remove the endothelium. After re-eversion the aorta was cut into 2–3 mm wide rings and mounted on parallel wires in 30 ml tissue baths containing ascorbic Krebs solution of the following composition (mM): NaCl 117.56, NaH₂PO₄ 0.89, NaHCO₃ 25.0, MgSO₄ 1.18, D-glucose 11.1, KCl 5.36, CaCl₂ 2.55 and L-ascorbic acid 1.1, maintained at 37°C and gassed with 95% O₂: 5% CO₂. Isometric tension changes were detected with a force transducer attached to the top wire and recorded on a chart recorder.

Experimental protocols

Aortic rings were equilibrated for approximately 60 min with frequent washing and re-adjustment to a resting tension of 2 g until a steady tension was maintained. They were then challenged with a maximally effective concentration of 5-HT (10 μ M) or angiotensin II (0.1 μ M) followed by acetylcholine (1 μ M) to confirm the absence of endothelium. Any rings that relaxed in response to acetylcholine were discarded. This initial constrictor response to 5-HT or angiotensin II was used as a reference to correct for differences in tension developed by different tissues. After washout and return to resting tension, rings were exposed to the irreversible α_1 -adrenoceptor antagonist, benextramine (10 μ M) and the cyclo-oxygenase inhibitor, indomethacin (3 μ M) for 30 min. The tissues were then washed four or five times before one of the following protocols was employed.

Fenoldopam affinity estimation These studies were carried out using a paired curve protocol. After construction of a control cumulative concentration-effect (E/[A], where E is the pharmacological effect and [A] is the concentration of the agonist) curve to 5-HT (0.01–10 μ M) followed by wash-out and return of tension to baseline, tissues were exposed to either incubation with the irreversible antagonist phenoxybenzamine (0.1 μ M for 30 min) followed by a second E/[A] curve to 5-HT (0.03–30 μ M), incubation with vehicle for 30 min followed by a second E/[A] curve to 5-HT (0.01–10 μ M) or a cumulative E/[A] curve to fenoldopam (0.1–100 μ M).

Antagonism of constrictor responses by ketanserin A single curve protocol was used in this series of experiments, as pilot experiments indicated that constrictor responses to fenoldopam were not readily reversed after washout. Cumulative E/[A] curves to 5-HT (0.01–300 μ M) or fenoldopam (0.1–300 μ M) were constructed 45 min after the addition of either vehicle or ketanserin (3–100 nM).

Interaction between fenoldopam and 5-HT These studies were also carried out with a single curve protocol. Cumulative E/[A] curves to 5-HT (0.01–100 μ M) or angiotensin II (0.1–30 nM) were constructed 45 min after the addition

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of vehicle or as soon as the initial constriction to fenoldopam (10–100 μ M) had plateaued.

Drugs used

Acetylcholine chloride, angiotensin II, benextramine hydrochloride, 5-hydroxytryptamine (creatinine sulphate complex) and indomethacin were purchased from Sigma Chemical Co. Ltd., Poole, Dorset. Ketanserin tartrate and phenoxybenzamine hydrochloride were purchased from Semat technical (UK) Ltd., St. Albans, Herts. Fenoldopam methane sulphate was a gift from SmithKline Beecham Ltd. Ketanserin, phenoxybenzamine and indomethacin were dissolved in ethanol, benextramine and 5-HT dissolved in Krebs solution and fenoldopam dissolved in acidified distilled water to give stock solutions of 30 mM. Angiotensin II was dissolved in distilled water to give a stock solution of 10 mM. Bath concentrations of ethanol did not exceed 0.43% and addition of the maximum vehicle volumes to the tissue bath produced no constrictor effects.

Analysis of data

Fenoldopam affinity estimation A combination of analytical methods was employed—the receptor inactivation method (Furchgott, 1966) to obtain the system parameters and a modification of the comparative method (Barlow *et al.*, 1967) to establish the affinity and efficacy of fenoldopam at the 5-HT₂ receptor:

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \quad (1)$$

in which E is the pharmacological effect, [A] is the concentration of the agonist, E_m is the maximum possible effect in the system, K_A is the agonist dissociation constant (estimated as the negative logarithm, that is, pK_A), τ is the efficacy of the agonist (estimated as a logarithm) and n is the slope of the occupancy-effect relation.

The comparative method for K_A estimation depends on the comparison of a partial agonist with a full agonist. In operation model terms a full agonist is needed to provide estimates of E_m and n in equation (1) so that K_A and τ for a partial agonist can be estimated (Leff *et al.*, 1989). 5-HT is not a full agonist in this tissue (Black *et al.*, 1985) and thus could not be used directly to analyse fenoldopam. However, it has earlier been shown that E_m and n estimates can be obtained in these circumstances by using the receptor inactivation method allowing the comparative analysis to proceed (Black *et al.*, 1985). Four curves, the E/[A] data for 5-HT, in the absence and presence of receptor inactivation, together with the E/[A] data for 5-HT and fenoldopam, were fitted simultaneously to the above equation providing a common estimate of E_m and n along with a value of τ for each curve in the set. The K_A for 5-HT was assumed to be the same for the three 5-HT curves: a different K_A was allowed for fenoldopam.

The data from the receptor inactivation method enabled E_m and n to be estimated whereas the data from the fenoldopam curve gave the additional information needed to produce the K_A of fenoldopam.

All data fitting procedures were carried out with the BMDP statistical package on a Vax 11/780 mainframe computer. Constrictor responses to 5-HT and fenoldopam were expressed as a percentage of the control 5-HT concentration-effect curve maximum. Results are expressed as mean $\pm$ s.e.mean.

Antagonism of constrictor responses by ketanserin, and full/partial interaction Tissue constrictor responses were expressed as a percentage of the initial constrictor response to 5-HT

(10 μ M) or angiotensin II (0.1 μ M), and the experimental data fitted to the following logistic equation:

$$E = \frac{\alpha [A]^m}{[A_{50}]^m + [A]^m} \quad (2)$$

where [A] is the concentration of the agonist, m is the slope parameter, α represents the asymptote and the $[A_{50}]$ is estimated as a logarithm.

The pK_B values for ketanserin and (in the full/partial interaction study) pA_2 values for fenoldopam were determined by fitting the computed log $[A_{50}]$ values to a linear form of the Schild equation as previously described (Leff & Martin, 1986). One way analysis of variance was used to test for treatment effects on E/[A] curve $[A_{50}]$'s, asymptotes and slopes, and statistical significance assigned at the $P < 0.05$ level. Results are expressed as mean $\pm$ s.e.mean.

Results

Fenoldopam affinity estimation

Both 5-HT and fenoldopam produced smooth monophasic E/[A] curves, with fenoldopam acting as a partial agonist in this system, approximately 30 times weaker than 5-HT (Figure 1). The constrictor responses to fenoldopam developed more slowly than those to 5-HT and were less readily reversed by wash-out.

Incubation with phenoxybenzamine produced a rightward shift and depression of the subsequent E/[A] curve to 5-HT, an interaction typical of an irreversible receptor antagonist (Figure 1). However, incubation with vehicle for 45 min did not significantly alter the agonist parameters of the second 5-HT E/[A] curve: the first 5-HT curve had a $p[A_{50}]$ of 6.88 ± 0.05 and a maximum of 100% and the second 5-HT curve a $p[A_{50}]$ of 6.80 ± 0.04 and a maximum of $106.4 \pm 2.3\%$ (data from 6 experiments).

Figure 1 Constrictor responses of rabbit aortic rings expressed as a percentage of the initial 5-hydroxytryptamine (5-HT) concentration-response curve maximum. Each point represents the mean of six experiments; vertical lines show s.e.mean. 5-HT control (○); 5-HT after incubation with phenoxybenzamine 0.1 μ M for 30 min (●); fenoldopam (■). The lines fitted through the data are calculated according to equation (1) in the text, E_m indicating the calculated maximum possible response in the system. The maximum constrictor response to 5-HT for all tissues was 5.56 ± 0.42 g.

Table 1 Parameter estimates for 5-hydroxytryptamine (5-HT) and fenoldopam at the rabbit aorta 5-HT₂ receptor

Parameter	Fenoldopam	5-HT
τ	0.57 ± 0.04	2.66 ± 0.41
pK_A	5.84 ± 0.04	6.65 ± 0.12

Curves were fitted to equation (1) expressing responses as a percentage of the control 5-HT concentration response curve maximum. The system had an E_m (system maximum) of $114.1 \pm 3.4\%$ and n (slope) of 2.37 ± 0.15 . Agonist dissociation constants are given as the negative logarithm of the K_A and efficacies as the logarithm of τ . The results are the mean $\pm$ s.e.mean of 6 experiments.

The system and agonist parameters, calculated according to equation (1) in the methods section, are summarized in Table 1. Note that in the case of 5-HT, its asymptote (100%) was less than the calculated E_m (indicating that it generated only 88% of the maximal possible response), confirming that 5-HT acts as a partial agonist in this system.

Antagonism of constrictor responses by ketanserin

Prior incubation with the 5-HT₂ receptor antagonist, ketanserin, produced a concentration-related rightward shift of the subsequent $E/[A]$ curve to 5-HT and fenoldopam. The Schild slopes were not significantly different from unity (0.89 ± 0.13 and 0.97 ± 0.32 for 5-HT and fenoldopam respectively), indicating that the antagonism was competitive in nature with pK_B values against 5-HT and fenoldopam of 8.83 ± 0.05 and 8.81 ± 0.06 respectively (pooled data from 3–4 experiments). The initial constrictor responses to 5-HT for all tissues used in 5-HT and fenoldopam experiments were 3.40 ± 0.38 g and 3.65 ± 0.18 g respectively.

Interaction between fenoldopam and 5-HT or angiotensin II

Prior incubation with fenoldopam (10–100 μ M) produced a concentration-related rightward shift of the subsequent $E/[A]$ curve to 5-HT (Figure 2). However, the interaction between fenoldopam and 5-HT indicated that fenoldopam was acting non-competitively at the 5-HT₂ receptor, as all concentrations of fenoldopam used produced a statistically significant reduction ($P < 0.05$) in the maximum response to 5-HT. This non-competitive behaviour precluded the quantitative estimate of a pK_B for fenoldopam: empirically Schild analysis gave an estimated pA_2 value for fenoldopam of 5.23 ± 0.23 and a slope of 1.20 ± 0.05 , significantly greater than unity ($P < 0.05$) (data from 6 experiments).

Prior incubation with fenoldopam (100 μ M) did not alter the $[A_{50}]$ or the maximum response of a subsequent angiotensin II $E/[A]$ curve, thus showing that the inhibition of the 5-HT response (Figure 2) was specific: the control angiotensin II $E/[A]$ curve had a $p[A_{50}]$ of 8.59 ± 0.10 and a maximum of $78.4 \pm 8.3\%$, and the post fenoldopam angiotensin II $E/[A]$ curve a $p[A_{50}]$ of 8.74 ± 0.11 and a maximum of $97.0 \pm 14.6\%$ (data from 5 experiments). The results are shown in Figure 3.

Discussion

In addition to acting as an antagonist at the peripheral D₁-receptor (Hahn *et al.*, 1982), fenoldopam (SKF 82526) shows significant antagonism at vascular α_2 -adrenoceptors (dog saphenous vein pA_2 of 7.78, Ohlstein *et al.*, 1985) with a lower affinity for vascular α_1 -adrenoceptors (rabbit aorta pA_2

Figure 2 Constrictor responses of rabbit aortic rings expressed as a percentage of the initial constrictor response to 5-hydroxytryptamine (5-HT). Cumulative concentration-response curves to 5-HT after incubation with either vehicle (○) or fenoldopam 10 μ M (●), 30 μ M (▲) or 100 μ M (■). Each point represents the mean of six experiments; vertical lines show s.e.mean. The lines fitted through the data are calculated according to equation (2) in the text. The initial constrictor response to 5-HT for all tissues was 4.20 ± 0.21 g.

Figure 3 Constrictor responses of rabbit aortic rings expressed as a percentage of the initial constrictor response to angiotensin II. Cumulative concentration-response curves to angiotensin II after incubation with either vehicle (□) or fenoldopam 10 μ M (■). Each point represents the mean of five experiments; vertical bars show s.e.mean. The lines fitted through the data are calculated according to equation (2) in the text. The initial constrictor response to angiotensin II for all tissues was 5.26 ± 1.45 g.

of 5.41, Ohlstein *et al.*, 1985). Following a recent report indicating that fenoldopam acts at the canine tracheal smooth muscle 5-HT receptor (Gretler *et al.*, 1992), we have shown that fenoldopam is also an agonist at the well-characterized rabbit aorta vascular 5-HT₂-receptor. The constrictor effects of fenoldopam are unlikely to be mediated by

D₁ agonism as dopamine was inactive in this preparation at up to 100 μ M (data not shown). This study extends the structure-activity relations reported for other benzazepines structurally related to fenoldopam such as SCH 23390 and SKF 83566 which are active as antagonists at the vascular 5-HT₂-receptor (Ohlstein & Berkowitz, 1985) and our observations with another benzazepine, SKF 38393 (Christie M.I. & Hollick L.J., unpublished), which acts as a weak, competitive 5-HT₂ antagonist with a pK_B of 4.76 ± 0.16 (data from 4 experiments). Furthermore this study confirms the finding with SKF 87516 (Le Monnier de Gouville *et al.*, 1991) that compounds in the benzazepine series may also act as agonists at the vascular 5-HT₂ receptor.

Our results with fenoldopam are qualitatively similar to those obtained in canine tracheal smooth muscle (Gretler *et al.*, 1992), in that constrictor responses are slow in onset and not readily reversed by washout. In both studies fenoldopam has a lower affinity and efficacy than the naturally occurring agonist 5-HT, such that any 5-HT₂ receptor agonism produced by fenoldopam will be dependent on the tissue receptor reserve as well as the concentration of fenoldopam. The results reported by Gretler *et al.* (1992) suggest that the canine tracheal smooth muscle has a higher 5-HT receptor reserve than the rabbit aorta as 5-HT appears more potent, with a $p[A_{50}]$ of 7.08. This may also explain the higher intrinsic activity of fenoldopam (ca. 84% of the maximum response to 5-HT) in the canine trachea. However, both studies are consistent with fenoldopam acting as a low efficacy agonist at the 5-HT₂ receptor.

We obtained two estimates of the affinity of fenoldopam for the 5-HT₂ receptor, one from the comparative analysis (pK_A of 5.84 ± 0.04) and one from Schild analysis of the full/partial interaction between 5-HT and fenoldopam (apparent pA_2 of 5.23 ± 0.23). The Schild result is likely to be an unreliable estimate of the affinity of fenoldopam for the 5-HT₂ receptor for three reasons: firstly fenoldopam caused depression as well as rightward shift of subsequent 5-HT curves and therefore did not satisfy the criteria for competitive antagonism, secondly the agonism caused by fenoldopam introduces a slight error in the pA_2 value (Kenakin, 1987) and thirdly, the steep transducer slope of this system introduces another source of error in full/partial interaction studies (Leff P., unpublished). We therefore believe that the pK_A value for fenoldopam obtained by the comparative method is the most accurate estimate of its affinity for the 5-HT₂ receptor.

The 5-HT₂ agonist activity of fenoldopam is confirmed by the blockade of the effects of fenoldopam by the 5-HT₂-receptor antagonist, ketanserin, with a pK_B value against fenoldopam of 8.81, similar to that obtained against 5-HT in this study (8.83), by Leff & Martin (1986) against 5-HT in the rabbit aorta (8.56 ± 0.09) and by Gretler *et al.* (1992) against fenoldopam in the canine trachea (8.37). Ketanserin also has affinity for α_1 -adrenoceptors, but the antagonism of fenoldopam by ketanserin is unlikely to be due to either direct or indirect (as occurs with 5-HT, due to a tyramine-like effect) α_1 -adrenoceptor antagonism, as the benextramine pretreatment used throughout this study effectively abolished the α_1 -adrenoceptor-mediated constrictor response to phenylephrine ($> 20,000$ fold rightward shift, data not shown).

Further confirmation that 5-HT and fenoldopam act at the same receptor was provided by the observation that fenoldopam produced a concentration-related rightward shift of subsequent 5-HT E/[A] curves. The reduction in the maximum response to 5-HT indicates that a component of the action of fenoldopam is non-competitive in nature, but whether this is due to desensitization of the 5-HT-mediated contractile response in this tissue (Ben-Harari *et al.*, 1991) or irreversible binding to the receptor is unclear. However, the lack of effect of fenoldopam on constrictor responses to angiotensin II indicates that the effects of fenoldopam are specific for the 5-HT₂ receptor.

In conclusion, these data confirm that fenoldopam is a low efficacy 5-HT₂-receptor agonist that displays characteristics of partial agonism in the rabbit aorta. Thus whilst fenoldopam acts primarily as a vasodilator *in vivo* (Hahn *et al.*, 1982), our evidence suggests that in states where the 5-HT₂ receptor-mediated constrictor response is functionally up-regulated, such as following endothelial loss (Cocks & Angus, 1983) or vascular smooth muscle damage (De Meyer *et al.*, 1990; Sobey *et al.*, 1991) fenoldopam may be capable of producing localized vasoconstriction via 5-HT₂ receptor agonism. Conversely in tissues where the 5-HT₂ receptor reserve is low, fenoldopam would be expected to behave as a 5-HT₂ receptor antagonist.

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