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Characterization of flufylline, fluprofylline, ritanserin, butanserin and R 56413 with respect to in-vivo α_1-, α_2- and 5-HT_2-receptor antagonism and in-vitro affinity for α_1-, α_2- and 5-HT_2-receptors: comparison with ketanserin

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The experimental drugs butanserin (R 53393), ritanserin (R 55667), R 56413, flufylline (Sgd 195/78) and fluprofylline (Sgd 144/80) were evaluated with respect to their antagonism at postjunctional α_1- and α_2-adrenoceptors and 5-HT_2-receptors in pithed rats. Moreoever, affinity for [^{3}H]mianserin, [^{3}H]prazosin and [^{3}H]yohimbine binding sites was assessed using rat brain preparations. In all experiments ketanserin was taken as a reference compound. It is concluded that of the compounds investigated butanserin is the most potent and selective α_1-adrenoceptor antagonist, whereas ritanserin was found to be a potent and selective 5-HT_2-antagonist. Of the other compounds, fluprofylline was a very selective though not very potent α_1-adrenoceptor antagonist. The other compounds were less active and less selective in this respect.

The experimental compounds butanserin (R 53393), flufylline (Sgd 195/78) and fluprofylline (Sgd 144/80), (for structures see Fig. 1) are potentially new cardiovascular drugs. Of these, butanserin displays potent α-adrenergic blocking activity and binds primarily to α_1-adrenergic sites (Niemegeers et al 1984), whereas the structurally related Sgd-compounds show antihypertensive properties in spontaneously hypertensive rats and display antagonism towards 5-HT receptors in various animal models (Thiele et al 1984). The pirenperone congeners R 56413 (2HCl) and ritanserin (R 55667), both show affinity for 5-HT_2 binding sites in-vitro (Leysen, unpublished results). In particular the latter of these compounds was a rather selective 5-HT_2 antagonist in cardiovascular and behavioural studies (Janssen 1985; Colpaert et al 1985).

The present study was designed to characterize the antagonistic properties of the above compounds at vascular post-junctional α_1- and α_2-adrenergic and 5-HT_2 receptors, mediating vasoconstriction in pithed, normotensive rats. Radioligand displacement studies were also carried out to determine affinity for central [^{3}H]prazosin, [^{3}H]yohimbine and [^{3}H]mianserin binding sites.

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METHODS

Antagonism at vascular postjunctional α_1- and α_2-adrenoceptors

Male, normotensive Wistar rats (200–300 g) were pithed by inserting a blunt needle into the spinal canal via the right orbit under hexobarbitone sodium anaesthesia (150 mg kg^{-1} i.p.). Subsequently, the animals were artifically ventilated by means of a Braun Melsungen positive pressure pump via a tracheal cannula. Body temperature (rectal) was maintained at about 37 °C by means of thermostat-controlled tables. The right jugular vein and common carotid artery were cannulated for administration of drugs and blood pressure measurements, respectively. Heparin was administered i.v. (150 iu kg^{-1}). Arterial blood pressure was continuously recorded via a Statham P23 dB pressure transducer, connected to a Hellige HE-19 device.

Cardiovascular parameters were allowed to stabilize for 15 min then the animals received saline or the antagonist via the intra-arterial route in a volume of 1 ml kg^{-1}. After a further 15 min, the maximal increase in diastolic pressure (mmHg) to intravenous bolus injections (0·5 ml kg^{-1}) of either amidephrine or B-HT 920, was measured. In the lower dose range, the pressor effects of the individual doses of

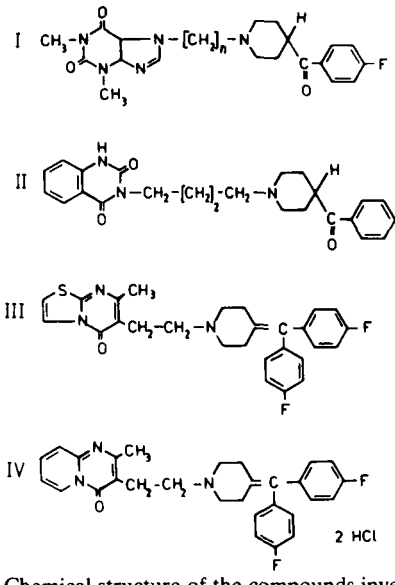

FIG. 1. Chemical structure of the compounds investigated. I, n = 2 flufylline, n = 3 fluprofylline; II, butanserin; III ritanserin; IV, R 56413.

the α-adrenoceptor agonists were measured, care being taken that no measurements were made before the diastolic pressure had returned to pre-injection values. Higher doses of the agonists were injected cumulatively.

One complete dose-response curve was obtained per individual animal, whereas each dose was studied in 4–6 animals. Antagonist potency was expressed as dose-ratio (DR) obtained by dividing ED50 values (=agonist dose at halfmaximal effect) for the antagonist-treated animals by that of saline-treated rats. DRs were calculated for doses of 10^{-5} mol kg^{-1} of the antagonists.

Antagonism of 5-HT-induced pressor responses in pithed rats

Male, normotensive Wistar rats (200–300 g) were pithed and prepared as described above. 15 min after injection of antagonist or vehicle the increase in diastolic pressure evoked by intravenous bolus injections of 5-HT was assessed. To avoid desensitization occurring after repeated administration of 5-HT and to deal with the depressor effect that follows the pressor response after bolus injection of 5-HT in pithed rats (see Fozard & Leach 1968), the following method for constructing dose-response curves was applied. Only two doses of 5-HT were evaluated in one preparation in the presence or absence of antagonist (one dose per preparation). Between the subsequent doses of 5-HT (in 0·5 ml kg^{-1}) a sufficiently wide time interval was allowed to achieve full recovery of diastolic pressure to pre-injection values. The effect of each separate dose of the agonist was evaluated in 5–6 different preparations. Accordingly, the results were pooled for the construction of dose-response curves.

Earlier experiments have shown that no parallel shifts are obtained for 5-HT dose-response curves when in-vivo $5HT_2$-antagonism was studied (e.g. Kalkman et al 1982), thus DRs could not be calculated. Therefore, ID50 values (dose of antagonist attenuating the pressor effect of a dose of $6{\cdot}28 \times 10^{-6}$ mol kg^{-1} 5-HT by 50%) were calculated by plotting the negative logarithm of the antagonist dose against the percentage of the control value of increase in diastolic pressure evoked by a dose of $6{\cdot}28 \times 10^{-6}$ mol kg^{-1} 5-HT.

Displacement of [^{3}H]prazosin, [^{3}H]yohimbine and [^{3}H]mianserin from their specific binding sites to rat brain homogenates

Binding studies were performed with [^{3}H]prazosin (33 Ci $mmol^{-1}$), [^{3}H]yohimbine (81·2 Ci $mmol^{-1}$) and [^{3}H]mianserin (55 Ci $mmol^{-1}$) as radioligands to label central α_1-, α_2- and 5-HT_2-binding sites, respectively. Rat brain membranes of normotensive, Wistar rats (250–300 g) were prepared according to standard procedures as described by Greenberg et al (1976) and modified by Timmermans et al (1981) ([^{3}H]yohimbine and [^{3}H]prazosin binding).

[^{3}H]Mianserin binding was performed using rat frontal cortex preparations as described by Peroutka & Snyder (1981) and Kalkman et al (1983).

In displacement experiments the specific binding of [^{3}H]prazosin (0·2 nM), [^{3}H]yohimbine (1·0 nM) and [^{3}H]mianserin (0·2 nM) was determined in the presence of various concentrations of non-radioactive competitors. Displaceable (specific) binding of the tritiated compounds was defined as the binding remaining after subtraction of non-displaceable (non-specific) binding determined in the presence of 10 μM unlabelled phentolamine ([^{3}H]yohimbine and [^{3}H]prazosin binding) or 0·3 μM unlabelled triprolidine ([^{3}H]mianserin binding). To prevent precipitation of the drugs (particularly ritanserin), 1% polysorbate (Tween) 80 was added to the incubation mixtures. The addition of the surfactant was found not to influence IC50 values (authors, unpublished observations).

The affinity of non-labelled competitors for the displaceable sites of the radioligands was expressed by the concentration inhibiting this binding by 50% (IC50). The IC50 values were determined from 4 experiments, each performed in duplicate.

Drugs used

(−)-Amidephrine mesylate (gift, Mead Johnson, USA); B-HT 920 2HCl (2-amino-6-allyl-5,6,7,8-tetrahydro [4*H*]thiazolo [4,5-*d*]azepine dihydrochloride; gift, Thomae, FRG); cyproheptadine (gift, Merck Co., USA); flufylline (Sgd 195/78) and fluprofylline (Sgd 144/80); gifts, Siegfried, Switzerland); heparin (NOVO, Denmark); hexobarbitone (O.P.G., The Netherlands); hydrocount (Baker, USA); ketanserin; butanserin (R 53393); ritanserin (R 55667); R 56413 (3-[2-[4-[bis(4-fluorophenyl)-methylene]piperido]ethyl]-2-methyl[4*H*]pyrido-[1,2-*a*]pyrimidin-4-one 2HCl; gifts, Janssen, Belgium); [^{3}H]mianserin (New England Nuclear, USA); [^{3}H]prazosin (gift, Pfizer, England); 5-hydroxytryptamine oxalate (Sigma USA); triprolidine (gift, Burroughs Welcome, England); [^{3}H]-yohimbine (New England Nuclear, FRG).

Flufylline, fluprofylline, ketanserin, butanserin, R 56413 and ritanserin were dissolved in 0·02 M tartaric acid. All other drugs were dissolved in saline.

All data are presented as means ± s.e.

Data handling

Data analysis was performed on an Apple IIe computer. For calculations of ID50 and IC50 values the program 'Totalfit' by Barlow (1983) was used. DR values were calculated using the 'APPALL' curve-fitting program as obtained from the SK & F software services, UK.

RESULTS

Antagonism of α_1- and α_2-adrenoceptor-mediated vasoconstriction in pithed rats

After pithing, dissection and equilibration, baseline diastolic pressure of the pithed normotensive rats before drug administration amounted to 40·7 ± 6·1 mmHg (n = 86). Pretreatment with the antagonists in doses of 10^{-5} mol kg^{-1} did not significantly influence baseline diastolic pressure.

All the antagonists studied displaced the log dose-response curve of amidephrine as well as that of B-HT 920 in a parallel fashion, without significantly affecting maximum pressor response to the agonists. DRs expressing antagonist potency of the compounds are displayed in Table 1.

A potency order of butanserin ≫ fluprofylline > ketanserin = flufylline > ritanserin = R 56413, resulted for the antagonism of amidephrine-induced increases in diastolic pressure. For all compounds, low antagonist potencies were found at post-junctional α_2-adrenoceptors. To indicate the preference of the antagonist in blocking either α-adrenoceptor subtype, a selectivity coefficient, defined as $DR_{\alpha_1}/DR_{\alpha_2}$ was introduced. A selectivity order of butanserin ≫ fluprofylline ≫ flufylline > ketanserin > ritanserin = R56413, resulted.

Table 1. Antagonistic properties of butanserin, ritanserin, R 56413, ketanserin, flufylline and fluprofylline against amidephrine- and B-HT 920-induced increases in diastolic pressure in pithed, normotensive rats.

Compound	α_1 DR[a]	α_1 s.e.[b]	α_2 DR	α_2 s.e.	Selectivity ratio $DR_{\alpha_1}/DR_{\alpha_2}$[c]	s.e.
Butanserin	7300	1200	4·2	1·3	1740	610
Ritanserin	2·3	0·9	1·0	0·3	2·4	1·2
R 56413	3·1	1·8	2·6	3·6	1·2	1·8
Flufylline	11·3	1·2	1·5	0·4	7·4	2·2
Fluprofylline	95	40	2·2	1·5	43	24
Ketanserin	10·4	2·1	2·4	0·8	4·3	1·4

[a] Dose-ratio, obtained by dividing ED50-values from log-dose response curves obtained for animals pretreated with 10^{-5} mol kg^{-1} of the antagonist or saline, respectively.
[b] Standard error (n = 4–6).
[c] Selectivity ratio obtained by dividing DRs for the antagonists against either amidephrine (α_1)- or B-HT 920 (α_2)-induced increases in diastolic pressure.

Antagonistic properties against 5-HT induced pressor responses in pithed rats

Mean diastolic pressure of pithed normotensive rats before application of drugs was 38·1 ± 5·2 mmHg (n = 330). After i.v. administration of 5-HT a dose-related rise in diastolic pressure of short duration, followed by a more persistent but relatively small depressor effect was observed. Only after pretreatment with low doses of the antagonists could parallel shifts of the log-dose pressor response curves to 5-HT be observed. However, pretreatment with higher doses of the antagonists resulted in a progressive decrease of both slope and maximum of the 5-HT log-dose pressor response curves (Fig. 2). For this reason calculation of DR values or in-vivo pA_2 values (Korstanje et al 1984) was inappropriate. Therefore, the potency of the drugs in attenuating 5-HT-induced increases in diastolic pressure was expressed as ID50 (see Methods).

−Log ID50-values for the six compounds investigated are listed in Table 2. This table shows that of these compounds ritanserin is the most potent attenuator of 5-HT-induced increases in diastolic pressure in rats. R 56413, butanserin, flufylline and ketanserin display moderate 5-HT-antagonistic properties, whereas fluprofylline is least active in this respect.

Inhibition of [^{3}H]prazosin, [^{3}H]yohimbine and [^{3}H]mianserin binding

[^{3}H]Prazosin and [^{3}H]yohimbine binding to rat brain membranes was found to be rapid and reversible and

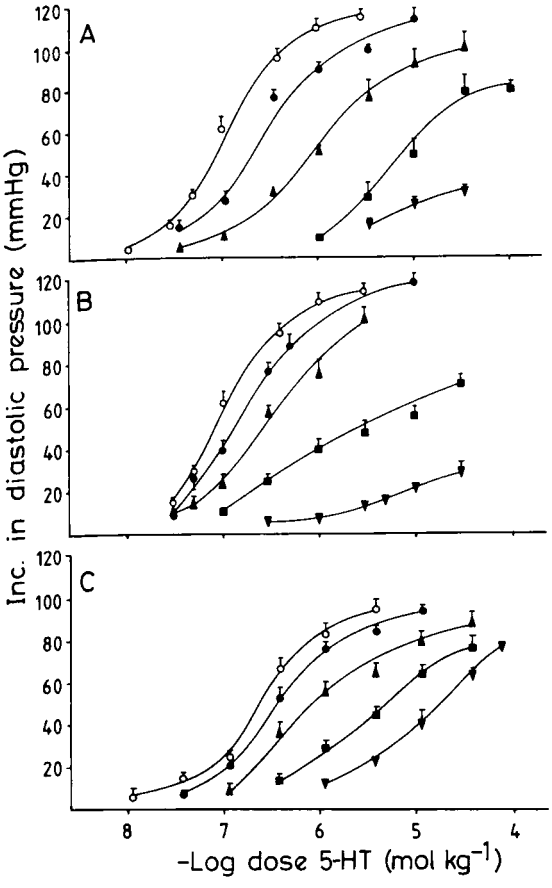

FIG. 2. Increase in diastolic pressure brought about by intravenous administration of 5-HT to pithed rats. Results were obtained after pretreatment with saline (○, all panels) or A. ritanserin: $6{\cdot}3 \times 10^{-10}$ (●); $2{\cdot}1 \times 10^{-9}$ (▲); $2{\cdot}1 \times 10^{-8}$ (■); $6{\cdot}3 \times 10^{-8}$ (▼) mol kg^{-1}, respectively. B. R 56413: $3{\cdot}0 \times 10^{-9}$ (●); $1{\cdot}0 \times 10^{-8}$ (▲); $3{\cdot}0 \times 10^{-8}$ (■); $1{\cdot}0 \times 10^{-7}$ (▼) mol kg^{-1}, respectively. C. flufylline: $1{\cdot}0 \times 10^{-8}$ (●); $3{\cdot}0 \times 10^{-8}$ (▲); $1{\cdot}0 \times 10^{-7}$ (■); $3{\cdot}0 \times 10^{-7}$ (▼) mol kg^{-1}, respectively. Results are expressed as mean values ± s.e.m. (n = 5–6).

with high affinity to single populations of α_1- and α_2-binding sites, respectively (see e.g. Timmermans et al 1981, 1982). Under the conditions applied, [^{3}H]mianserin was reported to label specifically 5-HT$_2$-binding sites (Peroutka & Snyder 1981; Kalkman et al 1983). Specifically bound [^{3}H]prazosin, [^{3}H]yohimbine and [^{3}H]mianserin were displaced by the non-labelled drugs yielding sigmoid displacement curves. IC50 values of the compounds investigated are listed in Table 3.

From the data in Table 3 it is clear that butanserin possesses the greatest affinity for central α_1-sites. For central α_2-sites it is obvious that none of these

Table 2. Attenuating effect of butanserin, ritanserin, R 56413, ketanserin, flufylline and fluprofylline with respect to the pressor effect of i.v. 5-HT in pithed rats.

Compound	ID50 ± s.e.[a] (mol kg^{-1})	pID50[b]	r[c]	n[d]
Butanserin	$(5{\cdot}3 \pm 1{\cdot}1) \times 10^{-8}$	7·3	0·998	5
Ritanserin	$(2{\cdot}2 \pm 0{\cdot}5) \times 10^{-9}$	8·6	0·991	3
R 56413	$(2{\cdot}6 \pm 0{\cdot}3) \times 10^{-8}$	7·6	1·000	4
Flufylline	$(5{\cdot}4 \pm 0{\cdot}7) \times 10^{-8}$	7·3	0·995	4
Fluprofylline	$(1{\cdot}3 \pm 0{\cdot}3) \times 10^{-6}$	5·9	0·988	5
Ketanserin	$(2{\cdot}6 \pm 0{\cdot}3) \times 10^{-8}$	7·6	0·997	4

[a] Dose of antagonist required to reduce a 50% attenuation of the pressor response evoked by $6{\cdot}28 \times 10^{-6}$ mol kg^{-1} 5-HT compared with control experiments; s.e. = standard error.
[b] Negative logarithm of ID50.
[c] Correlation coefficient for the fit of the straight line for the relation between the negative logarithm of the antagonist dose (mol kg^{-1}) and the % of the control value of increase in diastolic pressure evoked by a dose of $6{\cdot}28 \times 10^{-6}$ mol kg^{-1} 5-HT as obtained by the Totalfit program by Barlow (1983).
[d] Number of antagonist doses applied.

substances shows a pronounced affinity for [^{3}H]yohimbine-labelled sites, the least potent in this respect being fluprofylline. From the affinity for central 5-HT$_2$-sites, it is obvious that all compounds but fluprofylline show a comparable and high affinity for [^{3}H]mianserin-labelled sites. To classify the compounds according to their selectivity for either of the binding sites studied,

$$\frac{\text{IC50 [}^3\text{H]yohimbine}}{\text{IC50 [}^3\text{H]prazosin}} \quad \text{and} \quad \frac{\text{IC50 [}^3\text{H]mianserin}}{\text{IC50 [}^3\text{H]prazosin}}$$

ratios were calculated to express the selectivity for α_1- compared with α_2- and 5-HT$_2$-sites, respectively. As a result of this it became apparent that fluprofylline was the most selective compound with regard to α_1- over α_2-sites, followed by butanserin, while the other compounds were less selective. Fluprofylline was also the most selective to α_1- over 5-HT$_2$-sites, followed by butanserin, whereas R 56413, ritanserin, flufylline and ketanserin all showed high selectivity for central 5-HT$_2$- as compared to α_1-sites.

DISCUSSION

The pithed rat is appreciated as a suitable model to evaluate the potency of drugs in attenuating blood pressure responses mediated by vascular postjunctional α_1- and α_2-adrenoceptors (see e.g. Timmermans et al 1979) as well as 5-HT-receptors (Fozard & Leach 1968; Kalkman et al 1982).

In the present investigation we studied the antagonistic properties of five experimental compounds (butanserin, ritanserin, R 56413, flufylline, fluprofylline) with respect to the increase in diastolic pressure evoked by the agonists amidephrine (Flavahan & McGrath 1981; Mathy et al 1983) and B-HT 920 (Van Meel et al 1981; Kobinger & Pichler 1981) as selective stimulants of α_1- and α_2-adrenoceptors, respectively.

Table 3. Displacement of [^{3}H]yohimbine, [^{3}H]prazosin and [^{3}H]mianserin from their specific binding sites in rat frontal cortex tissue by unlabelled butanserin, ritanserin, R 56413, flufylline, fluprofylline and ketanserin.

Compound	[^{3}H]prazosin	−log IC50[a] [^{3}H]yohimbine	[^{3}H]mianserin	IC50 [^{3}H]yohimbine / IC50[^{3}H]prazosin	IC50 [^{3}H]mianserin / IC50 [^{3}H]prazosin
Butanserin	8·73	7·12	8·15	41	3·80
Ritanserin	6·72	6·20	8·26	3·3	0·029
R 56413	6·59	6·55	8·22	1·1	0·023
Flufylline	6·92	6·38	8·35	3·5	0·037
Fluprofylline	7·55	4·63	6·70	832	7·1
Ketanserin	7·14	6·53	8·50	4·1	0·044

[a] −Log concentration inhibiting 50% of specific binding of tritiated ligand.
Calculations of −log IC50 values are performed on an Apple IIe computer by a least-squares line fitting program ('Totalfit', Barlow 1983), calculated on basis of displacement curves using mean values of four separate determinations with s.e.m. < 5%.

It has been suggested that 5-HT$_2$-receptors play a role in blood pressure homeostasis in mammals (Van Nueten et al 1981). Later studies revealed that 5-HT$_2$-blocking compounds did not lower elevated blood pressure in various animal models (Fozard 1982; Kalkman et al 1982; Cohen et al 1983). However, the role of 5-HT$_2$-receptors in the maintenance of elevated blood pressure in man cannot be ruled out as yet, since the blood pressure-lowering effect of ketanserin in patients with essential hypertension (Wenting et al 1982), and with autonomic insufficiency (Wenting et al 1984), could not be completely reversed by infusion of the α_1-adrenoceptor agonist methoxamine. This is at variance with the results of Reimann & Frölich (1983), who demonstrated that in healthy volunteers the blood pressure-lowering effect of ketanserin could be ascribed to its α_1-adrenoceptor-blocking potency. It is stipulated, however, that under pathological conditions 5-HT$_2$-receptors would be involved in blood pressure regulation in man (Van Nueten et al 1984). 5-HT$_2$-antagonists with limited α-blocking properties would thus be of experimental interest. Therefore we also evaluated these compounds for their potency in attenuating 5-HT-induced increases in blood pressure in pithed rats. The effectiveness of these compounds, both in functional and binding studies, was compared with that of ketanserin.

The present study confirms the α_1-antagonistic activity and potency in attenuating 5-HT-induced pressor responses of ketanserin in pithed rats, as reported previously by Kalkman et al (1982). We observed a comparable affinity of ketanserin for [^{3}H]mianserin binding sites as that reported by Kalkman (1983), whereas the compound's affinity for [^{3}H]prazosin-labelled sites showed a close resemblance to that for [^{3}H]WB 4101-labelled sites as published by Leysen et al (1981). However, for [^{3}H]yohimbine-labelled sites a much greater affinity was found than for [^{3}H]clonidine as reported by Leysen et al (1981) and confirmed by us (unpublished observations). This obvious discrepancy cannot be easily explained. Although in-vitro studies have shown that yohimbine exhibits a considerable 5-HT$_2$-antagonist potency (Lattimer et al 1984), it was shown by Cheung et al (1982) that a Scatchard-plot of [^{3}H]yohimbine to rat isolated brain membranes revealed only a single binding site. A possible explanation could be the differences in agonist/antagonist binding as pointed out by Greenberg et al (1976), suggesting that α-noradrenergic agonists and antagonists act at non-identical, non-interconvertible sites. In view of their results and those of others (e.g. Glossmann et al 1981), caution is necessary, especially for mixed agonists/antagonists, when agonist and antagonist binding data are compared.

From both binding and in-vivo studies it is obvious that of the compounds studied butanserin and fluprofylline are most selective towards α_1-adrenoceptors. For reasons of potency, butanserin is favoured as an α_1-adrenoceptor antagonist. Comparing the present results with previous findings on other selective α_1-adrenoceptor blocking agents, it is clear that butanserin is about equipotent with prazosin, BE 2254 (2-[β-(4-hydroxyphenyl)-ethylaminomethyl]-tetralone) and AR-C239 (2-(2-[4-(*o*-methoxyphenyl)piperazin-1-yl]ethyl)-4,4-dimethyl[2*H*,4*H*]isoquinoline-1,3-dione) in pithed rats (Korstanje et al 1984), displaying a selectivity towards α_1-adrenoceptors comparable to that of WB4101 (2-[*N*-(2,6-dimethoxyphenoxyethyl)]-aminomethyl-1,4-benzodioxane; Drew 1982). This study shows that all compounds investigated, except fluprofylline, are potent inhibitors of 5-HT-induced

increases in diastolic pressure. When comparing [^{3}H]mianserin displacement data for the compounds tested the same pattern emerges; again fluprofylline shows much lower affinity for 5-HT$_2$-sites than the other compounds tested. Comparing the ratio as calculated from displacement experiments to bind either central α_1- or 5-HT$_2$-sites reveals a pronounced 5-HT$_2$-selectivity for both ritanserin, R 56413, flufylline and ketanserin. Considering its low blocking activity at vascular α_1- and α_2-adrenoceptors in-vivo and its strong attenuating effect on 5-HT-induced pressor responses in pithed rats, it is obvious that, among the compounds investigated, ritanserin is the most selective 5-HT$_2$-antagonist. Several other selective 5-HT$_2$-antagonists are also available now, e.g. Ly 53857 (4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*f,g*]quinoline maleate; Cohen et al 1983) and 1-(1-naphthyl)piperazine (Cohen et al personal communication). It will be interesting to compare their full pharmacological profiles.

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