Study of the in vivo and in vitro Cardiovascular Effects of Four New Analgeses of Ketanserin: Implication of 5-HT_{2A} and α₁ Adrenergic Antagonism in Their Hypotensive Effect

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The in vivo and in vitro cardiovascular effects of the novel 5-HT_{2A}/α_{1} H_{1} antagonist ketanserin analogues QF 0303B, QF 0307B, QF 0311B, QF 0313B were studied in anaesthetized normotensive rats (ANR) and in isolated rubbered rat aorta (IRRA). In ANR, 0.2 mg·kg^{-1}·l.v. of each compound produced a rapid, remarkable but short-lasting fall in mean arterial blood pressure (MAP) accompanied by bradycardia. All compounds significantly modified thepressor effects induced by 5-hydroxytryptamine (5-HT) and noradrenaline (NA). In IRRA, the compounds inhibited NA- and 5-HT-induced contractions in a competitive fashion. Furthermore, the analogues displayed lower H_{1}-antagonist activity than ketanserin. Compounds tested showed low 5-HT_{1B} affinity and no activity at muscarinic, nicotinic, or 5-HT_{3} receptors, nor any marked ability to produce smooth muscle relaxation via calcium entry blockade. There is a significant correlation between hypotension reached and inhibition of the 5-HT-induced pressor responses (but not for NA). A certain degree of correlation was observed between hypotensive effect endurance vs. α_{1}-adrenoceptor blockade (but not for serotonin). These results indicate that in this series the brief hypotensive activity in ANR is attributed to a 5-HT_{2A} receptor blockade and the duration of the effect is better attributed to an α_{1}-adrenoceptor blockade.

Key words: new ketanserin analogues; 5-HT_{2A} receptor; alpha1 adrenoceptor; noradrenaline; 5-hydroxytryptamine; antagonist

Hypertension is a serious risk factor for cerebrovascular disease and heart disease. It is commonly accepted that antihypertensive therapy improves the quality of life of the patients. Although there are several antihypertensive drugs clinically available, due to the different origins and pathologies of hypertension, it is difficult to control all types of hypertension through the use of only one drug, and each antihypertensive agent has its own side effects. Therefore, the development of new and effective antihypertensive strategies and drugs with different modes of action is still required. Ketanserin, a prototypic 5-HT_{2A} antagonist, is the first antiserotoninergic drug used in the treatment of hypertension. This drug lowers diastolic and systolic blood pressure in animals and hypertensive humans, while eliciting no compensatory tachycardia. It also reduces peripheral vascular resistance in a similar measure to α-adrenergic blockers or diuretics.

However, the mechanism of this hypotensive activity still remains unclear. It was reported that the mechanism of the acute and chronic antihypertensive effects of ketanserin is not clearly attributed to either peripheral vascular 5-HT_{3} or vascular α_{1}-adrenergic receptor blockade. It has also been pointed out that the α_{1}-adrenoceptor blocking ability of ketanserin is the more important mechanism in the rat, based on the observation that there is a significant blood pressure reduction to acute administration of ketanserin in a dose range where ketanserin blocks α_{1}-adrenoceptors. In fact, many other 5-HT_{2A} antagonists, such as cyproheptadine, mianserin, ritanserin, ICI 170809, and LY 53857 have been tried as antihypertensive agents, but with negative results. Interestingly, the novel compounds QF 0303B, QF 0307B, QF 0311B and QF 0313B share a p-fluorobenzoylpiperidine fragment (Fig. 1) and the only difference between ketanserin and these compounds is in the structural skeleton (where ketanserin has a quinazolinone moiety, while the new compounds present a cycloalkanone moiety (QF 0330B contains an indane ring, QF 0307B a tetralone ring, QF 0311B a benzosuberone ring and QF 0313B has a 5-fluorooindaneone)). In a previous paper, we demonstrated that these structural variations are responsible for the different affinity of the compounds on the 5-HT_{2A} receptors showing that the greater the planarity, the higher the affinity.

In this study we aimed to analyse whether the hypotension induced by ketanserin and these compounds on anaesthetized normotensive rats (ANR) at the dose used (0.2 mg·kg^{-1}·l.v.) was dependent on α_{1}-adrenoceptor, 5-HT_{2A} or 5-HT_{1B} receptor blockade and to investigate the hypotensive effect of these compounds to elicit the importance of the 5-HT_{2A} vs. α_{1}-receptor blockade in their vascular effects.

![Fig. 1. Structure of the New Compounds Analogaes of Ketanserin](image)

The compounds present a cycloalkanone moiety, with different substituents in R and n positions.
MATERIALS AND METHODS

Chemistry We have studied new compounds (2-[β-[4-(p-fluorobenzoyl)piperidin-1-yl]ethy]-1-indane, QF 0303B; 2-[β-[4-(p-fluorobenzoyl)piperidin-1-yl]ethyl]tetralone, QF 0307B; 2-[β-[4-(p-fluorobenzoyl)piperidin-1-yl]ethyl]benzosuberone, QF 0311B; and 5-fluoro-2-[β-[4-(p-fluorobenzoyl)piperidin-1-yl]ethyl]-1-indane, QF 0313B) as carbocyclic analogues of ketanserin.

These compounds have been prepared from cycloalkanone acetic acids according to the synthetic route illustrated in Chart 1 as previously described by us.14 With the ketanserin molecule, these compounds have a common 4-(p-fluorobenzoyl)piperidine fragment, but the quinazoline nucleus of the ketanserin has been replaced by an indane or 5-fluorindanone (QF 0303B; QF 0313B), a tetralone ring (QF 0307B) or a benzosuberone skeleton (QF 0311B).

In Vivo Experiments: Blood Pressure Measurements Normotensive male Sprague-Dawley rats weighting 250—300 g were anaesthesized by intraperitoneal injection of urethane (1.26 g·kg⁻¹ i.p.) and cannulated following the method described by Orallo.15 In the first set of experiments, after blood pressure and heart rate (HR) stabilization, saline (1 mg·kg⁻¹) or the tested compound (0.2 mg·kg⁻¹) was injected intravenously via the left femoral vein in order to observe the effects on blood pressure and HR.

In the second set of experiments, noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were administered. Once the haemodynamic parameters returned to basal values, a dose of saline, ketanserin (0.2 or 0.1 mg·kg⁻¹) or the tested compound (0.2 mg·kg⁻¹) was injected. After 10—15 min, the same dose of pressor agent was administered to test (a) the reproducibility of the cardiovascular effects induced by successive administrations of NA and 5-HT, (b) the modification of the cardiovascular responses induced by pressor agents in the treated preparations with the tested compounds.

In Vitro Experiments: General Preparation of Tissues Male Sprague-Dawley rats (250—300 g weight) and guinea-pigs (weighing 400—500 g) were sacrificed by decapitation after being anaesthetized with CO₂. The tissues were removed and mounted in organ baths containing Krebs solution (composition (ms): NaCl 119, KCl 4.7, CaCl₂·2H₂O 2.5, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.2, NaHCO₃ 25, glucose 11), thermoregulated at 37°C and bubbled with 95% O₂+5% CO₂. All the tissues were allowed to stabilize 60 min under 1 g (rat aorta and fundic strips) and 0.5 g resting tension (guinea-pig tissues). The assays were carried out as detailed below.

Isolated Rubbed Rat Aorta (5-HT₁, and 5-HT₃ Receptor) Vascular rings were prepared from the aorta of male rats essentially as described in Campos-Toimil et al.6 Cumulative concentration–response curves (CRCs) were constructed for 5-HT (30 nM to 100 μM) and NA (0.1 nM to 10 μM) by Van Rossum’s method.17 Three concentrations of QF 0303B...
(0.1—5 μm), QF 0313B (0.1—3 μm), QF 0307B, QF 0311B and ketanserin (1—10 μm) were incubated for 30 min prior to construction of a new concentration-effect curve. Cumulative relaxation curve to isometric contractions induced by NA (1 μM) or KCl (60 mM) were recorded. Once the plateau was reached, cumulative doses of the tested compounds were added.

**Isolated Rat Stomach Fundus (5-HT<sub>2B</sub> Receptor)**

Fundic tissue were cut following the method of Vane. The CRCs to 5-HT (30 nM—300 μM) were constructed as described above. Then, QF 0303B, QF 0311B (0.1—3 μm), QF 0307B, QF 0313B and ketanserin (1—10 μM) were incubated for 30 min prior to construction of a new 5-HT concentration-response curve (CRC).

**Isolated Guinea-Pig Ileum Longitudinal Muscle (5-HT<sub>3</sub> and H<sub>1</sub> Receptor)**

Longitudinal muscle strips from guinea-pig ileum were prepared using the method of Buchheit et al. In the experiments with 5-HT, methysergide 1 μM was used to block 5-HT, and 5-HT<sub>2A</sub> receptors. CRCs to 5-HT (1—100 μM) were constructed on a non-cumulative basis. A concentration of the test compound (0.1—1 μM) was then added to the organ bath 15 min before the agonist CRC was repeated. CRCs to HA (10 nM—30 μM) were constructed on a cumulative basis using the Van Rossum's method. A concentration of the test compound (1 μM) was added 25 min before the agonist CRC was repeated.

**Whole Isolated Guinea-Pig Ileum (Muscarnic and Nicotinic Receptors)**

In experiments with whole ileum, 3 cm length fragments were cut and were allowed to stabilize for 60 min. Non-cumulative concentration-response curve to bethanecol (100 nM—100 μM) or 1,1-dimethyl-4-phenylpiperazinium (DMPP) (1—100 μM) were constructed. In both cases, a concentration of the test compound (1—3 μM) was then added to the organ bath 15 min before a new agonist CRC.

**Data Presentation and Statistical Analysis**

Systolic blood pressure and heart rate values are expressed in millimeter of mercury (mmHg) and beats per minute, respectively. Mean arterial pressure (MAP) was calculated according to the formula: (2 diastolic pressure+ systolic pressure)/3. Unless otherwise specified, increases or decreases of mean blood pressure and HR values are expressed as absolute values with respect to basal values/values obtained in the presence of the compound tested.

In functional studies, from each individual CRC, EC<sub>50</sub> (agonist concentration required to elicit 50% of the maximal response) was calculated using the logistic equation:

\[ E = \frac{\alpha}{1 + \left(\frac{EC_{50}}{[A]}\right)^r} \]

in which E is the response, α is the maximum response, and [A] and s are the agonist concentration and the slope, respectively.

Agonist pD<sub>2</sub> values were obtained by calculating the negative log<sub>10</sub> of the EC<sub>50</sub>. Results shown are expressed as means±standard error mean (S.E.M.) [confidence interval 95%] where n is the number of assays and N the number of animals used in the experiments. Antagonist pD<sub>2</sub> values were calculated using the Van Rossum's method. pA<sub>2</sub> values were obtained according to Arunakrishna and Schild.

When only one concentration of the drug was used, the pA<sub>2</sub> values were obtained according to MacKay.

Statistical significance of differences between two means (p<0.05) was estimated by Student's two-tailed t test for paired data.

Correlation studies between in vitro and in vivo activities were carried out by comparing the in vitro antagonism (pA<sub>2</sub>) of each compound considered with a single dose (0.2 mg·kg<sup>-1</sup>) in vitro experiment. Pearson correlation coefficients and the corresponding bilateral statistical significations, were computed with the SPSS statistical package (SPSS Inc., Chicago, U.S.A.).

**Drugs and Chemicals**

The new compounds QF 0303B, QF 0307B, QF 0311B and QF 0313B were provided by the Department of Organic Chemistry (Section of Pharmaceutical Chemistry of the University of Santiago de Compostela). Other drugs used: (-)-noradrenaline bitartrate, histamine dihydrochloride, carbamyl-β-methylcholine chloride (bethanecol chloride), 1,1-dimethyl-4-phenylpiperazines iodide (DMPP) (from Sigma Chemical Co., St. Louis, MO, U.S.A.), serotonin hydrochloride, 2-methyl-serotonin (maleate), methysergide maleate and ketanserin tartrate (from RBI, Natick, MA, U.S.A.).

**RESULTS**

1. **Effects of QF Compounds in ANR**

The mean basal values for MAP and HR in anaesthetized rats (measured in control and treated groups before administration) were 80±3 mmHg and 351±8 beats·min<sup>-1</sup> (N=30), respectively. In control animals, these values were not significantly different throughout the period in which the measurements were carried out (p>0.05, n=5).

Administration of QF 0303B (Fig. 2A), QF 0307B, QF 0311B, QF 0313B and ketanserin (0.2 mg·kg<sup>-1</sup> i.v.) produced a rapid, remarkable but short-lasting fall in MAP: decrease from 30±4 (i.e. QF 0303B) to 24±4 (i.e. QF 0313B), p<0.01 with respect to control values, accompanied by a bradycardia, decrease in beats/min spans from 81±33 to 50±26, (p<0.01, n=5). The maximum effect in MAP was reached approximately 1 min after treatment and maintained only between 5—34 min after administration (Fig. 3). At higher doses (1 mg·kg<sup>-1</sup> i.v.), ketanserin produced a pronounced and sustained decrease in MAP (Fig. 4A).

5-HT (75 μg·kg<sup>-1</sup> i.v.) significantly modified MAP and HR with a characteristic triphasic response: (1) an initial decrease (52±3 and 95±10 for MAP and HR, respectively), (2) a fast increase (35±2 and 38±2), and (3) a long-lasting hypotensive effect (25±2 and 30±2 (Fig. 2C). After recovering the initial values of MAP and HR, a dose of the compound (0.2 mg·kg<sup>-1</sup> i.v.), was added. After 15 min, again, the same dose of 5-HT was administered and, as above, a characteristic triphasic response was produced (n=5). There were statistically significant differences (p<0.01) between the values of the increments of MAP (second phase) induced by 5-HT obtained before and after administration of QF 0303B (Fig. 2C), QF 0307B, QF 0311B, QF 0313B and ketanserin (Table 1). All compounds did not modify the first and third hypotensive phases of the triphasic response induced by 5-HT.

NA (5 μg·kg<sup>-1</sup> i.v.) produced an increase of MAP (48±4)
and HR (26±5). After recovering the initial values of MAP and HR, a dose of 0.2 mg·kg⁻¹ i.v. of the compounds (QF 0303B (Fig. 2B), QF 0307B, QF 0311B, QF 0313B) or ketanserin was injected. After 15 min, the same dosage of NA produced a lower increase of these two haemodynamic parameters in some of the tested compounds (n=5) (i.e. QF 0303B, QF 0307B and QF 0313B but not QF 0311B and ketanserin) (Table 1). At higher doses, however, ketanserin (1 mg·kg⁻¹ i.v.) strongly antagonized the pressor response induced by NA (Fig. 4B).

2.1. 5-HT₂A and α₁ Receptor Blockade in Isolated Rubbed Rat Aorta Rings The preparation lacked spontaneous activity. 5-HT elicited dose-related contractions in intact aorta rings with pD₂ of 5.71±0.04 [5.01—5.78] (n=80; N=20) and a maximal tension (mg) of 5660±210 [4335—6980]. All of the tested compounds shifted the CRC for 5-HT (0.3—100 μM) to the right without depression of the maximal response with pA₂ values and slope values shown in Table 2. QF compounds showed lower antagonist activity than ketanserin. These results are consistent with the concept of tested QF compounds being potent competitive antagonists at vascular 5-HT₂A receptors but not as potent as ketanserin.¹³

NA elicited concentration-related contractions in intact aorta rings. The pD₂ and maximal tension (mg) values were 7.40±0.06 [6.69—8.17] and 6525±325 [2460—10600] (n=72, N=18), respectively. QF 0303B, QF 0307B, QF 0311B, QF 0313B and ketanserin also shifted to the right without depression the maximal response of the CRCs to NA (0.1 nm—10 μM). Schild plot analysis yielded pA₂ (±S.E.M.) and slope values are shown in Table 2. None of the slope values differed significantly from the theoretical value of −1 (p>0.05) thus providing evidence for the competitive nature of the antagonist activity. Comparison of these pA₂ values with those obtained for ketanserin indicates that unlike the other compounds, QF 0303B is more potent as an α₁-adrenoceptor antagonist than ketanserin.

2.2. Effects on High KCl-Induced Contractions Neither ketanserin nor any of the reference antihypertensive compounds demonstrated any significant ability to inhibit the KCl-contracted rat aorta (pIC₅₀<5.1, n=40, N=10, Table 3).
These data suggest that ketanserin and the compounds have no calcium entry blocking ability.

2.3. 5-HT_{2B} Receptor Blockade in Isolated Rat Fundic Strip Serotonin (5-HT) elicited concentration-dependent contractions in rat fundic strips showed a pD_{2} value of 7.52±0.08 [7.36—7.68] and maximal tension of 2450±225 [1850—3050] (n=54, N=8). QF 0307B and ketanserin did not exhibit competitive antagonist activity at these receptors at concentrations equal or lower than 1 μM. On the contrary, QF 0303B, QF 0311B, and QF 0313B showed a moderate competitive antagonism (Table 2).

2.4. 5-HT_{3} and H_{3} Receptor Blockade in Isolated Guinea-Pig Ileal Longitudinal Muscle 5-HT and 2-methyl-5-HT produced a concentration-dependent contractile response in the guinea-pig ileal longitudinal muscle. Values of pD_{2}, were 5.57±0.07 [5.17—5.97] (n=34, N=7) and 5.44±0.06 [5.2—5.68] (n=30, N=7), respectively. All compounds, with the exception of ketanserin and QF 0313B that were non active, were weak non-competitive antagonists of contractile responses at 5-HT_{3} receptors (Table 3). The t-test did not find significant statistical differences between the potency of the two agonists used.

Histamine (HA) contracted guinea-pig ileal longitudinal muscle with a pD_{2} value of 6.59±0.07 [6.34—6.84] (n=20, N=7). Increasing concentrations of the new compounds and ketanserin produced parallel displacement to the right of the
Table 3. Effect of Compounds on KCl-Induced Contractions and on the Indicated Receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>pIC_{50} (KCl)</th>
<th>pA_{2} (H_{1})</th>
<th>pD_{2} (5-HT_{2A})</th>
<th>pD_{2} (5-HT_{2C})</th>
<th>PD_{2}</th>
<th>PD_{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>QF 0303B</td>
<td>4.97±0.12</td>
<td>7.29±0.13</td>
<td>5.80±0.05</td>
<td>6.10±0.04</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>QF 0311B</td>
<td>5.12±0.27</td>
<td>7.44±0.17</td>
<td>5.61±0.28</td>
<td>5.73±0.24</td>
<td>Inactive</td>
<td>5.36±0.22</td>
</tr>
<tr>
<td>QF 0313B</td>
<td>4.98±0.045</td>
<td>7.70±0.03</td>
<td>Inactive</td>
<td>n.d.</td>
<td>Inactive</td>
<td>4.86±0.02</td>
</tr>
<tr>
<td>QF 0307B</td>
<td>4.58±0.015</td>
<td>7.34±0.25</td>
<td>n.d.</td>
<td>5.64±0.20</td>
<td>Inactive</td>
<td>5.82±0.27</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>4.14±0.15</td>
<td>8.03±0.19</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

n.d.: not determined. a) KCl studies were elicited at rubbed rat aorta. b) pA_{2} values were obtained using one concentration of the antagonist using the Mackay method. c) Data were determined using serotonin as agonist. d) Data were determined using 2-methyl-serotonin as agonist. In all cases, data are expressed as mean±S.E.M. All PD_{2} values were calculated according to Van Rossum.

HA CRCs. Slopes of the treated curves were not significantly different with respect to slopes of the control curves suggesting competitive antagonist activity at H_{1}-receptors. All compounds were potent antagonists of HR in this preparation, although pA_{2} values were slightly lower than ketanserin (Table 3).

2.5. Muscarinic and Nicotinic Receptor Blockade in Whole Isolated Guinea-Pig Ileum
Bethanechol produced a concentration-dependent contraction in the guinea-pig ileum. The pD_{2} was 5.79±0.07 [5.65—6.03] (n=24, N=5). In this preparation, at concentrations ranging from 1—3 μM, the compounds and ketanserin (with the exception of QF 0311B) did not significantly block muscarinic receptors. QF 0311B displayed a very weak non-competitive antagonism with a pD_{2} value of 5.36±0.22 (p<0.01, n=4, N=3).

DMPP contracted guinea-pig ileum with pD_{2} value of 5.43±0.06 [5.13—5.73] (n=27, N=6). In this preparation, the compounds (with the exception of ketanserin and QF 0303B) produced a non-parallel shifting and additionally depressed the maximum effect of DMPP (Table 3), which suggests a non-competitive antagonism.

3. Correlation Studies
No significant correlation was observed between percent reduction of blood pressure and percent inhibition of NA induced responses (r=-0.53, p=0.36) neither between percent reduction of blood pressure nor the pA_{2} value of α_{1}-antagonism calculated in vitro (r=0.47, p=0.43). However, there was a significant correlation between percent reduction of blood pressure and the pA_{2} value of 5-HT_{2A}-receptor antagonism calculated in vitro (r=0.88, p=0.046). Duration of the hypertensive effect shows a correlation with the intensity of α_{1}-adrenergic blockade (r=0.64, p=0.2). Because of the high serotoninergic 5-HT_{2A} blockade (around 100% for all the compounds) found in vivo, it was not statistically possible to determine such a correlation. Because there was no 5-HT_{2A}-receptor antagonism for ketanserin and QF 0307B, it was not possible to calculate a correlation between the hypertensive action and this serotoninergic blockade.

DISCUSSION

The objective of this work was to determine the potential activity of the novel ketanserin analogues QF 0303B, QF 0307B, QF 0311B and QF 0313B on the cardiovascular system to offer new information and a deeper understanding of the hypertensive action of 5-HT_{2} blocker drugs. In addition, this work studies the correlation between the variation in the arterial pressure and the degree of α_{1}-adrenergic/serotoninergic 5-HT_{2A} blockade.

In vivo studies were performed to study the direct effect of the intravenous bolus injection of ketanserin and the new compounds, and after NA and 5-HT addition. In vitro, we have investigated the vasorelaxant effect of the four analogues in rat isolated aorta by evaluating their direct effect and their action on the NA and 5-HT-induced contractile responses to ascertain whether the in vivo cardiovascular action of these drugs is related to their in vitro receptor blocking profile.

In vivo, intravenous bolus injection of all of the tested compounds and ketanserin (0.2 mg·kg⁻¹ i.v.) produced a rapid and remarkable but not long-lasting fall in MAP accompanied by an intense decrease in HR. At higher doses, however ketanserin (1 mg·kg⁻¹ i.v.) produced a sustained hypotension. In this preparation, intravenous injection of NA increased systolic and diastolic pressures due to increases in peripheral vascular resistance, which involves vasoconstriction (through stimulation of postsynaptic α_{1}- and α_{2}-adrenoceptors) and an increase in HR (mediated by activation of β_{1}-adrenoceptors).

The results presented here show that all the ketanserin analogues did antagonize in a different degree the hypertensive response induced by NA although the effect of ketanserin (0.2 mg·kg⁻¹ i.v.) and QF 0311B did not reach statistical significance. However, the positive chronotropic effects were not affected, which indicates that: (i) vasodilatation induced by these drugs can be attributed (at least in part) to a blockade of the postsynaptic α_{1}- and/or α_{2}-adrenoceptors of smooth muscle cells and (ii) bradycardia is not due to a blockade of the β_{1}-adrenoceptors. On the other hand, it is interesting to note that ketanserin, at higher doses (1 mg·kg⁻¹ i.v.) strongly inhibited the pressor response produced by NA.

The results of our in vitro experiments show that the inhibition of the NA-induced contractions may be due to a competitive antagonism of the vascular postjunctional α_{1}-adrenoceptors, owing to the fact that: (i) QF compounds cause a shift to the right of the NA CRC without a depression of the maximal response and (ii) the slope value in the Schild plot is not significantly different from unity. Furthermore, the α_{1}-adrenoceptor antagonist activity in vivo showed the same rank of alpha blocking activity as in vitro assays of antagonism of NA CRCs (correlation estimates: r=0.95, p=0.016).

In ANR, the blood pressure response to intravenous 5-HT administration was triphasic: (1) a brief depressor phase immediately following the injection, initiated with the coronary chemoreflex (Bezold-Jarisch reflex) attributed to interaction with cardiac 5-HT_{1} receptors, which led to profound
bradycardia and hypotension; (2) a pressor phase, ascribed to the vasoconstriction mediated through 5-HT₂₅A receptors and due mainly to the direct effects of 5-HT in increasing total peripheral vascular resistance, HR and cardiac output and finally (3) within 1 or 2 min after the injection, a prolonged hypotensive phase due to the activation of other serotonin receptors (according to recent findings; 5-HT₁ᵦ,) 5-HT₁ᵦ, or 5-HT₂₅B receptors and release of endothelial vasodilator factors. QF compounds, like ketanserin, blocked in a similar degree the hypertensive pharmacological response to 5-HT on blood pressure or HR, but did not affect the first and third phase of the response to 5-HT, which suggests that these compounds do interact with the 5-HT₂₅A receptors.

The novel compounds demonstrate serotoninergic antagonist activity at vascular 5-HT₂₅A receptors. It is interesting to note that the 5-HT₂₅B antagonist activity (Table 2) of the analogues is lower (although higher than ketanserin, which is inactive) and inverse in the rank to the 5-HT₂₅A blocking activity. Therefore, in contrast to the findings of a parallel study at the 5-HT₂₅A receptor, the diminished planarity of the molecule seems to affect positively the binding to the 5-HT₂₅B receptor. The fact that the in vitro active compounds on 5-HT₂₅B receptors (rat stomach fundus) did not modify the third phase induced by 5-HT in anaesthetized rat suggests that the activation of the endothelial 5-HT₂₅B receptor does not play an important role in the prolonged hypotension produced by 5-HT in this preparation.

The potency of QF 0307B, QF 0313B, like of ketanserin, as 5-HT₂₅A antagonists is higher than their potency as α₁-antagonists. In contrast, QF 0303B and QF 0311B displayed a higher α₁-antagonist activity. (Table 2)

Since ketanserin and its analogues display little or no activity at muscarinic or nicotinic and 5-HT₁ receptors (Table 3), it is unlikely that effects at these receptors would be involved in either the in vivo hypotensive activity or any potential side effects.

Another notable feature of the compounds is that all of them possess a significant degree of HA₁-antagonist activity. This property is also displayed by ketanserin (Table 3). The high degree of antihistaminic activity for ketanserin has been documented by other researchers. Ketanserin has been reported to produce sedation as a side effect in clinical usage, leading to dosage limitations. This sedation is generally attributed to the presence of the significant antihistaminic activity. Therefore, ketanserin presented a higher affinity than the compounds based on the results outlined above and with respect to relative antihistaminic activity, the compounds could display less potential for the manifestation of sedation as a major side effect than ketanserin.

The question addressed in this study was whether the hypotension induced by ketanserin and the compounds on ANR at the dose used (0.2 mg·kg⁻¹ i.v.) was dependent on α₁-adrenoceptor blockade, 5-HT₂₅A-receptor blockade or 5-HT₂₅B receptor blockade. To answer this question, we studied the relationship between the α₁-adrenergic and 5-HT₂₅A/5-HT₂₅B blocking properties and the hypotensive activity of ketanserin and our four new ketanserin analogues with varying degrees of activity at vascular α₁-adrenoceptor and 5-HT₂₅A/5-HT₂₅B receptors. We found that 1) there was a clear correlation between hypotension and serotoninergic 5-HT₂₅A blockade in vitro (r=0.88, p=0.046). 2) In contrast, there was not such a clear correlation between the α₁ antagonist activity and the intensity of hypotension (r=0.47, p=0.43) neither the percent of reduction of blood pressure and the percent of inhibition of NA induced pressure responses (r=0.53, p=0.36). A possible reason is that ketanserin and QF 0311B, at the doses used in vivo, which had an important α₁-adrenergic blocking effect in vitro, only weakly inhibited the elevation of blood pressure by NA suggesting that these are not in vivo correlated with in vitro α₁ blocking properties. Perhaps, ketanserin presents selectivity for one of the α₁ subtypes found in rat aorta and the non-blockade of one of the subtypes is responsible for the reduced blocking effect shown in vivo. Whatever the explanation, it remains to be determined. 3) A correlation exists between the duration of the hypotensive action and the degree of adrenergic blockade (r=0.64, p=0.02). This conclusion is supported by the fact that ketanserin at higher doses (1 mg·kg⁻¹ i.v.), showed a remarkable in vivo α₁-adrenoceptor blocking activity and produced a long-lasting hypotension. The corresponding correlation between duration of hypotensive action and serotoninergic inhibition in vitro it does not exist, because the inhibition is around 100% for all the compounds. Regarding the third (hypotensive) phase of the response to serotonin in vitro, it is possible to exclude the participation of the 5-HT₂₅B receptors because the 5-HT₂₅B blockers did not produce any effect on this phase. This seems reasonable because the cardiovascular implication of these 5-HT₂₅B receptors was only found in mineralocorticoid hypertensive rats.

To summarize, our results indicate that: a) QF 0303B, QF 0307B, QF 0311B, QF 0313B and ketanserin after acute administration at the dose of 0.2 mg·kg⁻¹ i.v., have been characterised as agents with a short-lasting hypotensive activity in the ANR and present clear vasorelaxant effects on rat smooth muscle. The probability of sedative effects resulting from the presence of antihistaminic activity in our compounds may be expected to be less than experienced with ketanserin. b) The vasorelaxant action of all the compounds in isolated rat aorta can be attributed to α₁-adrenoceptors and 5-HT₂₅A blockade and c) the brief hypotensive activity in ANR at the dose of 0.2 mg·kg⁻¹ i.v. is better attributed to a 5-HT₂₅A blockade and the lasting of the effect would be attributed to an α₁ blockade.

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