Evidence that Blood Pressure Reduction by Serotonin Antagonists is Related to Alpha Receptor Blockade in Spontaneously Hypertensive Rats

MARLENE L. COHEN, PH.D., RAY W. FULLER, PH.D., AND KEN D. KURZ, PH.D.

SUMMARY In vitro affinity for vascular 5HT₂ and alpha receptors was determined for several compounds (spiperone, ketanserin, mianserin, trazodone, mepiprazole, benzoctamine, m-trifluoromethylphenylpiperazine, m-chlorophenylpiperazine, and 1-(1-naphthyl)piperazine) known to interact with serotonin receptors. All compounds competitively inhibited 5HT₂ and alpha receptors with differing degrees of selectivity. Based on these observations, ketanserin, benzoctamine, and 1-(1-naphthyl)piperazine were evaluated as antihypertensive agents in spontaneously hypertensive rats (SHR). Of these compounds, 1-(1-naphthyl)piperazine was a highly selective 5HT₂ receptor antagonist with a ratio of 5HT₂ to alpha receptor affinity of greater than 2000. The ratio of 5HT₂ to alpha receptor affinity for ketanserin and benzoctamine was 63 and 16, respectively. However, the order of affinity toward 5HT₂ receptors was ketanserin > 1-(1-naphthyl)piperazine > benzoctamine whereas the order of affinity toward alpha receptors was ketanserin > benzoctamine > 1-(1-naphthyl)piperazine. A similar order of potency toward both 5HT₂ and alpha receptors was found in pithed SHR based on antagonism of the pressor response to serotonin and methoxamine, respectively. In the SHR, maximum blood pressure reduction at a dose of 10 mg/kg i.p. was approximately 65 and 30 mm Hg for ketanserin and benzoctamine, respectively; 1-(1-naphthyl)piperazine did not affect blood pressure. Thus, blood pressure reduction more closely paralleled the in vitro and in vivo potency of these agents toward vascular alpha rather than 5HT₂ receptors. These data support the contention that alpha receptor blockade rather than selective 5HT₂ receptor blockade is responsible for the antihypertensive activity of "serotonergic antagonists" in the SHR. (Hypertension 5: 676-681, 1983)

KEY WORDS • ketanserin • trazodone • mepiprazole • phenylpiperazines • 1-(1-naphthyl)piperazine • dissociation constants for vascular 5HT₂ receptors • dissociation constants for vascular alpha receptors

BLOCKADE of peripheral vascular serotonergic receptors has been proposed as a useful mechanism for lowering blood pressure in animals and in humans. These vascular serotonergic receptors resemble those receptors defined as 5HT₂ receptors in the central nervous system and radiolabeled by [³H]spiperone. 2 Vascular receptors in rat aorta, 2 caudal artery, 4, 5 and jugular vein 6 have pharmacologic characteristics of 5HT₂ receptors. Ketanserin, a serotonin antagonist selective for 5HT₂ vs 5HT₁ receptors, lowered blood pressure in animals and in humans, and the mechanism proposed initially was block of vascular 5HT₂ receptors. 1, 7

Although ketanserin is a potent antagonist of 5HT₂ vascular receptors, 1, 4-6 it also possesses lower but still relatively high affinity, similar to that of phentolamine, for postsynaptic alpha₂ receptor sites. 8 Some investigators 9-12 have concluded that the antihypertensive activity of ketanserin is related to its postsynaptic alpha-receptor blocking activity rather than to its antagonism of vascular 5HT₂ receptors. As an additional approach to evaluating whether selective antagonism of peripheral 5HT₂ receptors can reduce blood pressure, we have compared a compound, 1-(1-naphthyl)piperazine, that more selectively antagonizes 5HT₂ receptors instead of alpha receptors than does ketanserin, with ketanserin and another serotonin antagonist, benzoctamine. Among these three compounds that antagonize 5HT₂ and alpha re-
blockade of 5HT₂ vascular receptors does not lower blood pressure in conscious SHR. These results argue that selective blockade of 5HT₂ vascular receptors does not lower blood pressure in the SHR.

Methods

Isolation of Vascular Tissue

Male Wistar rats (150–300 g) (Harlan Industries, Inc., Cumberland, Indiana) were killed by a blow to the head. External jugular veins and thoracic aortas were dissected free of connective tissue, cannulated in situ with polyethylene tubing (PE-50, outside diameter = 0.97 mm), and placed in Petri dishes containing Krebs’ bicarbonate buffer (see below). The tips of two 30-gauge stainless-steel hypodermic needles bent into an L-shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was attached with thread to a stationary glass rod and the upper one was tied with thread to the transducer. This procedure for ring preparations (circular smooth muscle) of blood vessels has been described previously.

Tissues were mounted in organ baths containing 10 ml of modified Krebs’ solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; CaCl₂, 2H₂O, 1.6; KH₂PO₄, 1.2; MgSO₄, 1.2; dextrose, 10.0; and NaHCO₃, 24.8. Tissue bath solutions were maintained at 37°C and aerated with 95% O₂-5% CO₂. An initial optimum resting force of 1 and 4 g was applied to the jugular vein and aorta, respectively. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph equipped with Statham UC-3 transducers and microscale accessory attachment. Tissues were allowed to equilibrate 1 to 2 hours before exposure to drugs.

Determination of Apparent Dissociation Constants

After control responses to serotonin in the jugular vein or to norepinephrine in the aorta were obtained, vessels were incubated with appropriate concentrations of antagonist for 1 hour, a procedure recommended by Furchgott. Responses to serotonin or norepinephrine were then repeated in the presence of antagonist. Contraction to serotonin was evaluated in the jugular vein since this tissue produces marked responses to serotonin in the absence of alpha receptors. The aorta was used to evaluate alpha receptor antagonist activity since the jugular vein does not contract to norepinephrine. Both tissues have minimal, if any, innervation.

Apparent antagonist dissociation constants (Kₐ) were determined for each concentration of antagonist according to the following equation:

\[ K_a = \frac{[B]}{[\text{dose ratio} - 1]} \]

where [B] is the concentration of the antagonist, and dose ratio is the ED₉₀ of the agonist in the presence of the antagonist divided by the control ED₉₀. These results were then expressed as the negative logarithm of the Kₐ (i.e., \(-\log K_a\)). Calculations were performed with the aid of a computer and digital plotter as previously described.

The data were also analyzed according to the procedure of Arunlakshana and Schild. The dose ratio was determined at various concentrations of antagonist. According to Arunlakshana and Schild, if blockade is competitive, under equilibrium conditions, a plot of the logarithm (dose ratio — 1) against the negative logarithm of the molar concentration of antagonist should yield a straight line whose slope is 1 and intercept along the abscissa is the pA₂ which is equal to \(-\log K_a\).

Conscious Rat

The effects of 5HT₂ receptor antagonism on blood pressure were determined in conscious SHR (325–425 g). Rats were anesthetized with halothane (2% in nitrous oxide and oxygen) and were implanted with femoral arterial and venous catheters (Tygon). The tips of arterial and venous catheters were positioned in the abdominal aorta below the renal arteries and lower abdominal vena cava, respectively. The catheters were routed subcutaneously to an exit point at the base of the skull and then through a small leather harness fastened around the forequarters of each animal. The animals were allowed a 3 to 4-day recovery period after surgery. On the day before an experiment, each rat was conditioned to the experimental surroundings for 6 hours. On the day of the experiment, the harness on the back of each animal was connected to a spring tether through which arterial and venous extension tubing was routed. The other end of the tubing was connected to a water tight swivel. This system permitted direct recording of blood pressure in conscious free moving animals. Mean arterial blood pressure was measured via a Statham strain gauge transducer (P23DB, Statham Instruments, Oxnard, California) and recorded on a multichannel oscillograph (Beckman Model R611, Beckman Instruments, Palo Alto, California).

A minimum 30-minute equilibration period was observed prior to the experimental protocol during which time the animals preened and blood pressure was quite labile. Afterward, the animals appeared to sleep and pressure was stable. Following a control blood pressure measurement, rats were dosed with ketanserin, benzoxcantine, 1-(1-naphthyl)piperazine, or vehicle i.p., and pressure was monitored at various time intervals thereafter.

Pithed Rat Studies

Serotonin and alpha receptor antagonism were evaluated in the pithed SHR. This model was selected because serotonin responses in the conscious animal
are multiphasic and difficult to interpret due to chemo- and baroreceptor stimulation; whereas those responses in the pithed preparation are primarily direct vascular effects. SHR were anesthetized with halothane (2% in nitrous oxide and oxygen), femoral arterial and venous catheters were implanted, and the trachea was cannulated. Rats were pithed by passing a steel rod through the right orbit and down the entire length of the spinal column where it remained for the duration of the experiment. Immediately after pithing, rats were ventilated with room air via a rodent respirator (Harvard, Model 680; tidal volume of 1 ml/100 g body weight, 60 cycles/min) which minimized anesthetic effects. An equilibration period of 15 minutes was observed prior to control measurements and intraperitoneal administration of drugs or vehicle. Increasing doses of serotonin or methoxamine were intravenously injected 15 minutes later. Blood pressure was allowed to recover to control levels before subsequent doses were given. The relatively specific alpha, receptor agonist, methoxamine, was used because ketanserin selectively blocks alpha, receptors.43

Drugs
Drugs used in this study were serotonin (Sigma Chemical Company, St. Louis, Missouri) prepared in doses of the free base, methoxamine (Burroughs Wellcome, Research Triangle Park, North Carolina), ketanserin (Janssen, Beerse, Belgium), benzoctamine (Ciba, Summit, New Jersey), and 1-(1-naphthyl)piperazine synthesized by B. B. Molloy in the Lilly Research Laboratories.

Test drug solutions were prepared fresh daily and administered i.v. in a volume of 1 ml/kg. Ketanserin was dissolved in 0.1 M tartaric acid solution, pH 3.1, and all other drug solutions were prepared in normal saline. The i.p. drug volume ranged up to 5 ml/kg depending on solubility characteristics.

Statistical Analysis
Statistical significance for mean changes in arterial blood pressure was determined by an analysis of variance followed by Dunnett’s test to compare differences of the mean from control.

Results
Since ketanserin was reported to have high affinity for both vascular 5HT₂ and alpha adrenergic receptors,4,5 we initially asked if alpha-receptor antagonist activity could also be demonstrated with other known 5HT₂ antagonists. Eight compounds (spiperone, ketanserin, mianserin, trazodone, mepiprazole, benzoctamine, m-trifluoromethylphenylpiperazine, and m-chlorophenylpiperazine) were evaluated for their ability to antagonize serotonin-induced contractions in the jugular vein and to block norepinephrine-induced contractions in the rat aorta. All eight compounds blocked both 5HT₂ and alpha receptors with varying degrees of selectivity (table 1).

![Figure 1](http://hyper.ahajournals.org/)

**FIGURE 1.** Structure and negative logarithm of the dissociation constant for interaction of 1-(1-naphthyl)piperazine with 5HT₂ and alpha receptors as determined in the rat jugular vein and aorta, respectively.

By systematic evaluation of several piperazine derivatives known to have 5HT₂ antagonist activity,20,21 we identified a compound unique in its specificity toward 5HT₂, vascular receptors. This compound, 1-(1-naphthyl)piperazine (fig. 1), showed high affinity for vascular 5HT₂ receptors (−log K_B = 8.75) but low affinity for postsynaptic alpha receptors (−log K_B = 5.38). Thus, this piperazine derivative showed a 2000-fold selectivity for antagonism of 5HT₂ receptors relative to alpha adrenergic receptors and provides a useful tool with which to examine the role of 5HT₂ vascular receptors in lowering blood pressure without the complication of alpha-receptor antagonist activity.

Three compounds with different affinity and selectivity toward 5HT₂ and alpha receptors (ketanserin, benzoctamine, and 1-(1-naphthyl)piperazine) were evaluated in the conscious SHR for their ability to lower mean arterial pressure (fig. 2). Values for ketanserin and the 15-minute time point for benzoctamine were significantly (p < 0.05) different from saline-treated SHR.

![Figure 2](http://hyper.ahajournals.org/)

**FIGURE 2.** Conscious SHR. Effect on mean arterial pressure produced by intraperitoneal administration of saline (n = 7); ketanserin (n = 4); benzoctamine (n = 9); or 1-(1-naphthyl)piperazine (n = 6) at a dose of 10 mg/kg. Values are means ± sem. All values for ketanserin and the 15-minute time point for benzoctamine were significantly (p < 0.05) different from saline-treated SHR.
### Table 1. Apparent Dissociation Constants of Antagonists for 5HT2 and Alpha Receptors Determined in the Rat Jugular Vein and Aorta, Respectively

<table>
<thead>
<tr>
<th>Serotonin Antagonists</th>
<th>Structure</th>
<th>5HT2 $-\log K_B \pm SE$</th>
<th>Alpha $-\log K_B \pm SE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiperone</td>
<td><img src="image" alt="Spiperone Structure" /></td>
<td>10.1 ±0.09 (8)</td>
<td>9.0 ±0.09 (13)</td>
</tr>
<tr>
<td>Ketanserin</td>
<td><img src="image" alt="Ketanserin Structure" /></td>
<td>9.7 ±0.07 (12)</td>
<td>7.9 ±0.04 (7)</td>
</tr>
<tr>
<td>Mianserin</td>
<td><img src="image" alt="Mianserin Structure" /></td>
<td>9.3 ±0.09 (14)</td>
<td>7.3 ±0.07 (6)</td>
</tr>
<tr>
<td>Trazodone</td>
<td><img src="image" alt="Trazodone Structure" /></td>
<td>8.7 ±0.09 (11)</td>
<td>6.8 ±0.12 (9)</td>
</tr>
<tr>
<td>Mepiprazole</td>
<td><img src="image" alt="Mepiprazole Structure" /></td>
<td>7.9 ±0.09 (6)</td>
<td>8.3 ±0.13 (13)</td>
</tr>
<tr>
<td>Benzoctamine</td>
<td><img src="image" alt="Benzoctamine Structure" /></td>
<td>7.8 ±0.08 (6)</td>
<td>6.6 ±0.08 (13)</td>
</tr>
<tr>
<td>m-Chlorophenylpiperazine</td>
<td><img src="image" alt="m-Chlorophenylpiperazine Structure" /></td>
<td>7.5 ±0.07 (9)</td>
<td>5.7 ±0.10 (11)</td>
</tr>
<tr>
<td>m-Trifluoromethylphenylpiperazine</td>
<td><img src="image" alt="m-Trifluoromethylphenylpiperazine Structure" /></td>
<td>7.5 ±0.07 (9)</td>
<td>6.43 ±0.24 (7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate number of rats studied.
benzoctamine, and 1-(1-naphthyl)piperazine) were selected for evaluation of antihypertensive activity in conscious SHR. The order of affinity toward 5HT₂ receptors in vitro was ketanserin > 1-(1-naphthyl)piperazine > benzoctamine whereas the order of affinity toward alpha receptors was ketanserin > benzoctamine > 1-(1-naphthyl)piperazine. At 10 mg/kg i.p., ketanserin produced the greatest fall in blood pressure; benzoctamine lowered blood pressure less effectively; and 1-(1-naphthyl)piperazine was ineffective as an antihypertensive agent in the conscious SHR (fig. 2). Therefore, blood pressure reduction paralleled in vitro antagonist potency or affinity for alpha receptors not 5HT₂ receptors.

To insure that the relative potency of these antagonists for vascular serotonin and alpha receptors as determined in vitro was similar to that which occurred in vivo, the effect of these antagonists on the pressor response to serotonin and methoxamine, a postsynaptic alpha receptor agonist, was evaluated in pithed SHR. The shift in the pressor response to serotonin was greatest for ketanserin followed by 1-(1-naphthyl)piperazine and then benzoctamine (fig. 3), paralleling their in vitro affinities for 5HT₂ receptors. The shift in the pressor response to methoxamine (fig. 4) paralleled the in vitro affinity toward alpha receptors. Thus, blood pressure reduction by the three agents in the SHR paralleled their alpha-adrenergic postsynaptic inhibitory effectiveness as demonstrated both in vitro and in vivo rather than their ability to block peripheral 5HT₂ receptors.

Discussion

An involvement of serotonin in vascular contraction related to elevations in blood pressure has been suggested. In initial reports, the mechanism of action for the antihypertensive activity of ketanserin was attributed to antagonism of peripheral vascular 5HT₂ receptors. More recently, however, the antihypertensive effectiveness of ketanserin in animals has been attributed to its antagonism of alpha adrenergic receptors (a known mechanism for reducing blood pressure).

In this report, we have used a different approach to compare the importance of 5HT₂ receptor blockade and alpha receptor blockade to blood pressure reduction in the SHR. We compared the antihypertensive activity of three compounds with differing profiles of inhibitory activity toward serotonin and alpha receptors. Blood pressure reduction by ketanserin, benzoctamine, and 1-(1-naphthyl)piperazine in the SHR paralleled alpha receptor blockade rather than 5HT₂ receptor antagonism. These results indicate that the antihypertensive activity of “selective 5HT₂ antagonists” may be more related to their alpha blocking properties, as recently suggested, and are in agreement with others who have concluded that ketanserin lowers blood pressure in SHR by alpha receptor blockade.

This conclusion is reinforced by the identification of a highly selective 5HT₂ receptor antagonist that did not lower blood pressure. This antagonist, 1-(1-naphthyl)piperazine, was unique in that it possessed high affinity for 5HT₂ receptors (\(-\log K_B = 8.75\)) with minimal affinity for postsynaptic vascular alpha receptors.

Figure 3. Pithed SHR. Log dose-pressor response to serotonin 15 minutes after intraperitoneal administration of saline (n = 13); ketanserin (1.0 mg/kg, n = 4); benzoctamine (10.0 mg/kg, n = 6); or 1-(1-naphthyl)piperazine (10.0 mg/kg, n = 6). Values are means ± SEM.

Figure 4. Pithed SHR. Log dose-pressor response to methoxamine 15 minutes after i.p. administration of saline (n = 5); ketanserin (10.0 mg/kg, n = 4); benzoctamine (10.0 mg/kg, n = 5); or 1-(1-naphthyl)piperazine (10.0 mg/kg, n = 3). Values are means ± SEM.

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(− Log \(K_{d}\) = 5.38). Using this compound to evaluate the importance of vascular serotonin to the elevation in blood pressure that occurs in SHR, we found that selective block of vascular 5HT₂ receptors is not an adequate stimulus to lower blood pressure.

Other piperazine derivatives, m-chlorophenylpiperazine and m-trifluoromethylphenylpiperazine, are vascular 5HT₂ receptor antagonists. However, 1-(1-naphthyl)piperazine showed approximately 30-fold higher affinity for vascular 5HT₂ receptors than the other two piperazines. In addition, m-chlorophenylpiperazine and m-trifluoromethylphenylpiperazine were only 63- and 6-fold (table 1) more selective toward 5HT₂ receptors relative to alpha receptors, respectively, whereas 1-(1-naphthyl)piperazine showed greater than 2000-fold selectivity. Thus, the naphthyl moiety conferred greater 5HT₂ receptor affinity and selectivity relative to the other phenylpiperazines.

Because of the high antagonist selectivity of 1-(1-naphthyl)piperazine toward 5HT₂ receptors, and the lack of peripheral vascular agonist activity, this compound is a useful tool with which to evaluate physiological or pathological states in addition to hypertension that may involve excessive activation of 5HT₂ receptors. In SHR, lack of an antihypertensive effect of 5HT₂ antagonists is unlike in other states. In SHR, lack of an antihypertensive effect of 5HT₂ antagonists may involve excessive activation of 5HT₂ receptors. Doses of ketanserin that lowered blood pressure in hypertensive patients did not antagonize the pressor response to phenylephrine, an alpha agonist. Furthermore, in humans, platelet-derived serotonin may play a greater role in blood pressure regulation, and the proposal that a selective 5HT₂ receptor antagonist may be more useful in an aged population remains to be evaluated.

**Addendum**
Reimann and Frolich (Lancet 1:703, 1983) have recently reported that ketanserin at antihypertensive doses in humans does antagonize methoxamine pressor responses, i.e., blocks alpha, adrenergic receptors in humans.

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*Hypertension*. 1983;5:676-681
doi: 10.1161/01.HYP.5.5.676

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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