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

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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

BLODARKADE of peripheral vascular serotoninergic receptors has been proposed as a useful mechanism for lowering blood pressure in animals and in humans. These vascular serotoninergic 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 vein⁶ 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.²⁷

Although ketanserin is a potent antagonist of 5HT₂ vascular receptors,¹,³⁻⁶ it also possesses lower but still relatively high affinity, similar to that of phentolamine, for postsynaptic alpha₂ receptor sites.⁸ Some investigators²⁹⁻¹² 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-
ceptors with markedly different selectivity, reduction of blood pressure paralleled alpha-receptor blocking potency but not 5HT₂-receptor blocking potency. 1-(1-naphthyl)piperazine antagonized vascular 5HT₂ receptors in vitro and the pressor response to injected serotonin in pithed SHR in vivo, but did 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.13

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 with Statham UC-3 transducers and microscale accessory attachment. Tissues were allowed to equilibrium 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.14 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.15 The aorta was used to evaluate alpha receptor antagonist activity since the jugular vein does not contract to norepinephrine.15 Both tissues have minimal, if any, innervation.16, 17

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 Kₐ). Calculations were performed with the aid of a computer and digital plotter as previously described.14

The data were also analyzed according to the procedure of Arunlakshana and Schild.19 The dose ratio was determined at various concentrations of antagonist. According to Arunlakshana and Schild,19 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 Kₐ.

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, benzoctamine, 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 chemoden 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.4,5

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 5HT, and alpha adrenergic receptors,4,5 we initially asked if alpha-receptor antagonist activity could also be demonstrated with other known 5HT, antagonists. Eight compounds (spiperone, ketanserin, mianserin, trazodone, meziprazole, 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 5HT, and alpha receptors with varying degrees of selectivity (table 1).

By systematic evaluation of several piperazine derivatives known to have 5HT, antagonist activity,20,21 we identified a compound unique in its specificity toward 5HT, vascular receptors. This compound, 1-(1-naphthyl)piperazine (fig. 1), showed high affinity for vascular 5HT, 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 5HT, receptors relative to alpha adrenergic receptors and provides a useful tool with which to examine the role of 5HT, vascular receptors in lowering blood pressure without the complication of alpha-receptor antagonist activity.

Three compounds with different affinity and selectivity toward 5HT, and alpha receptors (ketanserin,
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</th>
<th>Antagonists Structure</th>
<th>5HT₂ (-\log K_B \pm \text{se})</th>
<th>Alpha (-\log K_B \pm \text{se})</th>
</tr>
</thead>
</table>
| Spiperone | \[
\text{F} \quad \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{N} \quad \text{Y} \quad \text{I} \\
\]
| 10.1 ± 0.09 (8) | 9.0 ± 0.09 (13) |
| Ketanserin | \[
\text{F} \quad \text{O} \quad \text{C} \quad \text{N-CH} \quad \text{2-CH} \quad \text{-N} \quad \text{O} \quad \text{H} \\
\]
| 9.7 ± 0.07 (12) | 7.9 ± 0.04 (7) |
| Mianserin | \[
\text{N-CH} \quad \text{N-CH} \quad \text{3} \\
\]
| 9.3 ± 0.09 (14) | 7.3 ± 0.07 (6) |
| Trazodone | \[
\text{N} \quad \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{N} \quad \text{Y} \quad \text{I} \\
\]
| 8.7 ± 0.09 (11) | 6.8 ± 0.12 (9) |
| Mepiprazole | \[
\text{N} \quad \text{C} - \text{CH}_2 - \text{CH}_2 - \text{NH} - \text{CH}_3 \\
\]
| 7.9 ± 0.09 (6) | 8.3 ± 0.13 (13) |
| Benzoctamine | \[
\text{CH}_2 - \text{-NH} - \text{CH}_3 \\
\]
| 7.8 ± 0.08 (6) | 6.6 ± 0.08 (13) |
| m-Chlorophenyl-piperazine | \[
\text{HN} \quad \text{N} \quad \text{Cl} \\
\]
| 7.5 ± 0.07 (9) | 5.7 ± 0.10 (11) |
| m-Trifluoromethyl-phenylpiperazine | \[
\text{HN} \quad \text{N} \quad \text{CF}_3 \\
\]
| 7.5 ± 0.07 (9) | 6.43 ± 0.24 (7) |

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 5HT₁ 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 Kᵦ = 8.75) with minimal affinity for postsynaptic vascular alpha receptors.
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(-Log Kd = 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-trifluoromethylenylpiperazine, are vascular 5HT2 receptor antagonists.21 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-trifluoromethylenylpiperazine 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 1-(1-naphthyl)piperazine in rats at doses that block vascular 5HT2 receptors argues against the hypothesis that selective inhibition of vascular 5HT2 receptors is a mechanism by which pressure can be reduced. Furthermore, the demonstration that blood pressure reduction with three different agents (ketanserin, benzoxatamine, and 1-(1-naphthyl)piperazine), paralleled alpha receptor antagonist activity rather than 5HT2 receptor antagonist activity reinforces our conclusion that selective inhibition of vascular 5HT2 receptors is unlikely to lower blood pressure in SHR. However, in humans, Wenting et al.26 presented preliminary evidence that ketanserin lowered blood pressure without blocking alpha 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 proposal27 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.

References

Evidence that blood pressure reduction by serotonin antagonists is related to alpha receptor blockade in spontaneously hypertensive rats.
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