Sympathoinhibitory Responses to 2-Methylserotonin During Changes in Sodium Intake

Jørgen Søberg Petersen, Carmen Hinojosa-Laborde, and Gerald F. DiBona

The vagal-mediated reflex responses elicited by the selective serotonin type 3 receptor agonist 2-methylserotonin were examined by administration (6.25, 12.5, 25, and 50 μg/kg i.v.) of 2-methylserotonin to sinoaortic-denervated rats with either intact or sectioned vagi. To study the influence of dietary sodium intake on 2-methylserotonin-induced vagal reflex responses, we performed experiments in rats fed either a high or low sodium diet. Left ventricular end-diastolic pressure was significantly higher in animals on a high than low salt diet. However, mean arterial pressure and heart rate were similar in high and low salt groups. In rats with intact vagi, 2-methylserotonin produced a dose-dependent increase in afferent vagal nerve activity and a dose-dependent decrease in efferent renal sympathetic nerve activity, mean arterial pressure, and heart rate. The sympathoinhibitory responses of decreased efferent renal sympathetic nerve activity, mean arterial pressure, and heart rate were abolished by vagotomy and were not affected by changes in dietary sodium intake. We conclude that the sympathoinhibitory effect of 2-methylserotonin is due to stimulation of vagal afferents with inhibitory action on peripheral sympathetic nerve activity and that the sympathoinhibitory responses are unaffected by changes in dietary sodium intake. (Hypertension 1993;1:1000-1004)

KEY WORDS • serotonin • sodium • vagotomy • sympathetic nervous system • blood pressure • receptors, serotonin

The complex cardiovascular response of serotonin (5-hydroxytryptamine, 5-HT) has been ascribed to the action of 5-HT on several subtypes of 5-HT receptors with different cardiovascular effects. 1 5-HT subtype 3 (5-HT₃) receptor-mediated actions are defined as responses to 5-HT that are mimicked by the selective agonist 2-methyl-5-HT and blocked by selective antagonists such as MDL 72222 and ICS 205-930. 2-4 5-HT₃ receptors have been demonstrated on both afferent (vagus nerve, nodose ganglion, dorsal root ganglion, pain afferents) and efferent (superior cervical ganglion, cardiac sympathetic nerve endings, enteric neurons) neurons and at several locations in the brain. 5 Stimulation of neural 5-HT₃ receptors elicits an excitatory effect by stimulating an inward current of cations leading to depolarization. 6 Phenyl biguanide is known to mimic the effect of 5-HT on peripheral neurons, and the cardiovascular effects of phenyl biguanide are blocked by the selective 5-HT₃ receptor antagonist MDL 72222 or vagotomy, suggesting that the cardiovascular effects of peripheral 5-HT₃ receptor stimulation are mediated via vagal afferents. 7-10 In the present study, we tested this hypothesis by examining the sympathoinhibitory responses to peripheral administration of the selective 5-HT₃ receptor agonist 2-methyl-5-HT in rats with and without vagotomy.

Studies of cardiac vagal afferents have shown that they do not show similar responses to mechanical stimulation (e.g., volume expansion) and chemical stimulation (e.g., phenyl biguanide). 11 This has created the concept of two distinct types of unmyelinated vagal afferents, mechanosensitive and chemosensitive. Both types can affect efferent renal sympathetic nerve activity (ERSNA), but the physiological role of chemosensitive vagal afferents remains unknown. It is well documented that phenyl biguanide stimulates chemosensitive vagal afferents, but whether stimulation of these presumably 5-HT₃ receptor agonist-sensitive vagal fibers can interact with mechanosensitive vagal afferents in the control of ERSNA is unknown. Morgan et al. 12 provided evidence that serotonergic mechanisms are essential for the vagal reflex inhibition of ERSNA during acute severe hypotensive hemorrhage but not during acute intravenous volume expansion. However, whether vagal responsiveness to serotonergic stimuli is altered during conditions with...
Animals

Experiments were performed in male Sprague-Dawley rats weighing 327±3 g (Harlan Sprague Dawley, Indianapolis, Ind.). The animals were randomized to a dietary regimen allowing free access to either a high sodium rat chow (HNa; Na, <1 mmol/kg) or a low sodium rat chow (LNa; Na, <1 mmol/kg) and tap water ad libitum. All experimental procedures were in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of experimental animals.

Experimental Procedures

After a minimum of 2 weeks on LNa or HNa, the animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and placed on a heated micropuncture table maintaining rectal temperature at 37-38°C. Medical-grade Tygon catheters were inserted into the femoral artery and vein for measurement of arterial pressure and intravenous infusion, into the right jugular vein for intravenous administration of drugs, and into the left ventricle via the right carotid artery for measurement of left ventricular end-diastolic pressure. Isotonic saline (3 mL/hr) with pentobarbital (5 to 10 mg/kg per hour) was infused into the femoral vein catheter throughout the experiment. Catheter placement was evaluated by assessment of pressure waveform as determined by Statham P23XL pressure transducers coupled to a model 7E polygraph (Grass Instruments, Quincy, Mass.). HR was recorded by a linear cardiotachometer (Grass model 7P4) triggered by the arterial pressure waveform. An endotracheal tube was inserted, and the animal was paralyzed with pancuronium (1 mg/kg i.v., supplemented with 1 mg/kg as needed) while artificially ventilated with atmospheric air. Tidal volume and respiratory rate were adjusted to maintain arterial pH between 7.35 and 7.45 throughout the experiment. The left kidney was exposed via a retroperitoneal approach through a left flank incision. With the use of a dissection microscope (×25), a branch of renal nerve from the aortocaval ganglion was carefully isolated, and a bipolar platinum electrode was hooked on the renal nerve branch. The renal nerve activity was led through a Grass model HIP511 high-impedance probe and amplified (×3,000–20,000) and filtered (30–3,000 Hz) with a Grass model P511 bandpass amplifier. The amplified and filtered signal was led to an oscilloscope (model 5113, Textronix, Beaverton, Ore.) for visual representation, to an audio amplifier/loudspeaker (Grass model AM8 audio monitor) for audio representation, and to a rectifying voltage integrator (Grass model 7P10). The signal quality was evaluated by its pulse synchronous rhythmicity and by the decrease in renal nerve activity in response to either sinoaortic baroreceptor loading with phenylephrine (0.25 μg i.v.) or stimulation of vagal afferents by 2-methyl-5-HT (50 μg/kg i.v.). Phenylephrine at 0.25 μg i.v. was the highest dose without effect on AVNA. Both maneuvers elicited substantial inhibition of ERSNA. On establishment of an optimal recording, the recording electrodes were fixed to the renal nerve branch with a silicone adhesive (Sil-Gel 604, Wacker-Chemie, Munich, FRG). To eliminate afferent renal nerve activity from the recording signal, we cut the renal nerve bundle peripherally. The right cervical vagus was isolated, and the nerve sheath was carefully dissected away with sharp forceps under a dissection microscope (×25). Thin filaments were obtained and placed on a bipolar platinum electrode connected via a high-impedance probe (Grass model HIP511) to a bandpass amplifier (Grass P511), where the signal was filtered (30–3,000 Hz) and amplified (×15,000–30,000). The output from the amplifier was fed into an oscilloscope (Textronix 5113), to an audio amplifier/loudspeaker (Grass model AM8 audio monitor), and to a rectifying voltage integrator (Grass model 7P10). The quality of the signal was evaluated by its respiratory and pulse synchronous activity and by a marked stimulation of vagal nerve activity in response to intravenous injection of 50 μg/kg 2-methyl-5-HT. On establishment of a satisfactory recording, the recording electrodes were fixed to the nerve filaments by silicone adhesive. After hardening of the silicone adhesive, the vagal nerve filaments from the otherwise intact right vagal nerve were cut centrally to eliminate efferent vagal nerve activity.

The sinoaortic baroreceptors were denervated by bilateral cutting of the aortic depressor nerve, the superior laryngeal nerve, the pharyngeal nerve, the superior cervical ganglion, and the carotid sinus nerves. The effectiveness of sinoaortic denervation was confirmed by abolishment of the bradycardia and sympathoinhibitory response to phenylephrine (0.25 μg i.v.), whereas the response to 2-methyl-5-HT (50 μg/kg i.v.) was maintained.

The response to 2-methyl-5-HT was examined in groups of animals with either bilateral vagotomy or with intact vagal nerves. Effective bilateral vagotomy was confirmed by an abolished sympathoinhibitory response to 2-methyl-5-HT compared with the response before vagotomy. At the end of the experiment, the animal was killed by an overdose of pentobarbital (25 mg i.v.), and AVNA and ERSNA were continuously recorded for a further 30 minutes as a measure of background signal.

Drugs

Phenylephrine HCl, 1% (Elkins-Sinn, Inc., Cherry Hill, N.J.) was diluted in isotonic saline (2.5 g/L, pH 6.8) and stored at 5°C. 2-Methyl-5-HT maleate (Research Biochemicals Inc., Natick, Mass.) was dissolved in isotonic saline (50 mg/L, pH 6.3) and stored at −20°C. Injection volume was 1 mL/kg at all doses of 2-methyl-5-HT.
**TABLE 1. Baseline Hemodynamic Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low salt diet</th>
<th>High salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Vagotomy</td>
</tr>
<tr>
<td></td>
<td>(n=11)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>117±6</td>
<td>125±6</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>446±12</td>
<td>453±14</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>1.2±0.6</td>
<td>1.5±0.6</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; LVEDP, left ventricular end-diastolic pressure.

*p<0.05 vs. corresponding low salt group.

**Experimental Protocol**

The experimental protocol was started 30 to 60 minutes after the surgical preparation was completed. In all four groups (LNa/intact, n=11; LNa/vagotomy, n=10; HNa/intact, n=13; HNa/vagotomy, n=10), the dose-response relation of 2-methyl-5-HT on AVNA, ERSNA, MAP, and HR was evaluated by administering 6.25, 12.5, 25, and 50 μg/kg i.v. Doses were administered cumulatively, allowing 10 minutes of recovery between each dose. With this protocol, responses (ΔMAP, ΔHR, and ΔERSNA) to 50 μg/kg 2-methyl-5-HT were unaltered when repeated four times with 10-minute intervals (n=5), suggesting that tachyphylaxis did not develop after repeated administration of 2-methyl-5-HT.

**Data Analysis**

Integrated vagal and renal nerve activity was analyzed as mean integrated voltage per second. The postmortem background signal was subtracted from all measurements. Data for AVNA and ERSNA are expressed as percent change from control values. Overall statistical analysis was performed with one-way analysis of variance (when one-way classification) or repeated-measures analysis of variance (when two-way classification) followed by Student's t test with Bonferroni correction for multiple comparisons. Differences were considered significant at a value of p<0.05. All presented values are mean±SEM.

**Results**

Baseline values for hemodynamic parameters are presented in Table 1. There were no significant differences among groups in MAP or HR. Left ventricular end-diastolic pressure was significantly higher in animals on HNa diet than in animals on LNa diet, whereas vagotomy had no effect on left ventricular end-diastolic pressure.

2-Methyl-5-HT produced similar increases in AVNA at all doses in all four groups, and there were no signs of maximal response within the applied dose range (Figure 1). In animals with intact vagi, 2-methyl-5-HT produced dose-dependent reductions in MAP, HR, and ERSNA.

**FIGURE 1.** Line plots show dose-dependent effects of 2-methylserotonin (2-methyl-5-HT) on afferent vagal nerve activity (AVNA), mean arterial pressure (MAP), heart rate (HR), and efferent renal sympathetic nerve activity (ERSNA) in rats on low salt (LNa) and high salt (HNa) diets. Continuous lines indicate groups with intact vagi; broken lines indicate groups with bilateral vagotomy. *p<0.05 vs. LNa/vagotomy; tp<0.05 vs. HNa/vagotomy. bpm, Beats per minute.
that were abolished in rats with bilateral vagotomy (Figure 1). In intact rats, MAP decreased similarly in LNa and HNa rats, whereas it was not reduced in vagotomized rats. The reduction of HR in rats with intact vagi was significantly different from the response in vagotomized rats only after administration of 50 μg/kg 2-methyl-5-HT in animals on LNa diet. However, in both LNa and HNa groups, there was a significant interaction between dose and group (intact versus vagotomy) on HR values, without a significant effect of dose in the vagotomized groups. In intact rats, ERSNA decreased similarly in LNa and HNa rats, whereas it was not reduced in vagotomized rats.

When responses (ΔMAP, ΔHR, and ΔERSNA) were plotted as a function of the physiological stimulus (ΔAVNA), there were no differences between responses for LNa and HNa groups, whether intact or vagotomized (Figure 2).

**Discussion**

The selective 5-HT₃ receptor agonist 2-methyl-5-HT produced a dose-dependent increase in AVNA associated with a sympathoinhibitory response of decreased MAP, HR, and ERSNA in normal sinoaortic-denervated Sprague-Dawley rats. The sympathoinhibitory response was abolished after bilateral vagotomy, suggesting that 2-methyl-5-HT exerts its action through stimulation of vagal afferents and that spinal afferents do not contribute to the sympathoinhibitory response to 2-methyl-5-HT. Cardiovascular responses from 2-methyl-5-HT stimulation of aortic and carotid chemoreceptors were eliminated by sinoaortic denervation. These findings are in agreement with the observations that intravenous injection of 5-HT elicits hypotension and bradycardia, which is blocked by pretreatment with MDL 72222 or ICS 205-930, and that the sympathoinhibitory response of phenyl biguanide can be blocked by either selective 5-HT₃ antagonists or vagotomy.²–¹⁰

When stimulus–response curves were drawn by plotting the efferent responses (ΔMAP, ΔHR, ΔERSNA) as a function of the afferent stimulus (ΔAVNA), no differences were observed between LNa and HNa groups (Figure 2). This suggests that the gain of the central and the efferent limb of the 2-methyl-5-HT-sensitive chemoreflex was unaffected by changes in dietary sodium intake.

It is well known that the plasma concentrations of a variety of hormones (e.g., angiotensin II, aldosterone, atrial natriuretic peptide, epinephrine) and neurotransmitters (e.g., norepinephrine) change during alterations in dietary sodium intake. Because most of these substances can influence ERSNA by affecting either baroreceptor sensitivity or central sympathetic outflow,¹⁵–¹⁹ the sympathoinhibitory response mediated by the 2-methyl-5-HT-sensitive chemoreflex could be anticipated to be different in animals on LNa and HNa diets. However, 2-methyl-5-HT produced a dose-dependent increase in AVNA and a sympathoinhibitory response that was similar in LNa and HNa groups.

Because the significantly higher left ventricular end-diastolic pressure in rats on HNa diet presumably reflects higher intravascular volume in rats on HNa diet than in rats on LNa diet, the data suggest that the vagal response to 2-methyl-5-HT is unchanged within the range of intravascular volume obtained during alterations in dietary sodium intake. The unchanged vagal response to 2-methyl-5-HT during changes in dietary sodium intake is in contrast to the reduced vagal response to mechanostimulation produced by acute intravenous volume expansion in rats on HNa diet.¹³ The relative difference in vagal responsiveness to mechanostimulation and 2-methyl-5-HT stimulation during HNa intake is compatible with the differential control of

![FIGURE 2. Line plots show changes in mean arterial pressure (ΔMAP), heart rate (ΔHR), and efferent renal sympathetic nerve activity (ΔERSNA) as a function of 2-methylserotonin-induced increases in afferent vagal nerve activity (ΔAVNA). Continuous lines indicate groups with intact vagi; broken lines indicate groups with bilateral vagotomy. HNa, high salt diet; LNa, low salt diet; bpm, beats per minute.]()
ERSNA by mechanosensitive and 2-methyl-5-HT-sensitive vagal afferents.

In further support for differential pathways of mechanosensitive and 5-HT receptor-sensitive vagal afferents, intrapericardial administration of phenyl biguanide increases whereas acute intravascular volume expansion decreases adrenal nerve activity.²⁰

The effect of dietary sodium intake on the gain of the central and the efferent limb of the cardiopulmonary baroreceptor reflex has previously been examined by measuring MAP, HR, and ERSNA during electrostimulation of the central portion of the peripherally cut right cervical vagus nerve in rats on normal and high dietary sodium intake.²¹ Central vagal electrostimulation produced a frequency-dependent sympathoinhibitory response of decreased MAP, HR, and ERSNA that was identical in the two groups. In agreement with findings during electrostimulation-induced increases in AVNA, the sympathoinhibitory responses to 2-methyl-5-HT were similar at each level of AVNA, suggesting that reflex gain of the central and efferent limb of the 2-methyl-5-HT-sensitive chemoreflex was unaltered by changes in dietary sodium intake.

In conclusion, in rats with sinoaortic denervation, the selective 5-HT receptor agonist 2-methyl-5-HT elicits a sympathoinhibitory response of decreased MAP, HR, and ERSNA, which is abolished by vagotomy, suggesting that 2-methyl-5-HT stimulates vagal afferents with an inhibitory action on peripheral sympathetic nerve activity. In contrast to the reduced vagal responses to mechanostimulation in rats on HNa diet, the vagal responses to 2-methyl-5-HT are unchanged during alterations in dietary sodium intake.

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Sympathoinhibitory responses to 2-methylserotonin during changes in sodium intake.
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Hypertension. 1993;21:1000-1004
doi: 10.1161/01.HYP.21.6.1000

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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