Activation of 5-Hydroxytryptamine 1A Receptors Suppresses the Cardiovascular Response Evoked From the Dorsomedial Hypothalamic Nucleus

Jouji Horiuchi, Sonoe Wakabayashi, Roger A.L. Dampney

Abstract—The dorsomedial hypothalamic nucleus is a key component of the central pathways subserving the cardiovascular response to psychological stress, which is believed to be an important risk factor for hypertension. Previous studies indicate that 5-hydroxytryptamine 1A receptors can modulate the cardiovascular responses associated with stress. In this study, we determined in anesthetized rats the effects of systemic or intracisternal administration of 8-hydroxy-2-(di-n-propylamino)tetralin, a selective agonist of 5-hydroxytryptamine 1A receptors, and then subsequent administration of the selective antagonist WAY-100635 on the cardiovascular response evoked by activation of the dorsomedial hypothalamic nucleus (by microinjection of bicuculline). The increase in mean arterial pressure, heart rate, and renal sympathetic nerve activity (RSNA) evoked by bicuculline injection into the dorsomedial hypothalamic nucleus was greatly reduced (by 80% to 90%) by administration of 8-hydroxy-2-(di-n-propylamino)tetralin and then completely restored by subsequent administration of WAY-100635, whether administered systemically or intracisternally. In contrast, systemic administration of 8-hydroxy-2-(di-n-propylamino)tetralin had no significant effect on the baseline level or reflex changes in RSNA evoked by chemoreceptor or baroreceptor stimulation and resulted in only a modest reduction (12 mm Hg) in baseline mean arterial pressure. The results indicate that activation of 5-hydroxytryptamine 1A receptors in the brain stem causes a potent and selective suppression of the hypertensive and sympathoexcitatory response evoked by stimulation of the dorsomedial hypothalamic nucleus but has little effect on the tonic level or baroreceptor or chemoreceptor reflex control of RSNA. (Hypertension. 2005;46:173-179.)

Key Words: ■ hypothalamus ■ stress ■ baroreflex ■ chemoreceptors

Psychological stress is believed to be an important risk factor for hypertension and other cardiovascular disorders.1 Studies in conscious and anesthetized rats have demonstrated that the dorsomedial hypothalamic nucleus (DMH) plays a critical role in mediating the autonomic, neuroendocrine, and behavioral response to psychological stress, such as air jet stress.2 Activation of neurons in the DMH evokes anxiety-like behavior associated with a wide range of autonomic and hormonal responses, including increases in heart rate (HR; mediated via an increase in cardiac sympathetic nerve activity3,4), renal sympathetic nerve activity (RSNA), gastrointestinal motility, and in the secretion of adrenomedullary hormones and adrenocorticotropic hormone (corticotropin [ACTH]).2–8 Conversely, bilateral microinjections into the DMH of muscimol (a γ-aminobutyric acid type A [GABA_A] receptor agonist) or of kynurenate (an antagonist of ionotropic glutamate receptors) greatly reduces the increases in arterial pressure, HR, ACTH secretion, and anxiety-like behavior normally evoked by air jet stress.8–10

It has been shown recently that the DMH also plays an important role in generating thermoregulatory responses. Activation of neurons in the DMH evokes an increase in the activity of sympathetic nerves innervating brown adipose tissue (BAT), accompanied by increases in BAT temperature and end-tidal CO₂ as well as cutaneous vasoconstriction.11 These thermoregulatory effects may also be part of a stress response because an increase in body temperature can be induced by stress (so-called “stress hyperthermia”).12 In addition, bilateral injections of muscimol or kynurenate into the DMH greatly reduces the thermogenic and cardiovascular components of the febrile response generated by injection of prostaglandin E₂ (PGE₂) into the preoptic area of the hypothalamus.13,14 Thus, the DMH appears to be a component of the central pathways mediating the sympathetic changes accompanying fever, as well as those evoked by psychological stress.

The descending pathways that mediate the different components of the sympathetic response evoked by activation of the DMH have been shown to include synapses in the rostral ventrolateral medulla (RVLM) and in the region of the raphe pallidus (RPa) in the rostral midline medulla. In particular, the increase in RSNA evoked from the DMH is largely
dependent on a synapse in the RVLM, whereas the increase in HR, BAT sympathetic activity, and cutaneous vasoconstrictor activity is largely dependent on a synapse in the RPa.3,4,7,11,15,16 Consistent with the hypothesis that these descending pathways mediate the sympathetic changes accompanying psychological stress or fever, inhibition of neurons in RPa suppresses the tachycardia normally evoked by air jet stress in conscious rats17 as well as the tachycardia and thermogenic response evoked by injection of PGE2 into the preoptic area of anesthetized rats.15,18

Several lines of evidence suggest the possibility that 5-hydroxytryptamine 1A (5-HT1A) receptors in the brain may play a role in modulating autonomic responses evoked from the DMH. First, the RPa and RVLM contain 5-HT1A receptors,19 and these are also present in the DMH itself.20 Second, systemic administration of the selective 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetrinal (8-OH-DPAT) into conscious rabbits abolishes the cutaneous vasoconstriction and greatly reduces the thermogenic response associated with fever,21 which, as mentioned above, depends on activation of neurons in the DMH. These effects of 8-OH-DPAT are likely attributable to a central action of the drug because it is known that systemically administered 8-OH-DPAT affects sympathetic activity by an action on the central nervous system.22 Third, Morrison23 reported recently that microinjection of 8-OH-DPAT into the RPa reduced the BAT thermogenic response and tachycardia evoked by systemic injection of leptin, which acts, at least in part, on neurons in the DMH.24,25

In light of the above recent findings, the aim of this study was to test the hypothesis that central 5-HT1A receptors modulate the cardiovascular response evoked by activation of DMH neurons. For this purpose, we first determined the effect of activation of 5-HT1A receptors (by systemic or intracisternal administration of 8-OH-DPAT) on the changes in arterial pressure, HR, and RSNA evoked by disinhibition of the DMH. We then tested the effect of subsequent administration of WAY-100635, a highly potent and selective 5-HT1A receptor antagonist WAY-100635 (Sigma) was then administered (100 μg/kg IV), after which there was an additional waiting time of 5 to 10 minutes, followed by a third and final microinjection of bicuculline into the same site in the DMH. It has been demonstrated previously that microinjection of 10 pmol bicuculline into the DMH evokes a significant but submaximal increase in MAP, HR, and RSNA.27

To test the effects of intravenous 8-OH-DPAT and WAY-100635 administration on the chemoreceptor reflex, in 4 of the above experiments a bolus injection of sodium cyanide (NaCN) solution (Sigma; 100 μg/kg in 400 μL IV) was injected 10 minutes before and then 5 minutes after administration of 8-OH-DPAT (100 μg/kg IV) and then again 5 minutes after administration of WAY-100635 (100 μg/kg IV). All compounds injected intravenously were dissolved in physiological saline solution.

In 5 other experiments, the procedures were the same as the experiments in which 8-OH-DPAT and WAY-100635 were injected intravenously, except that in this case, the drugs were injected intracisternally in a dose that was 10X smaller (10 μg/kg in a volume of 3 to 5 μL). In these experiments, the drugs were applied bilaterally (5 μg/kg each side) to the exposed dorsolateral surface of the medulla, inside the dura mater. In 2 experiments, a similar volume of Evans’ Blue dye was injected in the same fashion. Subsequent examination of the brain stem demonstrated that the dye diffused to cover the dorsal and ventral medullary surface and the ventral surface of the caudal pons but did not spread to the midbrain or higher levels.

At the end of each experiment, the rat was euthanized with an overdose of pentobarbital sodium, the brain was removed, and after fixation in paraformaldehyde solution, coronal sections (50 μm) were cut on a freezing microtome and mounted onto glass slides. Injection sites were determined using a fluorescence microscope and mapped onto standard sections of the atlas by Paxinos and Watson.27

To determine the effects of administration of 8-OH-DPAT and WAY-100635 on the cardiac-related component of RSNA, the pulse-triggered average of the full-wave–rectified RSNA signal was determined as described previously.28 It is known that the cardiac-related component of RSNA arises from entrainment of the medullary, sympathetic burst-generating circuits by inhibitory inputs from baroreceptors.29 The magnitude of the cardiac-related component of RSNA after administration of 8-OH-DPAT and subsequent administration of WAY-100635 was measured with respect to the baseline RSNA just before administration of each of these drugs. Comparisons of the magnitudes of the changes in MAP (in mm Hg), HR (in bpm), and RSNA (measured as the percentage of the prestimulus baseline) evoked by microinjections of bicuculline into the DMH, or by chemoreceptor stimulation, were made using 1-way ANOVA. Pair-wise comparisons between the control responses and after administration of 8-OH-DPAT and after subsequent administration of WAY-100635 were made using the paired t test, with application...
TABLE 1. Baseline Levels of MAP, HR, and RSNA Before and After Intravenous Injections of 8-OH-DPAT or WAY-100635 Solution

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before 8-OH-DPAT</th>
<th>After 8-OH-DPAT</th>
<th>Before WAY-100635</th>
<th>After WAY-100635</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>102±2</td>
<td>90±2*</td>
<td>87±3</td>
<td>108±2*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>347±9</td>
<td>315±11*</td>
<td>324±9</td>
<td>346±8*</td>
</tr>
<tr>
<td>RSNA (% control)</td>
<td>100</td>
<td>100±3</td>
<td>100</td>
<td>115±4*</td>
</tr>
</tbody>
</table>

Values are means±SE. *P<0.05 vs before 8-OH-DPAT or WAY-100635.

of the Bonferroni procedure for multiple comparisons. A similar procedure was also used to compare the magnitudes of the cardiac-related component of the RSNA before and after administration of WAY-100635. A P value <0.05 was regarded as statistically significant. All values are presented as means±SEM.

Results
Effects of Systemic Administration of 8-OH-DPAT and WAY-100635
Baseline Variables
After intravenous administration of the 5-HT_{1A} selective agonist 8-OH-DPAT (100 µg/kg), the MAP and HR decreased by 12±1 mm Hg and 32±7 bpm, respectively (Table 1), reaching new stable levels by 5 to 10 minutes after the injection. In contrast, administration of 8-OH-DPAT had no significant effect on RSNA at this time (Table 1). After administration of WAY-100635 (100 µg/kg), the MAP, HR, and RSNA increased by 21±3 mm Hg, 22±5 bpm, and 15±4%, respectively, compared with the levels just before WAY-100635 administration (Table 1).

Responses Evoked From DMH
In 7 experiments, bicuculline (10 pmol in 20 nL) was injected into the DMH before and after intravenous administration of 8-OH-DPAT. The centers of the injection sites were confirmed to be located within the DMH (Figure 1). As described previously, 4,7 the MAP, HR, and RSNA began to increase within 10 to 20 seconds after injection of bicuculline in the DMH, reached a peak after 5 to 10 minutes, and then declined gradually, returning to baseline levels after ~30 minutes (Figure 2). After 8-OH-DPAT administration, the increases in MAP, HR, and RSNA evoked by microinjection of the same dose (10 pmol) of bicuculline into the same site in the DMH were greatly reduced (by 80% to 90%; Figure 2). After subsequent administration of WAY-100635, the cardiovascular response evoked by an additional microinjection of bicuculline (10 pmol) into the same site in the DMH was completely restored (ie, the evoked increases in MAP, HR, and RSNA were not significantly different [P>0.5 in all cases] from those evoked by bicuculline microinjection into the DMH before administration of 8-OH-DPAT [Figure 2]).

Chemoreceptor and Baroreceptor Reflex Changes in RSNA
In 4 of these 7 experiments, the chemoreceptor-sympathetic reflex was also tested by measuring the increase in RSNA evoked by a bolus injection of NaCN (100 µg/kg IV). In all experiments, a bolus injection of NaCN evoked short-lasting increases in MAP and RSNA, accompanied by a brief bradycardia (Figure 3). No such effects were observed when the same volume of the vehicle saline solution was injected intravenously. The magnitudes of the chemoreceptor reflex increase in RSNA after administration of 8-OH-DPAT or after subsequent administration of WAY-100635 were not significantly different from the control response (Figure 3; P>0.5 in both cases). Similarly, in 3 experiments (Figure 4), the amplitude of the cardiac-related component of RSNA after administration of 8-OH-DPAT was virtually unchanged (104±4% of the control amplitude before 8-OH-DPAT administration). However, after subsequent administration of WAY-100635 in these experiments, the cardiac-related component was significantly increased (P<0.05) to 130±5% of the control amplitude.

Effects of Intracisternal 8-OH-DPAT and WAY-100635
Intracisternal administration of 8-OH-DPAT (10 µg/kg) and WAY-100635 (10 µg/kg) had very similar effects on baseline levels of MAP, HR, and RSNA (Table 2) to those observed after intravenous administration of these drugs (Table 1). Similarly, the increases in MAP, HR, and RSNA evoked by bicuculline injections into the DMH in these experiments were greatly reduced by intracisternal 8-OH-DPAT and then subsequently restored by intracisternal WAY-100635 (Figure 5). Histological examination confirmed that the injection sites for these experiments were within the DMH.

Discussion
The results of the present study demonstrate that systemic injection of the selective 5-HT_{1A} receptor agonist 8-OH-
DPAT causes a profound suppression of the pressor, renal sympathoexcitatory and tachycardia response to disinhibition of the DMH, but had no effect on either the baseline tonic level of RSNA or on the phasic changes in RSNA reflexly evoked by chemoreceptor or baroreceptor stimulation. Furthermore, the inhibitory effect of 8-OH-DPAT on the DMH-evoked response was completely reversed by subsequent administration of the highly selective 5-HT\textsubscript{1A} receptor antagonist WAY-100635,\textsuperscript{26} which further indicates that the observed effects were mediated specifically by 5-HT\textsubscript{1A} receptors. Furthermore, intracisternal injections of 10-fold lower doses of 8-OH-DPAT and then WAY-100635 also caused almost complete suppression and then complete reversal of the DMH-evoked responses, indicating that activation of 5-HT\textsubscript{1A} receptors in the lower brain stem alone is sufficient to produce this suppression.

The dose of 8-OH-DPAT injected intravenously (100 \(\mu\)g/kg) was within the range (5 to 150 \(\mu\)g/kg) that has been shown previously to produce dose-dependent effects on arterial pressure and HR in rats,\textsuperscript{30} and was the same as the dose that, in conscious rabbits, causes inhibition of the cardiovascular and thermogenic responses associated with fever or cold stress.\textsuperscript{21,31} In agreement with previous studies,\textsuperscript{30,32} 8-OH-DPAT administration resulted in modest but significant falls in MAP and HR (12 mm Hg and 32 bpm, respectively). Although we found that the baseline level of RSNA was not altered by systemic administration of 8-OH-DPAT, the fact that the MAP and HR decreased suggests that there was inhibition of sympathetic nerves innervating other vascular beds and the heart. In fact, Nosjean and Guyenet\textsuperscript{32} found that in anesthetized rats, 8-OH-DPAT administration resulted in a decrease in lumbar sympathetic nerve activity of...
18%. Consistent with this, Lovick et al. found that microinjection of 8-OH-DPAT into the RVLM resulted in a small decrease in MAP and HR, accompanied by an increase in hindlimb vascular conductance and no change in renal vascular conductance. Thus, 8-OH-DPAT may have nonuniform effects on the sympathetic outflow, with greater effects on lumbar and cardiac sympathetic activity than on RSNA. However, the fact that the RSNA remained constant despite the decrease in MAP suggests that there was some inhibitory effect on the renal sympathetic outflow because otherwise, the RSNA would have increased reflexly as a consequence of baroreceptor unloading.

As mentioned above, systemic injection of 8-OH-DPAT abolishes or greatly reduces the cutaneous vasoconstrictor baroreceptor unloading. This is induced by cold, as well as the sympathetically mediated cutaneous vasoconstriction induced by cold, the former being dependent on lumbar and cardiac sympathetic activity than on RSNA. However, the fact that the RSNA remained constant despite the decrease in MAP suggests that there was some inhibitory effect on the renal sympathetic outflow because otherwise, the RSNA would have increased reflexly as a consequence of baroreceptor unloading.

Intracisternal administration of 8-OH-DPAT was sufficient to suppress the DMH-evoked response. In 2 experiments, intracisternal administration of a similar volume of dye indicated that the injectate did not spread more rostrally than the pons. Thus, although diffusion of the drug to higher brain regions cannot be ruled out, this suggests that the intracisternally administered 8-OH-DPAT acts mainly on neurons within the lower brain stem. Consistent with this, intracisternal administration of angiotensin II, in contrast to intracerebroventricular administration, acts mainly on neurons in the brain stem.

In the medulla, neurons in the RPa and RVLM are likely sites of action of 8-OH-DPAT. RPa neurons mediate the tachycardia, increased BAT sympathetic activity, and increased cutaneous sympathetic activity that can be evoked by activation of the DMH, which also contains 5-HT1A receptors. The RPa also contains a high density of 5-HT1A receptors, and microinjection of 8-OH-DPAT into the RPa has been shown to greatly inhibit the increased BAT sympathetic activity and tachycardia evoked by systemic injection of leptin, which acts, at least in part, on neurons in the DMH. Similarly, the increase in RSNA evoked by DMH activation is mediated primarily by neurons in the RVLM, which also contains 5-HT1A receptors. Furthermore, microinjection of 8-OH-DPAT into the RVLM suppresses the reflex increase in splanchnic sympathetic activity evoked by stimulation of somatic nociceptive afferent nerves. Thus, the DMH-evoked increase in RSNA (or in the activity of other sympathetic pathways mediated by the RVLM) could also be suppressed by activation of 5-HT1A receptors within the RVLM itself. It is very interesting to note that Miyawaki et al. also found, consistent with the findings in the present study, that 8-OH-DPAT microinjection into the RVLM did not inhibit the baroreceptor- and chemoreceptor-sympathetic reflexes.

Thus, it is possible that the suppression of the DMH-evoked increase in MAP, HR, and RSNA is attributable entirely to an action on 5-HT1A receptors within the key medullary nuclei (RPa and RVLM) that mediate the sympathetic components of the response. However, even if this is the case, 5-HT1A receptors at higher levels of the brain may also be capable of inhibiting all or part of the response. For example, the lateral periaqueductal gray in the midbrain mediates at least part of the response evoked from the DMH, and this region also contains 5-HT1A receptors, which, when activated, cause inhibition of periaqueductal gray neurons. Furthermore, there are 5-HT1A receptors within the DMH itself. Because the DMH response was evoked by blockade of GABAergic inhibitory receptors, it follows that the DMH sympatoexcitatory neurons that generate this response must receive tonic excitatory as well as tonic inhibitory synaptic inputs. Therefore, it is conceivable that the tonic excitatory inputs could be suppressed by activation of 5-HT1A receptors within the DMH, as has been
shown to occur in the locus coeruleus.\textsuperscript{39} Further studies are needed to determine the role of 5-HT\textsubscript{1A} receptors in supramedullary regions on the DMH-evoked response.

In general, activation of 5-HT\textsubscript{1A} receptors causes hyperpolarization and inhibition of neurons.\textsuperscript{26} Such receptors may be autoreceptors on serotonergic neurons, or else postsynaptic receptors on nonserotonergic neurons.\textsuperscript{26} It could be argued that 8-OH-DPAT suppresses the DMH-evoked increase in RSNA via direct inhibition of RVLM neurons, but if so, such neurons would have to be a separate population from RVLM neurons that mediate baroreceptor and chemoreceptor reflex responses. However, this seems unlikely because we found previously that 5 out of a sample of 6 identified barosensitive spinally projecting RVLM neurons were powerfully excited by DMH activation.\textsuperscript{7} Thus, there appears to be a powerful convergence of inputs from the DMH and baroreceptors onto RVLM sympathetic premotor neurons. A more likely possibility, then, is that 8-OH-DPAT acts presynaptically to selectively suppress DMH-evoked excitation of RVLM sympathetic premotor neurons, while leaving intact excitatory and inhibitory inputs from chemoreceptors and baroreceptors, respectively. As mentioned above, a similar selective action of 5-HT\textsubscript{1A} receptors in the RVLM, in inhibiting the somato-sympathetic reflex but not baroreceptor- or chemoreceptor-sympathetic reflexes, has been demonstrated previously by Miyawaki et al.\textsuperscript{35} These authors also proposed that this is likely to be a presynaptic effect, but further studies are needed to determine the precise cellular mechanism that underlies this selectivity.

**Perspectives**

There is much evidence, from studies in human and animal models, that 5-HT\textsubscript{1A} receptors have a major role in modulating behavioral and physiological changes associated with stress and anxiety,\textsuperscript{26} which is believed to be a significant risk factor for hypertension.\textsuperscript{1} 5-HT\textsubscript{1A} receptor agonists have an anxiolytic effect, as demonstrated in human and animal studies.\textsuperscript{40} The present study focused on the sympathoexcitatory response evoked by activation of DMH neurons, but our results also raise the important question as to whether other stress-induced responses that are mediated by the DMH, including respiratory effects and hormonal responses such as the release of ACTH,\textsuperscript{2} may also be suppressed by administration of 5-HT\textsubscript{1A} receptor agonists. In addition, a further question is whether the suppression of the DMH-evoked sympathoexcitatory response is specific to that particular nucleus or alternatively is a general effect on sympathoexcitatory responses evoked by activation of other hypothalamic nuclei, such as the paraventricular nucleus, which also play a role in mediating stress-induced responses.\textsuperscript{41} Future studies will also be needed to determine the precise sites and cellular mechanisms of action of 5-HT\textsubscript{1A} receptor agonists in modulating stress-induced physiological responses.

**Acknowledgments**

This study was supported by the National Health and Medical Research Council of Australia. We thank Suzanne Killinger for excellent technical assistance.

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Hypertension. 2005;46:173-179; originally published online June 6, 2005;
doi: 10.1161/01.HYP.0000169970.68151.17
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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