Activation of 5-hydroxytryptamine-1A receptors suppresses cardiovascular responses evoked from the paraventricular nucleus

Jouji Horiuchi, Alp Atik, Kamon Iigaya, Lachlan M. McDowall, Suzanne Killinger, and Roger A. L. Dampney

School of Medical Sciences (Physiology) and Bosch Institute, University of Sydney, New South Wales, Australia

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Horiuchi J, Atik A, Iigaya K, McDowall LM, Killinger S, Dampney RAL. Activation of 5-hydroxytryptamine-1A receptors suppresses cardiovascular responses evoked from the paraventricular nucleus. Am J Physiol Regul Integr Comp Physiol 301: R1088–R1097, 2011. First published July 13, 2011; doi:10.1152/ajpregu.00144.2011.—Activation of central 5-hydroxytryptamine-1A (5-HT1A) receptors powerfully inhibits stress-evoked cardiovascular responses mediated by the dorsomedial hypothalamus (DMH), as well as responses evoked by direct activation of neurons within the DMH. The hypothalamic paraventricular nucleus (PVN) also has a crucial role in cardiovascular regulation and is believed to regulate heart rate and renal sympathetic activity via pathways that are independent of the DMH. In this study, we determined whether cardiovascular responses evoked from the PVN are also modulated by activation of central 5-HT1A receptors. In anesthetized rats, the increases in heart rate and renal sympathetic nerve activity evoked by bicuculline injection into the PVN were greatly reduced (by 54% and 61%, respectively) by intravenous administration of (±)-8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT), an agonist of 5-HT1A receptors, but were then completely restored by subsequent administration of WAY-100635, a selective antagonist of 5-HT1A receptors. Microinjection of 8-OH-DPAT directly into the PVN did not significantly affect the responses to bicuculline injection into the PVN, nor did systemic administration of WAY-100635 alone. In control experiments, a large renal sympathoexcitatory response was evoked from both the PVN and DMH but not from the intermediate region in between; thus the evoked responses from the PVN were not due to activation of neurons in the DMH. The results indicate that activation of central 5-HT1A receptors located outside the PVN powerfully inhibits the tachycardia and renal sympathoexcitation evoked by stimulation of neurons in the PVN.

The PVN has long been known to have a crucial role in cardiovascular regulation, particularly in regard to the maintenance of fluid balance (6, 14). The PVN may also be involved in the long-term regulation of sympathetic activity and blood pressure (8), and there is now considerable evidence that it contributes to the sustained activation of renal and cardiac sympathetic activity that occurs in heart failure and neurogenic hypertension (1, 4, 14, 40). It has been proposed that the PVN is a potential target for treatment of sympathetic dysfunction in these conditions (14), and so it is important to identify the mechanisms that modify the regulation of sympathetic activity by the PVN. The aim of this study was to test whether activation of 5-HT1A receptors can modify the regulation of
arterial pressure, heart rate, and renal sympathetic nerve activity by the PVN.

MATERIALS AND METHODS

General procedures. Experiments were performed using male Sprague-Dawley rats (body wt 370–480 g) supplied by University of Sydney Laboratory Animal Services. All experimental procedures were approved by the Animal Ethics Committee of the University of Sydney and were carried out in accordance with the Guidelines for Animal Experimentation of the National Health and Medical Research Council of Australia. Anesthesia was initially induced by inhalation of isoflurane (2–3% in oxygen-enriched air). A thermo-regulated heating pad was used to maintain body temperature in the range 37–38°C as measured via a rectal probe. Catheters were placed in a femoral artery and a femoral vein for the recording of pulsatile arterial pressure and drug injection, respectively. After the surgery, the isoflurane anesthesia was gradually withdrawn while being replaced by urethane (1.3 g/kg iv with supplementary doses of 0.1 g/kg iv, if required). The adequacy of anesthesia was verified by the absence of the corneal reflex and a withdrawal response to nociceptive stimulation of a hind paw. A tracheotomy was performed to maintain an unobstructed airway, and the animals were allowed to breathe freely.

The rat was then mounted prone in a stereotaxic frame with the incisor bar at 19 mm below the interaural line. The dorsal surface of the cortex was exposed for approaching the PVN and DMH. The renal sympathetic nerve on the left side was isolated from surrounding connective tissue and its activity was recorded as described previously (16). The signal from the electrodes was amplified, passed through a band pass filter (100–1,000 Hz), and then rectified and integrated over successive 10-s intervals. At the end of the experiments, the baseline noise level of renal sympathetic nerve activity (RSNA) was determined by application of 2% lignocaine to the proximal end of the nerve. The mean arterial pressure (MAP) and heart rate (HR) signals were derived from the pulsatile pressure signal via a low-pass filter and rate meter, respectively. All signals were recorded on a computer using a PowerLab system (AD Instruments).

Microinjections were made using a glass micropipette held in a micromanipulator at an angle of 28 degrees (tip caudal). The compound injected was bicuculline methochloride (20 pmol or 10 pmol in 20 nl; Tocris Bioscience, Ellisville, MO). The vehicle solution was artificial cerebrospinal fluid adjusted to pH 7.4, and the drug solution contained 1% fluorescent microspheres to allow later histological determination of the injection sites. The tip of the micropipette was positioned stereotaxically (1.8 mm caudal to the bregma, 0.5–0.6 mm lateral to the midline, and 7.5–7.6 mm from the surface of the cortex).

In some experiments, the tip of the micropipette was positioned in the DMH (3.1 mm caudal to bregma, 0.5–0.6 mm lateral to the midline, and 8.5–8.6 mm ventral to the surface of the cortex) or at a site in between the PVN and DMH (2.5 mm caudal to bregma, 0.5–0.6 mm lateral to the midline, and 7.5–7.6 mm ventral to the surface of the cortex). Microinjections were made by pressure, and the volume injected was measured by the displacement of the meniscus in the pipette with respect to a horizontal grid viewed through an operating microscope.

Experimental procedures. In six rats, an initial microinjection of bicuculline (20 pmol in 20 nl) was made into the PVN. After a waiting period of 30–50 min, to allow for all cardiovascular variables to stabilize again, the selective 5-HT1A receptor agonist 8-OH-DPAT (Tocris Bioscience) was administered (100 µg/kg iv). After a further 5–10 min, a second microinjection of bicuculline was made into the same PVN site, and there was then a further waiting period of 30–50 min. The selective 5-HT1A receptor antagonist WAY-100635 (Sigma) was then administered (100 µg/kg iv) after which there was a further waiting time of 5–10 min followed by a third and final microinjection of bicuculline into the same site in the PVN.

In a second series of experiments in eight rats, the effects of WAY-100635 alone on the responses was also tested. In these experiments, a microinjection of bicuculline [either 20 pmol (n = 4) or 10 pmol (n = 4)] was first made into the PVN. After a waiting time of 30–60 min, WAY-100635 was administered (100 µg/kg iv) after which there was a further waiting time of 5–10 min followed by a second microinjection of bicuculline into the same site in the PVN.

In a third series of experiments in seven rats, the effect of blockade of 5-HT1A receptors within the PVN itself on the responses evoked by microinjection of bicuculline into the PVN was also tested. In these experiments, the procedure was the same as in the first series of experiments, except that instead of a systemic injection of 8-OH-DPAT, a microinjection of 8-OH-DPAT (1 nmol in 100 nl solution) was made at the same coordinates in the PVN as the site at which bicuculline was injected.

In a fourth series of experiments in 10 rats, the responses evoked by microinjections of bicuculline (20 pmol in 20 nl) in the PVN were compared with those evoked by microinjections into the DMH or into an intermediate region in between in the same experiment. In these experiments there was also a waiting period of 30–50 min between microinjections.

At the end of each experiment, the rat was euthanized with an overdose of pentobarbital sodium, the brain was removed, and after fixation in 4% paraformaldehyde solution, coronal sections (50 µm) were cut on a freezing microtome and mounted onto glass slides. The labeled microinjection sites were identified by examining the sections under a fluorescence microscope. In the third series of experiments, in which microinjections of bicuculline and of 8-OH-DPAT were made into the same coordinates in the PVN, each injectate contained microspheres with different colored fluorescent labels, so the injection sites could be distinguished. Injection sites were determined using a fluorescence microscope and mapped onto standard sections of the atlas by Paxinos and Watson (41).

Data analysis. The baseline MAP, HR, and RSNA were measured as the average values of these variables over the 2-min period preceding each microinjection into the PVN, DMH, or intermediate region. The maximum changes in MAP, HR, and integrated RSNA compared with their respective preinjection baseline levels were determined following each microinjection of bicuculline. One-factor ANOVA was used to compare the bicuculline-evoked peak changes in MAP, HR, and RSNA before and after 8-OH-DPAT administration, and after subsequent WAY-100635 administration, followed by paired comparisons using the Student’s t-test with application of the Holm step down procedure for multiple comparisons as appropriate (45). The same procedure was used to compare the increases in MAP, HR, and RSNA evoked by bicuculline microinjections into the PVN, DMH, and intermediate region. The time courses of the changes in MAP, HR, and RSNA following bicuculline microinjections under different conditions were compared using two-factor ANOVA, where the factors were treatment (control, after 8-OH-DPAT administration, or after subsequent WAY-100635 administration) and time (after injection). For the experiments in which the effects of WAY-100635 alone was tested on the peak responses evoked by microinjections of bicuculline (10 or 20 pmol) into the PVN, two-factor ANOVA was also used, where the factors were treatment (control or WAY-100635 administration) and dose of bicuculline. A value of P < 0.05 was taken as statistically significant. All values are presented as means ± SE.

RESULTS

Effects of 8-OH-DPAT and WAY-100635 on baseline variables. As previously reported (20, 24, 36), intravenous injection of the 5-HT1A receptor agonist 8-OH-DPAT (100 µg/kg) resulted in decreases in MAP and HR, reaching new stable levels by 5–10
The RSNA initially increased rapidly after 8-OH-DPAT injection but then declined to reach a new stable level by 5–10 min after the injection (Fig. 1), that was slightly but not significantly increased (by 18/110069%, *P* < 0.1) compared with its initial resting value (Table 1). The initial rapid increase in RSNA after injection of 8-OH-DPAT followed the initial decrease in MAP by 10.220.32.247 on July 6, 2017 http://ajpregu.physiology.org/ Downloaded from

min after the injection (Fig. 1, Table 1). The RSNA initially increased rapidly after 8-OH-DPAT injection but then declined to reach a new stable level by 5–10 min after the injection (Fig. 1), that was slightly but not significantly increased (by 18 ± 9%, *P* > 0.1) compared with its initial resting value (Table 1). The initial rapid increase in RSNA after injection of 8-OH-DPAT followed the initial decrease in MAP by 2–3 s, and thus may have been a baroreflex response to the decrease in MAP. After subsequent intravenous injection of WAY-100635, MAP decreased transiently but then increased to reach a new stable level within 5 to 10 min that was slightly greater than the initial resting level (Fig. 1, Table 1). The HR also increased after injection of WAY-100635 to reach a new stable level that was similar to the initial resting level (Fig. 1, Table 1). In contrast, the RSNA initially increased transiently, then decreased, and then increased again more gradually to reach a level that was not significantly different from the initial resting level (Fig. 1, Table 1). Again, the initial transient changes in RSNA after injection of WAY-100635 followed the changes in MAP, but in the opposite direction, and so may have been a baroreflex response to the changes in MAP.

In the experiments in which WAY-100635 was injected intravenously, without prior injection of 8-OH-DPAT, WAY-100635 injection had no significant effect on the resting levels of MAP or RSNA, but did result in a slight but significant increase in HR (Table 1).

**Table 1. Resting values of cardiovascular variables**

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RSNA, % baseline</th>
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<tr>
<td><strong>Effects of 8-OH-DPAT and subsequently WAY-100635</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>93 ± 5</td>
<td>333 ± 7</td>
<td>100</td>
</tr>
<tr>
<td>After 8-OH-DPAT</td>
<td>6</td>
<td>84 ± 4*</td>
<td>287 ± 8**</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>After WAY-100635</td>
<td>6</td>
<td>104 ± 3*</td>
<td>345 ± 7*</td>
<td>110 ± 10</td>
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<tr>
<td><strong>Effects of WAY-100635 alone</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>8</td>
<td>103 ± 4</td>
<td>356 ± 11</td>
<td>100</td>
</tr>
<tr>
<td>After WAY-100635</td>
<td>8</td>
<td>104 ± 4</td>
<td>373 ± 12*</td>
<td>106 ± 6</td>
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</table>

Values are means ± SE. Resting values were measured just before microinjection of bicuculline into the paraventricular nucleus (PVN), before (control) and after intravenous injection of (±)-8-hydroxy-2-(di-n-propylamino)tetrailin (8-OH-DPAT), and then after subsequent intravenous injection of WAY-100635, or before (control) and after intravenous injection of WAY-100635 alone. HR, heart rate; MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity. *P < 0.05, **P < 0.01 (compared with control).
Effects of 8-OH-DPAT and WAY-100635 on bicuculline-evoked responses from the PVN. In this series of experiments (n = 6), microinjections of bicuculline into the PVN evoked a large increase in HR and integrated RSNA and a modest increase in MAP (Fig. 1). The pattern of RSNA also changed, such that the amplitude of bursts increased, with periods of low activity between bursts (e.g., Fig. 1B). This was particularly exaggerated in one experiment, in which the periods of inactivity between intense bursts of RSNA were up to 1 s in duration (Fig. 2). This pattern of evoked RSNA in response to bicuculline microinjection into the PVN has been previously reported (26). The extreme bursting pattern of RSNA in this experiment resulted in a time course of integrated RSNA that was highly irregular (Fig. 2A) and quite different from that observed in all other experiments (e.g., Fig. 1A). For this reason, the RSNA data from this experiment were not included in the determination of the averaged time course of the responses as shown in Fig. 3.

After intravenous injection of 8-OH-DPAT, the maximum evoked increases in HR and RSNA were greatly reduced (Fig. 1) by 54 ± 6% (P = 0.0005) and 61 ± 7% (P = 0.0008), respectively. The evoked maximum increase in MAP was also decreased (by 55 ± 18%) but this did not reach statistical significance (P = 0.056). After subsequent intravenous injection of WAY-100635, the evoked maximum increases in MAP, HR, and RSNA recovered back to levels that were not significantly different from their respective control responses (P = 0.005 to 0.009).

Figure 3 shows the averaged time course of bicuculline-evoked responses before and after 8-OH-DPAT administration and after subsequent WAY-100635 administration. After 8-OH-DPAT administration, the bicuculline-evoked increases in MAP, HR, and RSNA measured at 1-min intervals up to 16 min after the injection were significantly reduced compared with the control responses (ANOVA, P < 0.0001 for all 3 variables) (Fig. 3). After subsequent administration of WAY-100635, however, the time courses of the evoked increases in MAP, HR, and RSNA were not significantly different from
their respective control responses (ANOVA, $P = 0.42, 0.11$ and $0.79$, respectively) (Fig. 3).

Apart from reducing the magnitude of the evoked RSNA response, administration of 8-OH-DPAT also largely restored the normal pattern of RSNA (e.g., Fig. 2B). After subsequent administration of WAY-100635, however, the bicuculline-evoked pattern of RSNA was very similar to that seen in the control response before 8-OH-DPAT administration, i.e., was characterized by high-amplitude irregular bursts (e.g., Fig. 2B).

**Effects of WAY-100635 alone on bicuculline-evoked responses from the PVN.** In this series of eight experiments, we tested the effects of intravenous injection of WAY-100635, without prior injection of 8-OH-DPAT, on the responses evoked by microinjections of either 20 pmol ($n = 4$) or 10 pmol ($n = 4$) of bicuculline into the PVN. Two different doses of bicuculline were used in case the higher dose evoked a maximal response and thus masked any facilitatory effect of WAY-100635 on the response. As shown in Fig. 4, however, although the magnitudes of the bicuculline-evoked increases in MAP, HR, and RSNA were dose dependent, they were not significantly changed after WAY-100635 injection ($P = 0.41–0.98$). As in the first series of experiments, bicuculline microinjection into the PVN evoked a pattern of RSNA characterized by high-amplitude bursts separated by periods of low activity, and this response pattern was also not altered after WAY-100635 administration (Fig. 4B).

**Effects of 8-OH-DPAT in the PVN on bicuculline-evoked responses from the PVN.** In this series of six experiments, we tested the effects of microinjection of 8-OH-DPAT (1 nmol in 100 nl) into the PVN on the response evoked by bicuculline microinjection (20 pmol) into the same site in the PVN (Fig. 5). Although the average bicuculline-evoked increases in MAP, HR, and RSNA after 8-OH-DPAT microinjection tended to be reduced compared with the control responses and the responses evoked after subsequent intravenous injection of WAY-100635 (Fig. 5B), these changes were not statistically significant ($P > 0.2$ in all cases).

**Comparison of responses evoked from PVN and nearby regions.** As mentioned in the introduction, we have previously demonstrated that systemic administration of 8-OH-DPAT also greatly reduced the cardiovascular responses evoked from the DMH (24). This raises the question as to whether, in the present study, the responses evoked by bicuculline microinjection into the PVN are primarily a consequence of disinhibition of neurons in the DMH, rather than the PVN. To test this possibility, in each of 10 experiments, microinjections of the same volume and concentration of bicuculline were made into a site in the PVN, in the DMH, and in a site in between the two (intermediate site). An example of the results from one experiment is shown in Fig. 6A, and the grouped results are shown in Fig. 6B. Although the increases in RSNA evoked from the DMH and PVN were of similar magnitude, the RSNA response...
evoked from the intermediate site was much less than that evoked from either the PVN or DMH (Fig. 6). Similarly, the increase in MAP evoked from the intermediate site was much less than that evoked from either the PVN or DMH, although the evoked HR response was not significantly different from that evoked from either the PVN or DMH (Fig. 6).

Figure 7 shows the locations of the centers of the bicuculline injection sites for all experiments. In the first, second, and third series of experiments (Figs. 7, A and B), the centers of the injection sites were located within the PVN or on its border. In the third series of experiments in which microinjections of bicuculline and 8-OH-DPAT were made at the same coordinates in the PVN, the centers of the 8-OH-DPAT microinjection sites (identified by different colored fluorescent microspheres) were either in the same location as the center of the bicuculline injection site, or within 0.3 mm of the center. In the fourth series of experiments (Fig. 7C), the centers of the injection sites were either in the PVN, the dorsal hypothalamic nucleus (DMN) that lies within the DMH (10), or in the intermediate region in between.

DISCUSSION

The results of this study show that the increases in HR and RSNA evoked by disinhibition of the PVN were greatly reduced by systemic administration of the 5-HT₁A receptor agonist 8-OH-DPAT. These effects were reversed by subsequent systemic administration of the highly selective 5-HT₁A receptor antagonist WAY-100635 (3, 15), which indicates that they are mediated specifically by 5-HT₁A receptors. In control experiments, microinjection of bicuculline into sites between the PVN and DMH evoked increases in MAP and RSNA that were much smaller than those evoked from either the PVN or DMH, thus demonstrating that responses evoked by bicuculline microinjection into the PVN are due primarily to disinhibition of neurons within that nucleus, rather than to disinhibition of neurons in the DMH. At the same time, we cannot rule out the possibility that activation of neurons in the region immediately surrounding the PVN may have contributed to the evoked responses. Systemically administered 8-OH-DPAT affects sympathetic activity by an action on the central nervous system (18, 29), so we therefore conclude that activation of central 5-HT₁A receptors powerfully inhibits cardiac and renal sympathoexcitatory responses evoked from the PVN.

Many previous studies have shown that activation of central 5-HT₁A receptors reduces the increases in HR and MAP evoked by acute psychological stressors (33–35, 48, 49), as well as the cutaneous vasoconstriction evoked by physical stressors such as cold exposure or fever (33, 37, 38). A common feature of all these stressors is that the central pathways that mediate the evoked cardiovascular responses include the DMH as a critical component (9–11, 32). The descending pathways from the PVN, however, projects to the spinal sympathetic outflow either directly or else via sympathetic premotor nuclei in the lower brainstem (19, 46), and so are independent of the DMH. Our findings, therefore, show that the inhibitory effects of activation of 5-HT₁A receptors are not limited to responses mediated via the DMH.
Methodological considerations. The dose of 8-OH-DPAT injected intravenously (100 μg/kg) is within the range (30–250 μg/kg) that has been shown to reduce the tachycardia and pressor response induced by psychological stressors (restraint or novel environment) in conscious rats (35, 49). This dose was also the same as that which in our previous study (24) powerfully suppressed DMH-evoked cardiovascular responses. It is higher, however, than the mean effective dose (ED50) for this compound, which has been estimated as close to 13.1 μg/kg (36). This raises the question as to whether the dose used may have resulted in desensitization of the 5-HT1A receptors. If that were the case, then the restoration of the bicuculline-evoked responses after WAY-100635 administration could be due simply to the absence of activation of 8-OH-DPAT on 5-HT1A receptors rather than a reversal of that activation. This seems unlikely, however, because intravenous injection of WAY-100635 reversed the effects of 8-OH-DPAT administration on MAP and HR, but had no effect on MAP and only a small effect on resting HR when injected without prior administration of 8-OH-DPAT (Table 1).

The compound 8-OH-DPAT has significant affinity for 5-HT7 receptors as well as 5-HT1A receptors (3). On the other hand, WAY-100635 has very low affinity for 5-HT7 receptors (17), and physiological responses that are inhibited by a selective 5-HT7 receptor antagonist are not altered by WAY-100635 (12). The observation that administration of WAY-100635 almost completely reverses the inhibitory effects of 8-OH-DPAT administration on responses evoked from the PVN therefore indicates that the latter effects are due primarily if not entirely to an action on 5-HT1A receptors.

Consistent with previous studies in conscious and anesthetized rats (20, 24, 35, 36) systemic injections of 8-OH-DPAT resulted in a moderate reduction in resting MAP and HR. The effect of 8-OH-DPAT in reducing the PVN-evoked pressor and tachycardic response is not a consequence of this reduction in resting MAP and HR, however, because systemic injections of the same or a higher dose of 8-OH-DPAT did not affect the cardiovascular responses to chemoreceptor stimulation or to cold exposure (24, 49).

We have previously shown that systemic injection of the same dose of 8-OH-DPAT as used in the present study did not affect the reflex sympathoexcitatory response to chemoreceptor stimulation or the sympathoinhibitory response to baroreceptor stimulation (24). Furthermore, Vianna and Carrive (49) showed that systemic injections of 8-OH-DPAT, even at a higher dose (250 μg/kg) than that used in the present study, did not reduce the pressor and tachycardic response to cold exposure in conscious rats. Therefore, the 5-HT1A receptor-mediated inhibition of the PVN-evoked sympathoexcitation is not simply due to a non-specific inhibition of sympathetic reactivity.

Effects of activation of 5-HT1A receptors on resting sympathetic activity. Previous studies in cats and rats have shown that systemic administration of 8-OH-DPAT generally reduces sympathetic activity via a central action (30, 31, 36, 42). In the present study, after injection of 8-OH-DPAT, the RSNA first increased and then decreased, reaching a stable level that was not significantly different from the preinjection baseline level. The initial increase in RSNA, however, occurred slightly later (by ~2–3 s) than the initial decrease in MAP, and is therefore likely to be mediated by the baroreceptor reflex, which we have previously shown is unaffected by systemic administration of 8-OH-DPAT (24). Thus, baroreflex-mediated sympathoexcitation may have masked the direct central sympathoinhibitory response to 8-OH-DPAT (24).

Effects of selective 5-HT1A receptor agonist and antagonist on cardiovascular responses. Previous studies in cats and rats have shown that selective 5-HT1A receptor agonists and antagonists have different effects on cardiovascular responses (30, 31, 36, 42). In the present study, after injection of 8-OH-DPAT, the RSNA first increased and then decreased, reaching a stable level that was not significantly different from the preinjection baseline level. The initial increase in RSNA, however, occurred slightly later (by ~2–3 s) than the initial decrease in MAP, and is therefore likely to be mediated by the baroreceptor reflex, which we have previously shown is unaffected by systemic administration of 8-OH-DPAT (24). Thus, baroreflex-mediated sympathoexcitation may have masked the direct central sympathoinhibitory response to 8-OH-DPAT (24).

Fig. 6. A: example of the effects of bicuculline microinjection into the PVN, dorsomedial hypothalamus (DMH), and intermediate region (Int) between the PVN and DMH in 1 experiment. B: grouped data showing changes (means ± SE, n = 10) in MAP, HR, and RSNA evoked by bicuculline microinjection into the PVN, Int, and DMH. *P < 0.05, **P < 0.01 vs. response from PVN; ##P < 0.01, vs. response from DMH.
The effect of activation of central 5-HT$_{1A}$ receptors that has been described previously (30, 31, 36, 42).

Possible location of site(s) at which 5-HT$_{1A}$ receptors suppress the PVN-evoked responses. Since blockade of GABA receptors on PVN neurons evokes a cardiovascular response, the neurons that drive that response must receive tonic excitatory as well as tonic inhibitory inputs. The PVN contains neurons that express 5-HT$_{1A}$ receptors (28), and activation of 5-HT$_{1A}$ receptors inhibits adenylyl cyclase and can also open K$^+$ channels, leading to inhibition of neuronal activation (3). Thus 8-OH-DPAT may act on 5-HT$_{1A}$ receptors located on PVN neurons to inhibit responses evoked by disinhibition of PVN neurons. It has been shown that serotonin attenuates glutamate-evoked excitation of neurons in the locus coeruleus (2), and so it is possible that 5-HT$_{1A}$ receptors may mediate a similar attenuation of tonic excitatory inputs to PVN neurons, resulting in a reduced response to disinhibition of these neurons. Our results showed, however, that microinjection of a high concentration (1 nmol in 100 nl) of 8-OH-DPAT into the PVN did not significantly reduce the responses to bicuculline microinjections into the same site. This dose of 8-OH-DPAT, when microinjected into the medullary raphé pallidus, has been shown to be as effective in reducing stress-evoked tachycardia as a systemic injection of 100 $\mu$g/kg of 8-OH-DPAT (35). Thus, although we cannot rule out the possibility that activation of 5-HT$_{1A}$ receptors in the PVN may have contributed to the inhibition of the bicuculline-evoked response, the results indicate that the inhibition is dependent on activation of 5-HT$_{1A}$ receptors outside the PVN.

There is a major direct projection from the PVN to the RVLM (19), which contains sympathetic premotor neurons that have a critical role in regulating the sympathetic outflow (7, 21). RVLM sympathetic premotor neurons also contain 5-HT$_{1A}$ receptors (22) and are thus a potential site at which 8-OH-DPAT could cause inhibition of PVN-evoked sympathoexcitation. Similarly, there are also major descending projections from the PVN to the intermediolateral cell column in the spinal cord and to the nucleus tractus solitarius (NTS) (19, 44, 46), both of which also contain 5-HT$_{1A}$ receptors (27, 47). The RVLM, NTS, and intermediolateral cell column are also all essential components of the central pathways mediating baroreceptor and chemoreceptor reflexes (7, 21). As mentioned above, we have previously shown that systemic administration of 8-OH-DPAT did not alter the reflex renal sympathetic responses to baroreceptor or chemoreceptor stimulation (24). It therefore follows that the RVLM, NTS, and intermediolateral cell column are unlikely to be sites at which activation of 5-HT$_{1A}$ receptors suppresses PVN-evoked sympathoexcitation, unless the 5-HT$_{1A}$ receptors involved are located on neurons within these regions that are not essential components of the baroreceptor or chemoreceptor reflex pathways, or else are located presynaptically on nerve terminals within one or more of these regions.

The raphé pallidus in the medulla also contains a high density of 5-HT$_{1A}$ receptors (22), and previous studies have shown that activation of 5-HT$_{1A}$ receptors in this region attenuates the increase in HR evoked by psychological stress in rats and rabbits (33, 35). Consistent with this, previous func-
tional and anatomic studies indicate that the tachycardia evoked from the DMH is mediated to a large extent via a direct descending pathway to cardiac sympathetic premotor neurons in the raphé pallidus (5, 23, 43). DiMicco et al. (10) have proposed that the descending pathway from the DMH to cardiac sympathetic neurons in the medullary raphé pallidus is a critical component of the central pathways mediating the tachycardic response to psychological stress. In contrast to the DMH, however, there is only a very sparse direct innervation of the raphé pallidus from neurons in the PVN (19). It therefore seems unlikely that the inhibition of the PVN-evoked tachycardia following administration of 8-OH-DPAT is due to activation of 5-HT₁A receptors within the raphé pallidus.

Finally, an alternative possibility is that activation of 5-HT₁A receptors inhibits neurons that do not receive inputs from the PVN, but which provide a tonic facilitatory input to neurons that are part of the descending pathway from the PVN to the renal or cardiac sympathetic outflow. Future studies will be required to define the precise locations of 5-HT₁A receptors that inhibit the cardiac and renal sympathoexcitatory response to PVN activation.

**Tonic effects of endogenous activation of 5-HT₁A receptors on baseline variables and evoked responses.** Blockade of 5-HT₁A receptors with WAY-100635 without prior administration of 8-OH-DPAT had little effect on resting MAP or RSNA, but did cause a modest but significant increase in HR, suggesting that endogenous activation of 5-HT₁A receptors may tonically inhibit central neurons that normally act to increase HR. A possible site of action for this effect is the raphé pallidus, which, as mentioned above, contains neurons that increase HR and which can be inhibited by activation of 5-HT₁A receptors (33, 35). Alternatively, there are direct projections from the PVN to the nucleus ambiguus and dorsal motor nucleus of the vagus (19), regions that also contain 5-HT₁A receptors (25), and so it possible that endogenous activation of these receptors may tonically excite cardiac preganglionic neurons within these nuclei.

Disinhibition of the PVN increased overall RSNA but also altered the pattern of RSNA, such that the bursts were of greater amplitude and were separated by periods of low activity, as has been previously reported (26). Activation of 5-HT₁A receptors with 8-OH-DPAT reduced the peak RSNA response to PVN disinhibition and also restored the normal pattern of activity. This raises the question as to whether the normal pattern of RSNA may depend upon tonic activation of 5-HT₁A receptors. To test this possibility, in one series of experiments we determined the effects of blockade of 5-HT₁A receptors alone, without prior activation with 8-OH-DPAT. The results showed that both the ongoing resting pattern of RSNA, as well as the change in RSNA evoked by disinhibition of the PVN, were unchanged after blockade of 5-HT₁A receptors. These findings therefore indicate that that there is not a tonic suppression by 5-HT₁A receptors of either resting RSNA or evoked RSNA responses, at least in the anesthetized preparation.

**Perspectives and Significance**

The PVN has long been known to be a key brain region regulating the autonomic outflow, via direct and indirect connections to sympathetic and vagal preganglionic neurons (46). It also receives multiple inputs from many other nuclei in the hypothalamus (e.g., subfornical organ, median preoptic nucleus, suprachiasmatic nucleus) and brainstem (e.g., periaqueductal gray, lateral parabrachial nucleus, tractus solitarius, ventrolateral medulla), which in turn relay signals from a variety of peripheral receptors (e.g., baroreceptors, cardiopulmonary receptors, chemoreceptors, osmoreceptors) as well as inputs that respond to changes in the levels of circulating hormones such as angiotensin II (14, 39). There is also much evidence that the PVN has an essential role in generating the sustained activation of renal and cardiac sympathetic activity that occurs in heart failure and neurogenic hypertension (1, 4, 14, 39, 40). The present study demonstrates clearly that sympathoexcitatory responses generated by activation of PVN neurons can be powerfully inhibited by activation of 5-HT₁A receptors, raising the possibility that this may be a potential therapeutic tool for reducing the sympathoactivation that occurs in some forms of hypertension and heart failure (13, 40). Future studies will be required to determine the precise sites at which activation of 5-HT₁A receptors reduces sympathetic activation evoked from the PVN, as well as the precise mechanisms of this effect.

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**DISCLOSURES**

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