5-HT-mediated inhibition of cardiovagal baroreceptor reflex response during defense reaction in the rat


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5-HT-mediated inhibition of cardiovagal baroreceptor reflex response during defense reaction in the rat. Am J Physiol Heart Circ Physiol 287: H1641–H1649, 2004. First published May 27, 2004; 10.1152/ajpheart.01204.2003.—Previous studies showed that the cardiac response of the baroreceptor reflex (bradycardia) is inhibited during the defense reaction evoked by direct electrical or chemical stimulation of the periaqueductal gray (dPAG) in the rat. Whether central serotonin and nucleus tractus solitarius (NTS) serotonin (5-HT) receptors might participate in this inhibition was investigated in urethane-anaesthetized and atenolol-pretreated rats. Our results showed that both electrical and chemical stimulation of the dPAG produced a drastic reduction of the cardiovagal component of the baroreceptor reflex triggered by either intraventricular administration of phenylephrine or aortic nerve stimulation. This inhibitory effect of dPAG stimulation on the baroreflex bradycardia was not observed in rats that had been pretreated with p-chlorophenylalanine (300 mg/kg ip daily for 3 days) to inhibit serotonin synthesis. Subsequent 5-hydroxytryptophan administration (60 mg/kg ip), which was used to restore serotonin synthesis, allowed the inhibitory effect of dPAG stimulation on both aortic and phenylephrine-induced cardiac reflex responses to be recovered in p-chlorophenylalanine-pretreated rats. On the other hand, in nonpretreated rats, the inhibitory effect of dPAG stimulation on the cardiac baroreflex response could be markedly reduced by prior intra-NTS microinjection of granisetron, a 5-HT3 receptor antagonist, or bicuculline, a GABA A receptor antagonist. These results show that serotonin plays a key role in the dPAG stimulation-induced inhibition of the cardiovagal baroreceptor reflex response. Moreover, they support the idea that 5-HT3 and GABA A receptors in the NTS mediated the inhibitory effect of dPAG stimulation on the baroreflex response caused by dPAG stimulation.

nucleus tractus solitarius; serotonin receptors; GABA A receptors; defense/attack

MUCH EVIDENCE indicates that serotonin (5-hydroxytryptamine; 5-HT) plays a modulatory role in the central control of cardiovascular parameters (28, 32), notably through actions at the level of the nucleus tractus solitarius (NTS) (13, 15), a key structure for the integration of baroreceptor and other peripheral and central messages involved in the homeostatic control of blood pressure (BP) and heart rate (HR) (9, 11, 19). In particular, serotonin3 (5-HT3) receptor stimulation specifically in the NTS was shown to elicit a transitory increase in BP and to block the cardiovascular component (bradycardia) of the baroreceptor reflex (15, 25). Interestingly, transient hypertension and inhibition of the baroreceptor reflex response are also the cardiovascular changes that accompany the defense reaction and other behavioral responses to various stressful conditions (16, 17).

Inhibition of the baroreceptor reflex bradycardia caused by NTS 5-HT3 receptor stimulation seems to be mediated through the activation of a local GABAergic system because it could be abolished by prior intra-NTS application of bicuculline, a GABA A receptor antagonist (15). Interestingly, the latter treatment was also found to prevent the inhibition of NTS barosensitive neurons that normally occurs during the defense reaction (10).

All of these data led us to postulate that both 5-HT3 and GABA A receptors in the NTS are implicated in the defense reaction-induced inhibition of the baroreceptor reflex. In line with this idea, we recently showed that the baroreflex inhibition normally observed during electrical stimulation of structures that elicit the defense reaction, including the dorsal part of the periaqueductal gray (dPAG; Refs. 2 and 3), could be prevented by intra-NTS microinjections of granisetron, a specific 5-HT3 receptor antagonist (8), as well as bicuculline (26).

However, to really establish that serotoninergic receptors actually mediate the defense reaction-induced inhibition of the baroreflex, it is also necessary to demonstrate that their stimulation by endogenous 5-HT is a key component of underlying inhibitory mechanisms. To this goal, we analyzed the effects of dPAG stimulation on the baroreflex bradycardia in rats that had been pretreated with p-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor (12). For these experiments, the baroreceptor reflex was triggered by either systemic injection of phenylephrine or electrical stimulation of the aortic depressor nerve. In addition, we investigated whether 5-HT3 and/or GABA A receptors in the NTS mediated the inhibitory effect of electrical and chemical dPAG stimulation on the baroreflex cardiac response to phenylephrine administration. For this purpose, both receptors were specifically blocked by intra-NTS microinjections of selective antagonists.

MATERIALS AND METHODS

General Procedures

Experiments were performed on 116 male Sprague-Dawley rats weighing 330–370 g. Animals were kept under controlled environmental conditions (ambient temperature: 21 ± 1°C, 60% relative humidity, food, and water ad libitum, alternate 12:12-h light-dark cycles) for at least 1 wk after receipt from the breeding center (CER Janvier; Le Genest-St. Isle, France). Procedures involving animals and their care were all conducted in conformity with the institutional guidelines, which are in compliance with national and international...
lows and policies [Council Directive no. 87-848, 19 October 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 75-116 (to M. Hamon) and no. 75-117 (to R. Laguzzi)].

Rats were anesthetized with urethane (1.5 g/kg ip), and the depth of anesthesia was regularly assessed by pinching a hindpaw and monitoring the stability of BP and HR. In case of withdrawal reflex and/or variations of cardiovascular parameters, a supplementary dose of urethane was given (0.1–0.2 g/kg iv). A cannula was inserted into the femoral vein for administration of drugs and/or additional doses of urethane. Systemic BP, mean BP (MBP), and HR were monitored (Pressure Processor and DC Amplifier, Gould; Courtaboeuf, France) through a catheter inserted into the femoral artery. ECG was recorded with the use of stainless steel pins placed subcutaneously into fore- and hindpaws; signals were amplified and filtered (Universal Amplifier, Gould). The R wave of ECG was discriminated with a window discriminator and used to generate pulses. Arterial BP and ECG pulse signals were relayed to a 1401 interface (1401 Plus, CED) connected to a computer running Spike 2 software (CED; Cambridge, UK). HR signals were relayed to a 1401 interface (1401 Plus, CED) connected to a computer running Spike 2 software (CED; Cambridge, UK). HR was automatically computed from R wave pulses and displayed as mean frequency per minute (bin size: 1 s). Rectal temperature was maintained at 37°C with a thermostatically controlled heating blanket.

**Procedures for dPAG Stimulation and Intra-NTS Microinjections**

The rats were placed in a stereotaxic frame with the head fixed in horizontal position. A craniotomy was performed, and either a bipolar stimulating electrode or a single-barrel glass micropipette (<100 µm external diameter), connected to a Hamilton microsyringe filled with bicuculline in saline), was lowered into the dPAG using stereotaxic coordinates (P 6.7, L 0.7, V 3.5–4.5) from Paxinos and Watson’s atlas (20). In both cases, dPAG was identified by observing body reactions and cardiorespiratory responses typical of the defense reaction caused by local electrical stimulation (50 Hz, 1 ms pulse duration, 200 µA, Ref. 26) or bicuculline microinjection (100 pmol in 100 nl); mydriasis, viussaie and body movements, tail erection, rise in BP, tachycardia, and hyperventilation. In addition, stimulation of the dPAG by bicuculline (but not by electrical pulses) produced a slight and nonsignificant delayed increase in the variability of MBP that occurred 2–5 s after BP had reached its maximal value. In some rats, the left aortic depressor nerve was dissected out from the vagus nerve by a lateral approach and placed on silver bipolar hook electrodes for electrical stimulation.

For microinjections of neuroactive substances into the NTS, the dorsal surface of the brain stem was exposed through a limited occipital craniotomy. A single-barrel glass micropipette (<100 µm external diameter), connected to a Hamilton microsyringe filled with drugs or saline, was lowered into the commissural NTS, 0.5 mm lateral to the calamus scriptorius and 0.5 mm beneath the dorsal surface of the medulla (26). Microinjections (100 nl) were made over 2 s with a pneumatic microinfusion pump and the micropipette was removed 15 s after the injection. The same micropipette was used for bilateral microinjections of a given drug. The time interval between two symmetrical microinjections was <1 min.

**Atenolol Administration**

Because our purpose was to analyze the effects of dPAG stimulation on reflex vagal bradycardia, all rats used in the present study were pretreated with atenolol (1 mg/kg iv; 20 min before phenylephrine injection or aortic nerve stimulation), a β-adrenergic receptor antagonist to eliminate both the influence of the defense reaction-induced sympathetic activation on HR and the sympathetic cardioinhibitory component of the baroreflex (29). Systemic administration of atenolol produced a rapid and significant decrease in HR (ΔHR: ~42 ± 5 beats/min from a baseline of 348 ± 4 beats/min, paired Student’s t-test, P < 0.05, n = 116) without affecting BP (ΔMBP = −2 ± 1 mmHg from a baseline of 81 ± 1 mmHg, n = 116). HR and MBP baseline values then remained stable for at least 2 h. In addition, this treatment blocked for >1 h the increase in HR normally evoked by dPAG stimulation and induced a slight but nonsignificant reduction (~5%) of baroreflex bradycardia.

**Baroreflex Activation**

The baroreflex was triggered either by a bolus injection of phenylephrine at a dose (10 µg/kg iv) sufficient to elevate MBP by 50–60 mmHg, or by electrical pulses (20 Hz, 1 ms pulse duration) applied to left aortic depressor nerve. The intensity of aortic nerve stimulation was adjusted (100–150 µA) to elicit the maximal aortic cardioevoked response (ΔHR: −170 to −190 beats/min) that could be obtained without producing nerve damage, according to the criteria fixed by Sévoz-Couche et al. (26).

**Quantification of Cardiac Baroreflex Response**

In phenylephrine-injected rats, the cardiac reflex response (PECR) was assessed from the ratio of the maximal increase in HR (ΔHRmax) over the maximal increase in MBP (ΔMBPmax) caused by the drug. In addition, we drew the curve of phenylephrine-induced baroreflex gain and calculated the cardiac baroreceptor sensitivity from its rectilinear part by fitting the data with a common sigmoid curve equation (4)

\[
Y = X_{min} + \left(\frac{X_{max} - X_{min}}{1 + \exp(MPT \times SSC)}\right)
\]

where X is the change in BP, Y is the change in HR, Xmin is set to zero as the minimal change in BP, MPT is midpoint X, and SSC is the sigmoid slope coefficient. The data obtained from the rectilinear part of the resulting sigmoid curves were plotted against the pressure changes (between 25 and 50 mmHg) and a regression analysis allowed calculation of the baroreflex slope for each experimental conditions (4).

In rats whose aortic depressor nerve was stimulated, the evoked cardiac response (ACR) was expressed as the ratio of ΔHRmax caused by the stimulation over its corresponding baseline value (HR baseline).

**Inhibition of Cardiac Baroreflex Responses by dPAG Stimulation**

To assess the effects of dPAG stimulation on cardiac reflex responses normally evoked by phenylephrine administration, PECR were first determined after injection of the α1-adrenoreceptor agonist alone (“PECR control”). Then, 10 min later, “PECR experimental” was determined in response to a second injection of phenylephrine made simultaneously with electrical stimulation of dPAG or when the increase in MBP induced by intra dPAG microinjection of bicuculline (chemical stimulation) reached its maximum value. Electrical dPAG stimulation could be performed several times in each rat. In these conditions, three successive determinations of PECR control-PECR experimental could be made (within 30 min). Corresponding values were considered as stable when they differed by <10%. Only the last one was taken into account for statistical analysis.

In contrast, only one chemical stimulation of dPAG could be made in a given rat. Therefore, only one determination of PECR control-PECR experimental was performed in rats microinjected with bicuculline within dPAG.

To quantify the inhibition of the aortic cardiac response caused by dPAG stimulation, ACR was first evoked by aortic nerve stimulation during 4 s (“ACR control”). Five minutes later, when MBP and HR had returned to baseline levels, electrical stimulation of dPAG was turned on and the aortic nerve was stimulated again (“ACR experimental”) for 4 s when the cardiovascular changes associated with the evoked defense reaction were maximal (i.e., ~3 s after the beginning of dPAG stimulation). Stimulation of dPAG was then turned off 3 s after the end of aortic nerve stimulation. Three successive determinations of “ACR control-ACR experimental” were performed (within 10
min). Corresponding values were considered stable when they differed by <10%. Only the last one was used for statistical analysis.

**Pharmacological Treatments**

*Systemic treatments with PCPA and 5-HTP.* Two groups of rats were treated daily (at 8 PM), during 3 consecutive days, with PCPA at the dose of 300 mg/kg ip. In these animals, either PECR (n = 15) or ACR (n = 15) was determined 12 h after the last PCPA injection. Such determinations were carried out under basal conditions and during electrical stimulation of dPAG (as described above). Paired controls received saline instead of PCPA and were used for PECR (n = 6) or ACR (n = 6) determinations also under baseline and dPAG stimulating conditions. In addition, in a fraction of PCPA-treated rats, the effects of dPAG stimulation on PECR (n = 6 out of 15) and ACR (n = 6 out of 15) were also tested before and after (60 min) administration of the 5-HT precursor 5-HTP (60 mg/kg ip).

*Intra-NTS microinjections of granisetron and bicuculline.* These treatments were performed in combination with either electrical or chemical stimulation of dPAG. Electrical stimulation (n = 36) was performed in three different groups of rats (groups A, B, and C) for the determinations of PECR control (before stimulation) and experimental (during stimulation) before and after (15 and 60 min) bilateral intra-NTS microinjections of the following: group A, saline (n = 6); group B, granisetron (250 pmol, n = 15), or group C, bicuculline (5 pmol, n = 15) (see Table 1).

In case of chemical stimulation by intra-dPAG injection of bicuculline (n = 26), a first group of naive rats (n = 7) was used to determine PECR control and experimental, and corresponding couples of PECR values were also quantified in three other groups of rats (groups D, E, and F) after (15 min) bilateral microinjections of: group D, saline (n = 6), group E, granisetron (250 pmol, n = 7), or group F, bicuculline (5 pmol, n = 6), into the NTS (see Table 2).

**HPLC analyses: measurements of tissue levels of 5-HT and 5-hydroxyindoleacetic acid.** Some of the PCPA-treated rats (n = 6, see above) and paired saline-treated controls (n = 6) were decapitated and the brain stem was immediately dissected at 0°C, weighed, and homogenized in 250 μl of 0.1 N HClO4 supplemented with 0.05% Na2S2O3 and 0.05% disodium EDTA. Homogenates were centrifuged at 30,000 g for 15 min at 4°C and the supernatants were neutralized with 2 M KH2PO4/K2HPO4, pH 7.4. After a second centrifugation as above, the clear supernatants were saved and aliquots (10 μl) were injected directly into a HPLC column (UltraspHERE IP, 25 cm, 0.46 cm outside diameter, 5 μm) protected with a Brownlee precolumn (3 cm, 5 μm). The mobile phase consisted of 70 mM KH2PO4, 2 mM triethylamine, 0.1 mM disodium EDTA, 0.62 mM octane sulfonic acid, and 16% methanol, adjusted to pH 3.02 (6). The electrochemical detection system (model 5011, ESA; Bedford, MA) comprises an analytic cell with dual coulometric monitoring electrodes (+50 and +350 mV). Signals corresponding to eluted 5-HT and 5-hydroxyindoleacetic acid were quantified with the use of a computing integrator (Millenium-Waters; St. Quentin-en-Yvelines, France).

**Histology.** At the end of the experiments, electrolytic lesions (50 Hz, 4 mA, 20 s) were made at dPAG stimulation sites, and methylene blue (0.1 μl) was microinjected into the injection sites within NTS and dPAG. Rats were then perfused intracardially with saline and a solution of 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4. After perfusion, the tissue was fixed in the skull, and coronal sections (60 μm) were cut and stained with neutral red.

**Statistical Calculations**

Absolute values are expressed as means ± SE of n rats. Statistical analyses were performed using paired and unpaired Student’s t-test or two-way ANOVA as appropriate (see RESULTS). A difference was considered significant at P < 0.05.

**Drugs**

Atenolol (Sigma-Aldrich; St. Quentin-Fallavier, France), bicuculline methiodide (Sigma-Aldrich), dl-p-chlorophenylalanine methyl ester (Sigma-Aldrich), granisetron (Glaxo Smith Kline; Harlow, UK), 5-hydroxy-L-tryptophan (Sigma-Aldrich), phenylephrine (Chibret; Paris, France), and urethane ethyl-carbamate (Sigma-Aldrich) were dissolved in saline. The pH of all solutions microinjected into the dPAG or the NTS was adjusted to 7.4.

**RESULTS**

**Effects of dPAG Stimulation on Baroreflex Cardiac Responses in Naive Rats**

In naive rats, administration of phenylephrine (10 μg/kg iv, n = 62) induced an increase in BP of +53 ± 1 mmHg from a baseline of 79 ± 3 mmHg, which triggered a cardiac reflex...

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**Table 1. Cardiac baroreflex responses induced by phenylephrine administration alone (PECR control) or in combination with dPAG stimulation (PECR experimental) in rats: effects of prior bilateral microinjections of saline, granisetron, or bicuculline into NTS**

<table>
<thead>
<tr>
<th>Intra-NTS Microinjections</th>
<th>PECR Control, beats/min⁻¹−mmHg⁻¹</th>
<th>PECR Experimental, beats/min⁻¹−mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.05±0.03</td>
<td>0.52±0.01</td>
</tr>
<tr>
<td>After 15 min</td>
<td>2.06±0.03</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>After 60 min</td>
<td>2.02±0.04</td>
<td>0.48±0.02</td>
</tr>
<tr>
<td>Granisetron (n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.04±0.03</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>After 15 min</td>
<td>2.05±0.04</td>
<td>1.74±0.03*</td>
</tr>
<tr>
<td>After 60 min</td>
<td>2.02±0.03</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>Bicuculline (n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.09±0.08</td>
<td>0.58±0.04</td>
</tr>
<tr>
<td>After 15 min</td>
<td>2.16±0.07</td>
<td>1.81±0.05*</td>
</tr>
<tr>
<td>After 60 min</td>
<td>2.11±0.06</td>
<td>0.60±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. PECR, maximal heart rate change over maximal increase in blood pressure for phenylephrine (PE) administration. PECR control determined 15 min after microinjections of saline, granisetron, or bicuculline was not significantly (paired Student’s t-test) different from that quantified before each nucleus tractus solitarius (NTS) microinjection. However, at the difference of saline, intra-NTS microinjections of granisetron (250 pmol) and bicuculline (5 pmol) prevented the attenuation of PECR during dorsal part of periaqueductal gray (dPAG) stimulation (PECR “experimental” obtained 15 min after drug microinjection was compared with those obtained before and 60 min after microinjections, two-way ANOVA, *P < 0.05).

**Table 2. Cardiac baroreflex responses induced by phenylephrine administration alone or in combination with dPAG chemical stimulation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PECR Control, beats/min⁻¹−mmHg⁻¹</th>
<th>PECR Experimental, beats/min⁻¹−mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive (n = 7)</td>
<td>2.02±0.02</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>Saline (n = 6)</td>
<td>2.04±0.03</td>
<td>0.79±0.01</td>
</tr>
<tr>
<td>Granisetron (n = 7)</td>
<td>2.00±0.03</td>
<td>1.76±0.02*</td>
</tr>
<tr>
<td>Bicuculline (n = 6)</td>
<td>2.02±0.04</td>
<td>1.70±0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. PECR control determined after microinjections of saline, granisetron, or bicuculline was not significantly (paired Student’s t-test) different from that found in naive rats; however, at the difference of saline, intra-NTS microinjections of granisetron (250 pmol) and bicuculline (5 pmol) prevented the attenuation of PECR that normally occurred (i.e., in naive rats) during chemical stimulation of dPAG. *P < 0.05, unpaired Student’s t-test, PECR experimental after each intra-NTS microinjections was compared with the respective value in naive rats.
response of $-117 \pm 5$ beats/min from a baseline HR of 304 $\pm 4$ beats/min (paired Student’s $t$-test, $P < 0.05$). Electrical stimulation of dPAG (Fig. 1A) performed before any intra-NTS microinjections increased BP (MBP = 101 $\pm 3$ vs. 80 $\pm 2$ mmHg, $+26\%$; paired Student’s $t$-test, $n = 36, P < 0.05$) and markedly reduced the cardiac response normally induced by phenylephrine (PECR control and experimental: 2.07 $\pm 0.03$ and 0.55 $\pm 0.02$ beats $\cdot$ min$^{-1}$ $\cdot$ mmHg$^{-1}$, respectively, $-73\%$, paired Student’s $t$-test, $n = 36, P < 0.05$) (Fig. 2A). Consequently, dPAG electrical stimulation produced a flattening of the baroreflex gain curve (Fig. 3). In the same manner, dPAG chemical stimulation by local microinjection of bicuculline (Fig. 1B) induced an increase in BP (110 $\pm 3$ vs. 80 $\pm 4$ mmHg, $+37.5\%$, paired Student’s $t$-test; $n = 26, P < 0.05$) and significantly reduced phenylephrine-evoked cardiac response (PECR control and experimental: 2.01 $\pm 0.03$ and 0.80 $\pm 0.01$, respectively, $-60\%$, $n = 7$, paired Student’s $t$-test, $P < 0.05$) (Fig. 2B).

On the other hand, the baroreflex cardiac response to aortic depressor nerve stimulation (ACR = $-135 \pm 3$ from a baseline of 308 $\pm 5$ beats/min, giving an ACR of $-0.44 \pm 0.03; n = 12$) was also significantly inhibited by concomitant electrical stimulation of the dPAG (Fig. 2C).

Effects of dPAG Stimulation on Baroreflex Cardiac Responses in PCPA-Treated Rats

Biochemical controls showed that PCPA treatment almost totally depleted 5-HT (36 $\pm 2$ vs. 539 $\pm 39$ ng/g in paired saline-treated controls, $-93\%$, $n = 6$ in each group, unpaired Student’s $t$-test; $P < 0.05$) and 5-hydroxyindoleacetic acid (15 $\pm 1$ vs. 338 $\pm 32$ ng/g in PCPA and saline-treated rats, respectively, $-96\%$, $n = 6$ in each group, $P < 0.05$) levels in the brain stem. In these animals, as well as in paired saline-treated controls, the increase in BP normally produced by dPAG electrical stimulation (PCPA: $+20 \pm 3$ mmHg from a baseline of 81 $\pm 4$ mmHg, $n = 30$; saline: $+25 \pm 5$ mmHg from a baseline of 78 $\pm 4$ mmHg, $n = 12$), and both PECR (PCPA: 2.09 $\pm 0.03$, $n = 15$; saline: 2.05 $\pm 0.03$ beats $\cdot$ min$^{-1}$ $\cdot$ mmHg$^{-1}$, $n = 6$) and ACR (PCPA: 0.39 $\pm 0.02$ beats $\cdot$ min$^{-1}$ $\cdot$ mmHg$^{-1}$, $n = 15$; saline: 0.41 $\pm 0.03$ beats $\cdot$ min$^{-1}$ $\cdot$ mmHg$^{-1}$, $n = 6$) did not significantly differ from those observed in naive untreated rats (see above). In contrast, treatment with PCPA but not saline ($n = 6$ for each evoked cardiac response) prevented the reduction of PECR (Fig. 4A, $n = 15$) and ACR (Fig. 4B, $n = 15$) normally produced by dPAG electrical stimulation.

As illustrated in Fig. 4, 5-HTP administration (60 mg/kg ip) in PCPA-treated rats restored the inhibitory effect of dPAG stimulation on PECR ($n = 6$) and ACR ($n = 6$) to the same level (around $-75\%$) as that observed in control rats.

![Frontal brain sections showing the localization of sites where electrical stimulation and microinjections were performed in the dorsal part of the periaqueductal gray (dPAG) and nucleus tractus solitarius (NTS).](http://ajpheart.physiology.org/)

**Fig. 1.** Frontal brain sections showing the localization of sites where electrical stimulation and microinjections were performed in the dorsal part of the periaqueductal gray (dPAG) and nucleus tractus solitarius (NTS). A: photomicrograph showing a representative electrode track (*) for electrical stimulation (50 Hz, 1 ms) of the dPAG inducing inhibition of phenylephrine (PE)-induced baroreflex bradycardia. B: serial coronal camera lucida drawings of sections displaying the area (shaded region) where chemical (microinjections of bicuculline, 100 pmol, $n = 26$) stimulations of dPAG produced inhibition of phenylephrine-induced baroreflex bradycardia. C: sections displaying the areas in the NTS where bilateral microinjections of granisetron (250 pmol, solid region, $n = 28$) or bicuculline (5 pmol, hatched region, $n = 21$) were performed. Anteroposterior levels (in mm) are from bregma, according to Paxinos and Watson’s atlas (20). AP, area postrema; DG, dentate gyrus; DVN, dorsal vagal motor nucleus; LRN, lateral reticular nucleus; NAmb, nucleus ambiguous; mPAG, medial part of the periaqueductal gray; pyx, pyramidal decussation; Sp5C, spinal trigeminal nucleus; tfp, transverse fibers pons; 12, hypoglossal nucleus.
Effects of Intra-NTS Microinjections of Granisetron on Inhibition of Phenylephrine-Induced Bradycardia by dPAG Stimulation

Bilateral microinjections of granisetron (250 pmol) into the commissural NTS (Fig. 1C) did not significantly modify cardiovascular parameters ($\Delta$MBP = +2.1 ± 1.0 mmHg from a baseline of +81 ± 2 mmHg; $\Delta$HR = +11 ± 5 beats/min from a baseline of 296 ± 6 beats/min, $n = 28$) and PECR (Table 1). However, such microinjections, but not those of saline in paired control rats ($n = 6$, Tables 1 and 2, Fig. 5A), markedly reduced (by about -80%) for -45 min the inhibitory effects of both electrical ($n = 15$, Table 1) and chemical ($n = 7$, Fig. 5B, Table 2) stimulation of dPAG on PECR. Consequently, the flattening effect of dPAG stimulation on the baroreflex gain curve was no longer observed in rats that had been microinjected with granisetron (Fig. 3A). As expected, the effect of intra-NTS microinjection of granisetron and that of systemic administration of PCPA were not additive (PECR control and experimental were 2.00 ± 0.02 and 1.79 ± 0.03, respectively, in PCPA-treated rats before granisetron, and 2.02 ± 0.03 and 1.81 ± 0.04, respectively, in PCPA-treated rats after granisetron, $n = 6$).

Effects of Intra-NTS Microinjections of Bicuculline on Inhibition of Phenylephrine-Induced Bradycardia Elicited by dPAG Stimulation

Bilateral microinjections of a low dose of bicuculline (5 pmol) into the commissural NTS (Fig. 1C) produced no significant changes in HR ($\Delta$HR = −2.0 ± 1.9 beats/min, from a baseline of 295 ± 5 beats/min, $n = 21$) and MBP ($\Delta$MBP =
2.0 mmHg, from a baseline of 79 ± 3 mmHg, and did not affect PECR (Table 2). However, the decrease in PECR as well as the flattening of the baroreflex gain curve normally caused by dPAG electrical stimulation were prevented by intra-NTS administration of bicuculline (n = 15, Table 1 and Fig. 3B). Similarly, the latter treatment (n = 6) markedly attenuated PECR inhibition normally caused by dPAG chemical stimulation (Table 2 and Fig. 5C). Such effects of intra-NTS bicuculline microinjections lasted ~35 min.

DISCUSSION

The data reported herein showed that serotonin plays a key role in the inhibitory effect of dPAG stimulation on the cardiovagal component of the baroreceptor reflex triggered by phenylephrine administration. Moreover, microinjection experiments provided further support to the conclusion that 5-HT3 receptors in the NTS participate in this 5-HT-mediated control of the baroreceptor reflex (26).

In our previous studies (26) demonstrating that dPAG stimulation was able to inhibit baroreflex bradycardia, the baroreflex was triggered by direct electrical stimulation of the aortic depressor nerve and its inhibition was evaluated by comparing the magnitude of the maximal reflex responses before (ACR control) versus during (ACR experimental) the latter stimulation. The use of phenylephrine to trigger the baroreflex in the present study permitted us not only to compare the magnitude of the maximal reflex responses (PECR experimental vs. PECR control) but also, as explained in MATERIALS AND METHODS, to determine the slope of the baroreceptor gain curve under control versus experimental conditions. Thus comparison of

Fig. 3. Curves of cardiac baroreceptor gain during dPAG electrical stimulation, before and after intra-NTS microinjections of granisetron and bicuculline. Reflex fall in HR (ΔHR, y-axis) in response to increase in BP [mean BP (ΔMBP) x-axis] triggered by PE administration. Sigmoidal function stimulus-response curves (see MATERIALS AND METHODS) are drawn from raw data points obtained in 8 animals for each experimental condition (PE alone, PE + dPAG, PE + dPAG/intra-NTS granisetron, and PE + dPAG/intra-NTS bicuculline). Slopes were calculated by regression analysis of the rectilinear part of the curves (pressure changes between 25 and 50 mmHg). The curves in A and B show that reflex bradycardia was attenuated when PE was administered simultaneously with dPAG electrical stimulation (PE + dPAG). Prior (10 min) microinjections of granisetron (250 pmol, PE + dPAG/intra-NTS granisetron, A) or bicuculline (5 pmol, PE + dPAG/intra-NTS bicuculline, B) into the NTS prevented the attenuating effect of dPAG stimulation.

Fig. 4. Effects of dPAG electrical stimulation on baroreflex cardiac responses in rats treated with saline, PCPA or PCPA + 5-HTP. Baroreflex cardiac responses (PECR, ACR, open bars) to PE administration (A; n = 6) or aortic depressor nerve stimulation (AS, B; n = 6) were significantly reduced by dPAG electrical stimulation (PE + dPAG and AS + dPAG, respectively, solid bars) in rats pretreated with ip saline (see MATERIALS AND METHODS), as in naive rats. Pretreatment with PCPA at the dose of 300 mg/kg ip daily for 3 days (n = 15 in A and B, respectively) almost completely suppressed the inhibitory effect of dPAG stimulation on both baroreceptor cardiac responses (PCPA + 5-HTP, n = 6 in A and B, respectively). *P < 0.05 compared with PE or AS alone (paired Student’s t-test).
respective slopes allowed quantitative estimates of the inhibitory effects of dPAG stimulation on sensitivity of the cardiac baroreflex response. The data reported herein clearly demonstrated that electrical stimulation of the dPAG triggered a marked reduction in both PECR and the sensitivity of the cardiac baroreceptor reflex response. Because phenylephrine produces a general vasoconstriction, the evoked cardiac reflex response in fact results from both aortic and carotid sinus baroreceptor stimulation. Thus if dPAG stimulation had no effect on the cardiac response to carotid sinus receptor stimulation, the resulting attenuation of PECR would have been less than that of ACR. Indeed, our data showed that the magnitude of the inhibition of PECR and ACR by dPAG stimulation was actually similar, thereby indicating that the latter stimulation affected not only aortic but also carotid baroreceptor cardiac responses.

Previous studies (17) have suggested that dPAG stimulation could exert an inhibitory influence on the cardiac component of the baroreceptor reflex through the local activation of not only cell bodies but also fibers “en passage.” However, chemical stimulation by bicuculline excites cell bodies only, and our observations that electrical and chemical stimulation of the dPAG produced similar inhibition of PECR strongly support the idea that cell bodies in the dPAG but not fibers en passageme are implicated in the inhibition of the baroreflex during the defense reaction.
Data reported herein showed that 5-HT synthesis inhibition by PCPA almost totally prevented the inhibitory effects of dPAG stimulation on the cardiac response elicited by either phenylephrine administration or aortic nerve stimulation. Moreover, 5-HT implication in the inhibitory influence of dPAG stimulation is confirmed by the fact that 5-HTP administration, to restore 5-HT synthesis in PCPA-pretreated rats, allowed recovery of this negative control of both aortic and phenylephrine-induced cardiac reflex responses as the same level as that observed in naive rats. However, PCPA treatment did not affect the increase in BP induced by dPAG electrical stimulation, further confirming that 5-HT is not involved in this defense reaction-induced response (26).

Anatomic data support the idea that the NTS might be one of the structures where serotoninergic neurotransmission is implicated in at least some of the autonomic changes associated with behavioral reactions to stressful conditions. Thus numerous projections from the dPAG and the hypothalamus to different raphe nuclei have been described (7, 22, 31), and the latter nuclei are well known to be a major source of serotoninergic fibers terminating within the NTS (24, 28, 30). Our previous studies (26) suggested the participation of NTS 5-HT receptors in the electrical dPAG stimulation-induced inhibition of the cardiac component of the aortic baroreceptor reflex. The data reported herein actually confirmed and extended them. Indeed, we showed here that intra-NTS administration of granisetron, a selective 5-HT receptor antagonist (8), prevented the inhibition of PECR normally observed during either electrical or chemical stimulation of the dPAG. Accordingly, it can be inferred that NTS 5-HT receptors are critically involved in the dPAG-mediated inhibition of the reflex bradycardia normally evoked by general baroreceptor activation at the periphery. Furthermore, the fact that 1) granisetron and PCPA prevented to the same extent the inhibitory effect of dPAG stimulation on the baroreflex bradycardia, and 2) there was no additional effect of granisetron in PCPA-pretreated rats strongly support the idea that the action of endogenous 5-HT in such an inhibitory control is mediated through the stimulation of NTS 5-HT receptors.

The data reported herein also confirmed and extended those of our previous studies (15, 26), which suggested that inhibition of the baroreceptor reflex bradycardia by NTS 5-HT receptor stimulation involves a local GABAergic system. Indeed, we found that the inhibitory effect of dPAG electrical or chemical stimulation on PECR could be blocked not only by intra-NTS microinjections of a 5-HT receptor antagonist, granisetron, but also by local microinjections of a GABA receptor antagonist, bicuculline. Taking into account these and previous data, it can be hypothesized that the inhibition by dPAG stimulation of the cardiovascular component of the baroreceptor reflex implies the following sequence: 1) excitation (by electrical or chemical stimulation) of dPAG cell bodies triggers the release of endogenous 5-HT within the NTS and consequently the activation of local presynaptic 5-HT receptors (21, 23); 2) stimulation of these receptors on vagal afferent fibers promotes the local release of glutamate (1), which, in turn, activates GABAergic interneurons; and 3) the resulting stimulation of GABA receptors finally exerts an inhibitory influence on NTS neurons at the origin of the aortic and carotid cardiac responses of the baroreceptor reflex.

Histological controls performed in the present and previous (26) studies revealed that methylene blue microinjected into the NTS did not reach the area postrema but spread to the dorsal vagal nucleus (data not shown). Although the latter nucleus contains 5-HT receptors as well as preganglionic vagal motoneurons, its contribution to the defense reaction-induced inhibition of the baroreflex bradycardia is unlikely because the vagal motoneurons, which participate in the reflex control of HR, are almost exclusively located in the nucleus ambiguus in rats (14, 27).

In conclusion, the data reported herein show that serotoninergic neurotransmission plays a key role in the reduction of the aortic and carotid baroreceptor cardiac reflex response produced by electrical and chemical dPAG stimulation in the rat. Moreover, these data confirm and complete previous results (26), which already suggested that the inhibitory influence of endogenous 5-HT triggered by dPAG stimulation is actually mediated through the stimulation of 5-HT receptors in the NTS, and that local GABA receptors contribute downstream to this effect.

Altogether, these data contribute to the elucidation of mechanisms underlying inhibition of the cardiac component of the baroreceptor reflex during stressful conditions. Such mechanisms are especially important because the resulting cardiovascular adaptations allow performance of appropriate behavioral reactions in stressed animals (17). Physiopathological relevance can also be emphasized because the defense reaction in the rat is known to share the same central pathways as panic disorders in humans (5).

ACKNOWLEDGMENTS

The authors are grateful to Glaxo SmithKline Laboratories (Harlow, UK) for the generous gift of granisetron.

GRANTS

This research was supported by grants from Institut National de la Santé et de la Recherche Médicale and the Bristol-Myers-Squibb Foundation (Unrestricted Biomedical Research Grant Program).

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