Activation of GABA$_A$ but not GABA$_B$ receptors in the NTS-blocked bradycardia of chemoreflex in awake rats

JOÃO CARLOS CALLERA,$^1$ LEINI G. H. BONAGAMBA,$^1$
ANNE NOSJEAN,$^2$ RAUL LAGUZZI,$^2$ AND BENEDITO H. MACHADO$^1$

$^1$Department of Physiology, School of Medicine of Ribeirão Preto,
University of São Paulo, 14049-900 Ribeirão Preto, São Paulo, Brazil;
and $^2$Institut National de la Santé et de la Recherche Médicale Unité 288,
Centre Hospitalier Universitaire Pitié-Salpêtrière, 75634 Paris Cedex 13, France

Callera, João Carlos, Leni G. H. Bonagamba, Anne Nosjean, Raúl Laguzzi, and Benedito H. Machado. Activation of GABA$_A$ but not GABA$_B$ receptors in the NTS blocked bradycardia of chemoreflex in awake rats. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1902–H1910, 1999.—In the present study we analyzed effects of bilateral microinjections of muscimol (a GABA$_A$ agonist) and baclofen (a GABA$_B$ agonist) into the nucleus tractus solitarius (NTS) on bradycardic and pressor responses to chemoreflex activation (potassium cyanide, 40 µg/rat iv) in awake rats. Bilateral microinjections of muscimol (25 and 50 pmol/50 nl) into the NTS increased baseline mean arterial pressure (MAP): 119 ± 8 vs. 107 ± 2 mmHg (n = 6) and 121 ± 8 vs. 103 ± 3 mmHg (n = 6), respectively. Muscimol at 25 pmol/50 nl reduced the bradycardic response to chemoreflex activation 5 min after microinjection; with 50 pmol/50 nl the bradycardic response to chemoreflex activation was reduced 5, 15, 30, and 60 min after microinjection. Neither muscimol dose produced an effect on the pressor response of the chemoreflex. Effects of muscimol (50 pmol/50 nl) on basal MAP and on the bradycardic response of the chemoreflex were prevented by prior microinjection of bicuculline (a GABA$_A$ antagonist, 40 pmol/50 nl) into the NTS. Bilateral microinjections of baclofen (12.5 and 25 pmol/50 nl) into the NTS produced an increase in baseline MAP [137 ± 9 vs. 108 ± 4 (n = 7) and 145 ± 5 vs. 105 ± 2 mmHg (n = 7), respectively], no changes in basal heart rate, and no effects on the pressor response; 25 pmol/50 nl only attenuated the pressor response to chemoreflex activation. The data show that activation of GABA$_A$ receptors in the NTS produces a significant reduction in the bradycardic response, whereas activation of GABA$_B$ receptors produces a significant reduction in the pressor response of the chemoreflex. We conclude that 1) GABA$_A$ but not GABA$_B$ plays an inhibitory role in neurons of the lateral commissural NTS involved in the parasympathetic component of the chemoreflex and 2) attenuation of the pressor response of the chemoreflex by activation of GABA$_B$ receptors may be due to inhibition of sympathoexcitatory neurons in the NTS or may be secondary to the large increase in baseline MAP produced by baclofen.

heart rate; autonomic nervous system; cardiovascular regulation; peripheral chemoreceptors; GABAergic receptors; muscimol; baclofen; bicuculline

The Nucleus Tractus solitarius (NTS) is the site of termination of the cardiovascular afferent fibers from the arterial baro- and chemoreceptors as well as from the cardiopulmonary receptors (28, 33). Therefore, the NTS plays a critical role in the processing of the autonomic adjustments to the heart and vessels in response to the activation of these cardiovascular reflexes. Several studies have demonstrated that the excitatory amino acid l-glutamate is the putative neurotransmitter of the cardiovascular afferents in the NTS (23, 37, 41). However, the NTS contains other neurotransmitters that also seem to be involved in the processing of the baro- and chemoreflex afferent messages (20, 42). In particular, the NTS presents a high density of GABA-containing nerve terminals (24, 31) and GABA$_A$ and GABA$_B$ receptors (3, 15). Moreover, local microinjections of GABA and of GABA$_A$- or GABA$_B$-receptor agonists in anesthetized rats elicit an increase in mean arterial pressure (MAP), which seems to be the consequence of the blockade of the sympathoinhibitory component of the baroreflex (7, 12, 30, 38, 40). Microinjection of muscimol, a GABA$_A$ agonist, into the NTS reduced the cardiovagal component of the baroreflex (27) as well as the hypotensive and bradycardic responses to electrical stimulation of the aortic depressor nerve (19). Microinjection of baclofen, a GABA$_B$ agonist, into the NTS also increased the baseline MAP and attenuated the baroreceptor reflex (12, 30, 31). Therefore, several lines of evidence indicate that GABA$_A$ and GABA$_B$ receptors in the NTS are involved in the inhibition of the baroreflex (12, 20, 32, 36).

In relation to the cardiac component, the responses to baro- and chemoreflex activation in unanesthetized rats are characterized by a bradycardic response (10, 13, 14, 17, 22, 23). Moreover, pharmacological studies suggest that the parasympathetic component of these two reflexes shares similar NTS neurochemical mechanisms, because in both cases the reflex bradycardia was blocked by N-methyl-D-aspartate (NMDA)-receptor antagonists (17, 22, 23). In addition, previous studies from our laboratories have documented that bilateral microinjection of serotonin (5-HT$_3$) agonists into the NTS of conscious or anesthetized rats inhibited the cardiovagal component of the baro- and chemoreflex, an effect that was blocked by previous microinjection of bicuculline into the NTS, suggesting that the inhibitory effect of 5-HT$_3$ agonists on the cardiovagal component of the baro- and chemoreflex was mediated by GABA$_A$ receptors (5, 6, 33, 34). However, the effect of the activation of GABA$_A$ and GABA$_B$ receptors in the NTS on the autonomic responses to the chemoreflex activation has not been evaluated. For this reason, in the...
present study we investigated the effects of microinjections of muscimol or baclofen into the lateral aspect of the commissural NTS on the bradycardic (parasympathetic component) and pressor (sympathoexcitatory component) responses to chemoreflex activation in unanesthetized rats.

METHODS

Male Wistar rats weighing 280–300 g were used in the present study. Four days before the experiments, rats under 2% tribromoethanol (1 ml/100 g ip; Aldrich Chemical) anesthesia were placed in a stereotaxic apparatus (David Kopf, Tujunga, CA), and the technique described by Michelini and Bonagamba (25) was used to implant bilateral guide cannulas in the direction of the NTS, in accordance with the coordinates of Paxinos and Watson (29). Additional tribromoethanol was injected when the rat reacted to a frequent toe pinch during the stereotaxic surgery. To implant the cannulas, a small window was opened caudal to the lambda, through which a 15-mm-long stainless steel cannula (22 gauge) was introduced perpendicularly 14 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.9 mm below the skull surface of the bregma. The tip of each guide cannula was placed in the cerebellum 1.0 mm above the dorsal surface of the brain stem. The guide cannula was fixed to the skull with methacrylate and watch screws and closed with an occluder until the day of the experiments. The needle (33 gauge) used for microinjection into the NTS was 1.5 mm longer than the guide cannula and was connected by PE-10 tubing to a 1-µl syringe (Hamilton, Reno, NV).

The needles for microinjection of drugs into the NTS were carefully inserted into the guide cannula, and manual injection was initiated 30 s later. For bilateral microinjection, the first microinjection was initially performed in one side, the needle was withdrawn and repositioned on the contralateral side, and then the second microinjection was made; microinjections were therefore made ~1 min apart. At the end of each experiment, 50 nl of 2% Evans blue were microinjected into the same sites for histological analysis. The animals were then submitted to intracardiac perfusion with saline (0.9%) followed by 10% buffered Formalin under ether anesthesia. The brains were removed and stored in buffered Formalin for 2 days, and serial coronal sections (10 µm) were cut and stained by the Nissl method. Only the rats in which the site of microinjection was located bilaterally in the lateral portion of the commissural NTS (0.5 mm lateral to the midline) were used for data analysis.

One day before the experiments, under tribromoethanol anesthesia, a catheter (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta through the femoral artery for measurement of pulsatil arterial pressure, MAP, and heart rate (HR). A second catheter was inserted into the femoral vein for drug administration. Both catheters were tunneled and exteriorized through the back of the neck to be connected to the pressure transducer under conscious freely moving conditions. Pulsatile arterial pressure and MAP were measured with a pressure transducer (model CDX III, Cobe Laboratories, Lakewood, CO) connected to a polygraph (Narco trace 80, Narco Bio-Systems, Austin, TX). HR was quantified with a Narco Biotachometer Coupler (model 7302) and recorded with the same polygraph.

The chemoreflex was activated by injection of potassium cyanide (KCN, 40 µg/rat iv) in accordance with previous studies by Franchini and Krieger (13, 14) and Haibara et al. (17). The cardiovascular responses to KCN injection are essentially due to the activation of the arterial chemoreceptors, because in previous studies it was demonstrated that bilateral ligation of the carotid body arteries abolished the cardiovascular and respiratory changes produced by KCN injection (14, 17). After a control recording of the cardiovascular parameters for ~20 min, a functional identification of the lateral commissural NTS was performed with microinjections of L-glutamate (1 nmol/50 nl), because the microinjection of this excitatory amino acid into the NTS of unanesthetized rats produces pressor and bradycardic responses (10, 11, 21). The experiments were performed using the following drugs: muscimol HBr, (+)-baclofen, and (+)-bicuculline (Research Biochemical International, Natick, MA) and KCN (E. Merck, Darmstadt, Germany). All microinjections into the lateral commissural NTS were performed in a volume of 50 nl. The solutions were freshly dissolved in 0.9% saline, and sodium bicarbonate was added to adjust the pH to 7.0. At 15 min after functional identification of the NTS by microinjection of L-glutamate, saline (50 nl) or the different doses of muscimol or baclofen were bilaterally microinjected into the NTS, and the changes in MAP and HR were monitored for the next 60 min. In the series of experiments related to the chemoreflex, we used muscimol at 25 and 50 pmol/50 nl or baclofen at 12.5 and 25 pmol/50 nl, because in preliminary experiments we observed that doses higher than these produced significant changes in the baseline ventilatory pattern, including apnea (data not shown), and for this reason the maximal doses of muscimol (50 pmol/50 nl) and baclofen (25 pmol/50 nl) were those producing an increase in MAP but no effect on the baseline ventilatory pattern.

Five groups of rats were used to evaluate the effects of bilateral microinjection of saline (50 nl, n = 6), muscimol (25 pmol/50 nl (n = 6) and 50 pmol/50 nl (n = 6)), and baclofen (12.5 pmol/50 nl (n = 7) and 25 pmol/50 nl (n = 7)) into the lateral commissural NTS on the bradycardic and pressor responses to chemoreflex activation. The carotid chemoreceptors were activated by administration of KCN (40 µg·0.1 ml−1·rat−1 iv) (13, 14, 17) before and 5, 15, 30, and 60 min after the bilateral microinjections of the vehicle or GABAergic agonists. A sixth group of rats received bilateral microinjections of bicuculline (40 pmol/50 nl, n = 6) into the NTS 2 min before the local bilateral microinjection of muscimol (50 pmol/50 nl), and the chemoreflex was tested before and 5, 15, 30, and 60 min after microinjection of muscimol into the NTS.

Values are means ± SE, and results were analyzed using the paired Student’s t-test. The data related to the effects of muscimol or baclofen on the chemoreflex responses were evaluated by one-way ANOVA followed by Tukey’s post test. Differences were considered significant at P < 0.05.

RESULTS

Cardiovascular responses to microinjections of muscimol or baclofen into the NTS of unanesthetized rats. Figure 1 presents typical tracings of rats representative of their respective groups that received bilateral microinjection of muscimol (50 pmol/50 nl) or baclofen (25 pmol/50 nl). Bilateral microinjection of both doses of muscimol (25 and 50 pmol/50 nl) into the NTS produced a significant increase in MAP and no changes in the baseline HR (Table 1, Figs. 1 and 2). The pressor response to muscimol lasted 15 min for 25 pmol/50 nl (Fig. 2) and 15 min for 50 pmol/50 nl (Figs. 1 and 2). Bilateral microinjection of both doses of baclofen (12.5 and 25 pmol/50 nl) into the NTS also produced a
significant increase in MAP and no changes in the baseline HR (Table 1, Figs. 1 and 2). The magnitude and the duration of the pressor response to both doses of baclofen (Fig. 2B, top) were greater than the changes produced by both doses of muscimol (Fig. 2A, top). Microinjection of the vehicle (0.9% saline) into the NTS produced no changes in baseline MAP or HR (Table 1, Fig. 2).

Effect of microinjection of muscimol into the NTS on the chemoreceptor reflex responses. Bilateral microinjections of the vehicle (saline 0.9%) into the NTS produced no changes in the pressor or bradycardic responses to chemoreflex activation (Table 2, see Figs. 4 and 5), whereas microinjection of 25 pmol/50 nl of muscimol produced a significant reduction (~50%) in the bradycardic response to the chemoreflex activation only at 5 min after microinjection. A greater reduction in the bradycardic response to the chemoreflex activation was observed 5 min after microinjection of muscimol (50 pmol/50 nl), and the response continued to be significantly reduced for ~60 min after microinjection (Figs. 3A and 4, bottom). Neither dose of muscimol produced any effect on the pressor response of the chemoreflex (Fig. 4, top). Table 2 summarizes the changes in MAP and HR in response to chemoreflex activation before and after bilateral microinjections of muscimol (50 pmol/50 nl, n = 6) into the NTS.

Microinjection of bicuculline into the NTS blocked the effect of muscimol. Bilateral microinjections of bicuculline (40 pmol/50 nl) into the NTS produced no effect on the basal MAP or HR (data not shown). However, 2 min after pretreatment with bicuculline the bilateral microinjection of muscimol (50 pmol/50 nl, n = 6) into the NTS produced no significant increase in basal MAP (104 ± 4 and 110 ± 12 mmHg) and suppressed the inhibitory effect of muscimol on the bradycardic component of the chemoreflex observed at different time intervals in untreated rats (Table 2).

Effect of microinjection of baclofen into the NTS on the chemoreflex responses. Microinjection of 12.5 (n = 7) or 25 pmol/50 nl (n = 7) of baclofen into the NTS produced no changes in the bradycardic responses to chemoreflex activation (Figs. 3B and 5, bottom). Baclofen at 12.5 pmol/50 nl produced a significant reduc-

**Table 1.** Baseline MAP and HR before and 5 min after bilateral microinjection of vehicle, muscimol, and baclofen into commissural NTS of awake rats

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>5 min</td>
</tr>
<tr>
<td>0.9% Saline (50 nl)</td>
<td>6</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>Muscimol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 pmol/50 nl</td>
<td>6</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>50 pmol/50 nl</td>
<td>6</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Baclofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5 pmol/50 nl</td>
<td>7</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>25 pmol/50 nl</td>
<td>7</td>
<td>105 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats in each group. MAP, mean arterial pressure; HR, heart rate; NTS, nucleus tractus solitarius. *P < 0.05 compared with basal.
tion of the pressor response of the chemoreflex only at 15 min after the microinjections (Fig. 5, top), whereas 25 pmol/50 nl produced significant reduction of the pressor response at 5, 15, and 30 min after microinjection (Fig. 5, top). It is important to note that the activation of the chemoreflex after 12.5 pmol/50 nl of baclofen and particularly after 25 pmol/50 nl of baclofen occurred when baseline MAP was significantly increased (Figs. 2 and 3), which may explain why the pressor response was attenuated.

Histology. Only the rats with histologically confirmed bilateral microinjections into the lateral commissural NTS were considered for the data analysis. Figure 6 is a photomicrograph of a 10-µm coronal section of the brain stem of one rat representative of the groups showing the tract of the injectors and the sites of microinjections into the NTS bilaterally.

DISCUSSION

Several studies have shown that the parasympathetic component of the baroreflex is inhibited by GABA_A and GABA_B agonists microinjected into the NTS (12, 24, 27, 28, 39), but no study has previously evaluated the effect of GABA agonists on the parasympathetic component of the chemoreflex in unanesthetized rats. The present study shows that bilateral microinjection of muscimol into the lateral commissural NTS, in contrast to baclofen, produced a blockade of the bradycardic response to chemoreflex activation, indicating that GABA_A but not GABA_B receptors are involved in the inhibitory neuromodulation of the parasympathetic component (cardiovagal) of the chemoreflex. The inhibitory effect of muscimol on the bradycardic response to chemoreflex activation was blocked by previous microinjection of bicuculline into the NTS, confirming that the effect of muscimol was due to selective activation of GABA_A receptors.

The pattern of the bradycardic response to chemoreflex activation differs from the bradycardic response of baroreflex activation, because the magnitude of the bradycardic response to chemoreflex activation with KCN is greater than the bradycardic response to barore-

Table 2. Changes in MAP and HR in response to chemoreflex activation (KCN) before and after bilateral microinjection of vehicle (saline) and muscimol and before and after sequential bilateral microinjection of bicuculline and muscimol into commissural NTS

<table>
<thead>
<tr>
<th></th>
<th>Control (KCN)</th>
<th>Time After Microinjection, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>0.9% Saline (50 nl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ MAP</td>
<td>−183 ± 13</td>
<td>−182 ± 26</td>
</tr>
<tr>
<td>Δ HR</td>
<td>−195 ± 23</td>
<td>−37 ± 11*</td>
</tr>
<tr>
<td>Muscimol (50 pmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ MAP</td>
<td>40 ± 4</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>Δ HR</td>
<td>−207 ± 10</td>
<td>−186 ± 42</td>
</tr>
<tr>
<td>Bicuculline (40 pmol) + muscimol (50 pmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ MAP</td>
<td>43 ± 3</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Δ HR</td>
<td>−207 ± 10</td>
<td>−186 ± 42</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats in each group. KCN, potassium cyanide (40 µg/0.1 ml/rat iv). *Significantly different from respective control (P < 0.05).

Fig. 2. A: MAP and HR before (control, C) and 5, 15, 30, and 60 min after bilateral microinjection of vehicle (0.9% saline, □, n = 6) or muscimol [25 pmol/50 nl (●) or 50 pmol/50 nl (○)], n = 6 each) into lateral commissural NTS. B: MAP and HR before and 5, 15, 30, and 60 min after bilateral microinjection of vehicle (0.9% saline, □, n = 6) or baclofen [12.5 pmol/50 nl (●, n = 6) or 25 pmol/50 nl (○, n = 7)] into lateral commissural NTS. * and ** Different from respective control (P < 0.05).
flex activation with phenylephrine injection in conscious rats (6). Despite the difference in magnitude, previous studies from our laboratories have documented that the blockade of NMDA receptors or the activation of 5-HT₃ receptors in the NTS inhibited the cardiovagal component of the chemoreflex (6, 17, 33) as well as the cardiovagal component of baroreflexes and Bezold-Jarisch reflexes (34). Taken together, these data suggest that the parasympathetic component of the cardiovascular reflexes share similar neurochemical mechanisms in the NTS. However, the fact that in the present study baclofen produced no effect on the bradycardic response to chemoreflex activation opens new and interesting perspectives indicating that the parasympathetic component of the baro- and chemoreflexes are modulated by different inhibitory mechanisms. Additional studies involving the effect of microinjection of GABAₐ and GABAₐ agonists into the NTS on the baroreflex in unanesthetized rats are required to confirm this hypothesis.

Previous electrophysiological studies by Ruggeri et al. (32) have shown that muscimol and baclofen inhibit NTS barosensitive neurons. However, the inhibitory effects of muscimol were always exerted on the whole neuronal discharge, independently of its correlation to the cardiac cycle, whereas baclofen significantly affected the peaks of neuronal discharge synchronous with the pulse. These data by Ruggeri et al. suggest that the inhibition of the neuronal firing by muscimol was mediated by hyperpolarizing actions through postsynaptic GABAₐ receptors located on barosensitive NTS neurons and also that the specific inhibition elicited by baclofen may have been due to the activation of presynaptic GABAₐ receptors located in vagal afferent fibers. Our data showing that muscimol but not baclofen affects the bradycardic response to chemoreflex activation suggest that GABAₐ but not GABAₐ receptors are postsynaptically located in neurons of the NTS involved in the cardiac parasympathetic component of the chemoreflex.

The effect of baclofen on the pressor response to chemoreflex activation suggests that the neurons of the NTS projecting to the rostral ventrolateral medulla, which are probably involved in the sympathoexcitatory component of the chemoreflex (1, 18), may contain GABAₐ receptors, because baclofen produced a reduction in the pressor responses to chemoreflex activation. However, the large increase in baseline MAP produced by baclofen, probably due to inhibition of NTS neurons involved in the sympathoinhibitory pathways of the baroreflex, makes this possibility unlikely. Microinjections of 12.5 pmol/50 nl of baclofen into the NTS produced a significant increase in baseline MAP for ≥ 30 min after microinjection, whereas with 25 pmol/50

Fig. 3. A: tracings of 1 rat representative of group showing changes in HR, PAP, and MAP in response to chemoreflex activation (potassium cyanide (KCN), 40 µg/rat iv) before and 5, 15, 30, and 60 min after bilateral microinjection of muscimol (50 pmol/50 nl) into NTS (arrows). B: tracings of 1 rat representative of group showing changes in HR, PAP, and MAP in response to chemoreflex activation (KCN, 40 µg/rat iv) before and 5, 15, 30, and 60 min after bilateral microinjection of baclofen (25 pmol/50 nl) into NTS (arrows).
the baseline MAP remained significantly elevated until 60 min after the microinjection. With 12.5 pmol/50 nl of baclofen, the MAP was 137–139 mmHg at 5 and 15 min after microinjection, whereas with 25 pmol/50 nl the MAP was between 145 and 142 mmHg at the same time points. Although microinjections of muscimol produced a significant increase in baseline MAP, these increases were 115–120 and 120–125 mmHg, respectively, for 25 and 50 pmol/50 nl. Therefore, it is possible that the significant reduction in the pressor response to chemoreflex activation 5 min after 12.5 pmol/50 nl of baclofen or 5, 15, and 30 min after 25 pmol/50 nl of baclofen was due to the large increase in baseline MAP. The increase in the baseline MAP was smaller with muscimol than with baclofen, and this fact may explain why the pressor response of the chemoreflex after muscimol was not significantly different. Therefore, the inhibition of NTS neurons involved in the sympathoinhibitory projections of the baroreflex by GABA agonists allows an increase in the sympathetic nerve discharge and a consequent increase in baseline MAP. Under these circumstances, especially after 25 pmol/50 nl of baclofen, the activation of the chemoreflex produces an additional increase in the sympathetic nerve discharge, which is smaller than the control, probably because the activity of the basal sympathetic nerve discharge is near the maximal increase compared with the control condition.

The data of the present study showing that the parasympathetic component of the chemoreflex was blocked only by the GABA_A agonist are in accordance with previous studies from our laboratories showing that the stimulation of presynaptic 5-HT_3 receptors with chlorophenylbiguanide or 2-methylserotonin in the NTS elicits, similar to stimulation of GABAergic receptors, an increase in MAP and inhibits the cardiovalvular component of the chemoreflex (6, 33). On the other hand, pretreatment with bicuculline blocked the inhibitory effect of the 5-HT_3 agonist on the parasympathetic component of the cardiovascular reflexes, indicating that GABA_A receptors play a key role in this inhibitory neuromodulation (33). In addition, pretreatment with bicuculline produced no effect on the increase in baseline MAP produced by 5-HT_3 agonists and no changes in the pressor response to chemoreflex activation (33, 34). These data indicate that GABA_A receptors play an important role in the inhibitory neuromodulation of the cardiovalvular component of the chemoreflex and also suggest that the sympathoexcitatory component of this reflex is not modulated by GABA_A receptors. Therefore, the data of the present study suggest that muscimol activated GABA_A receptors located only in the neurons of the NTS projecting to preganglionic parasympathetic neurons in the nucleus ambiguus and/or dorsal motor nucleus of the vagus, producing hyperpolarization and consequent blockade of the cardiovalvular component of the reflex.

An important aspect verified in the present study refers to the increase in baseline MAP produced by the microinjection of muscimol or baclofen into the NTS, a
finding consistent with previous studies performed on anesthetized animals (2, 7, 12, 27, 38, 40). The fact that several studies support the concept that L-glutamate is the putative neurotransmitter involved in the parasympathetic (cardiovagal) and the sympathoinhibitory components of the baroreflex in the NTS (22, 23, 41) suggests that muscimol may be acting on GABA<sub>A</sub> receptors located postsynaptically on neurons involved in the sympathoinhibitory projection of the baroreflex. In relation to the effect of baclofen on baseline MAP, we may also suggest that the activation of GABA<sub>B</sub> receptors presynaptically located in the terminals of the baroreceptor afferents in the NTS may inhibit the release of the excitatory amino acid L-glutamate, resulting in the removal of the tonic sympathoinhibitory activity of the baroreceptor afferents.

In the present study using unanesthetized rats, microinjections of muscimol or baclofen produced no increase in the basal HR, as also reported by Sved and Sved (38, 40), whereas Florentino et al. (12) found an increase in HR in response to bilateral microinjection of baclofen into the NTS. Under anesthesia the activation of these receptors inhibits the baroreceptor reflex arc (12, 24, 27, 30, 39), and thus the increases in MAP and HR produced by such procedures were interpreted as an expression of this inhibition. The data showing that in unanesthetized rats both agonists increase MAP, but not HR, raise questions about whether the cardiac baroreflex control in conscious rats is really affected by GABA receptor agonists. Therefore, further studies about the role of GABA receptors in the baroreflex of unanesthetized rats are required to explain why baseline HR was not affected by muscimol or baclofen.

The present data showing that activation of GABA<sub>A</sub> receptors affected the bradycardic but not the pressor response of the chemoreflex support the concept of an autonomic dissociation in the processing of the cardiovascular afferents at the NTS level. A series of studies from our laboratories showing that the stimulation of 5-HT<sub>3</sub> receptors or the blockade of NMDA receptors inhibits specifically the cardiovagal component of the chemoreflex constitute a body of evidence in favor of this dissociation (5, 6, 17, 33, 34). Similar evidence was obtained in other pharmacological studies in which we demonstrated a similar dissociation in the NTS neurochemical mechanisms controlling the cardiovagal and the sympathetic component of the baroreflex, chemoreflex, and Bezold-Jarisch reflex (8, 17, 22, 23). The findings of the present study are additional evidence in favor of the concept of dissociation between the processing of the two autonomic components of the chemoreflex in the NTS, i.e., the GABA<sub>A</sub> agonist blocked the bradycardia but produced no effect on the pressor response of the chemoreflex. However, the interpretation of the present data is restricted to the lateral commissural NTS, where our microinjections were performed, and further studies, including microinjections of muscimol and baclofen into the medial commissural NTS, are required, because previous studies have suggested that neurons involved in the signaling of the baro- and chemoreflex can be found at different subregions of the NTS (9, 26).

We conclude that the neurons of the NTS involved in the parasympathetic component of the chemoreflex in the lateral commissural NTS are inhibited by activation of GABA<sub>A</sub> but not by GABA<sub>B</sub>-receptors. We also suggest that the attenuation of the pressor response of the chemoreflex by a GABA<sub>B</sub>-receptor agonist may be the consequence of 1) the blockade of the sympathoexcitatory neurons in the NTS involved in the chemoreflex pathways or 2) the large increase in baseline MAP secondary to the blockade of sympathoinhibitory neurons of the NTS involved in the baroreflex pathways.

**Perspectives**

The data showing that the activation of GABA<sub>A</sub> receptors affects the bradycardic but not the pressor response of the chemoreflex are additional evidence in favor of a possible dissociation in the processing of the autonomic pathways of the cardiovascular reflexes at the NTS. Therefore, further studies on the role of GABA receptors in the neurotransmission/neuromodulation of the baroreflex and Bezold-Jarisch...
reflex in the NTS as well as on the neurotransmission of projection from other important areas involved in the cardiovascular adjustments, e.g., hypothalamic defense area, periaqueductal gray, parabrachial nucleus, and raphe nucleus, to the NTS are required to establish a better understanding of the inhibitory neuromodulation of the autonomic nervous system in the NTS. On the other hand, the evidence that the sympathoexcitatory component of the chemoreflex (pressor response) was affected by the activation of GABA$_B$ receptors, probably because of the large increase in the baseline MAP, suggests that this sympathoexcitatory pathway in the NTS, when excited, is not under GABA$_A$ergic inhibitory influence. This possibility raises a question about whether the continuous or frequent activation of this sympathoexcitatory system under any physiopathological circumstances contributes to the development of hypertension. Additional studies using experimental models of hypertension and exploring the role of GABA receptors in the neuromodulation of sympathoexcitatory projections in the NTS may bring new insights to the understanding of the neurogenic mechanisms of essential hypertension.

We thank Rubens F. de Melo for histological technical assistance. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo Grants 95/4685-8 and 95/9562-1, Conselho Nacional de Desenvolvimento Científico e Tecnológico Grant 522150/95-0, the Programa de Apoio aos Grupos de Excelência, Comité Français d’Evaluation de la Cooperation Universitaire avec le Brésil/Universidade de São Paulo Grant U/C 38/97, and the Institut Franc¸ais d’Evaluation de la Cooperation Universitaire avec le Bre ´sil/95-0, the Programa de Apoio aos Grupos de Excele ˆncia, Comite ´ Nacional de Desenvolvimento Cientı´fico e Tecnolo´gico Grant 522150/"}

REFERENCES


