

The bradycardic and hypotensive responses to serotonin are reduced by activation of GABA_A receptors in the nucleus tractus solitarius of awake rats

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Abstract

We investigated the effects of bilateral injections of the GABA receptor agonists muscimol (GABA_A) and baclofen (GABA_B) into the nucleus tractus solitarius (NTS) on the bradycardia and hypotension induced by *iv* serotonin injections (5-HT, 2 µg/rat) in awake male Holtzman rats. 5-HT was injected in rats with stainless steel cannulas implanted bilaterally in the NTS, before and 5, 15, and 60 min after bilateral injections of muscimol or baclofen into the NTS. The responses to 5-HT were tested before and after the injection of atropine methyl bromide. Muscimol (50 pmol/50 nl, N = 8) into the NTS increased basal mean arterial pressure (MAP) from 115 ± 4 to 144 ± 6 mmHg, did not change basal heart rate (HR) and reduced the bradycardia (-40 ± 14 and -73 ± 26 bpm at 5 and 15 min, respectively, vs -180 ± 20 bpm for the control) and hypotension (-11 ± 4 and -14 ± 4 mmHg, vs -40 ± 9 mmHg for the control) elicited by 5-HT. Baclofen (12.5 pmol/50 nl, N = 7) into the NTS also increased basal MAP, but did not change basal HR, bradycardia or hypotension in response to 5-HT injections. Atropine methyl bromide (1 mg/kg body weight) injected *iv* reduced the bradycardic and hypotensive responses to 5-HT injections. The stimulation of GABA_A receptors in the NTS of awake rats elicits a significant increase in basal MAP and decreases the cardiac Bezold-Jarisch reflex responses to *iv* 5-HT injections.

Key words

- Muscimol
- GABA receptors
- Nucleus tractus solitarius
- Parasympathetic component
- Bezold-Jarisch reflex
- Awake rats

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Research supported by FAPESP
(No. 01/06246-4).

Part of these data was reported in
abstract form (see Ref. 39).

Received June 24, 2004
Accepted March 9, 2005

Introduction

The activation of cardiopulmonary vagal afferent C-fibers originating in the heart and lungs by intravenous (*iv*) or intra-atrial injections of different substances such as serotonin (5-hydroxytryptamine, 5-HT), phenyl-

biguanide or veratrum alkaloids increases parasympathetic efferent activity and inhibits sympathetic efferent activity, resulting in hypotension and bradycardia. This phenomenon was first described by von Bezold and Hirt in 1867 and characterized further by Jarisch and Richter in 1939 and is known as

the Bezold-Jarisch reflex (1,2).

The nucleus tractus solitarius (NTS) receives primary afferent input from a wide variety of visceral receptors important for the control of autonomic nervous system functions (3,4). The vagal afferent fibers originating in the heart and lungs terminate in two distinct areas localized in the dorso-medial NTS (5) and lateral to the NTS (6) where their first synapse is located (3,7).

Studies have shown that the neurotransmission of cardiovascular reflexes in the NTS is influenced by different neurotransmitters or neuromodulators, among them 5-HT (8-11) and γ -aminobutyric acid (GABA) (12-18). In previous studies we observed that bilateral injections of serotonergic 5-HT₃ agonists into the lateral NTS of anesthetized (9) or awake rats (11) inhibited the parasympathetic component (bradycardia) of the Bezold-Jarisch reflex induced by *iv* 5-HT injections. This inhibition was reversed by injection of bicuculline, a GABA_A receptor antagonist (19), into the NTS, suggesting that the inhibitory effect of serotonergic 5-HT₃ agonists on the parasympathetic component of the Bezold-Jarisch reflex is mediated by GABA receptor activation (9,20). However, the direct effect of GABA receptor agonists injected into the lateral NTS on the bradycardic and hypotensive responses to *iv* injection of 5-HT is not known.

The effects of GABA can be mediated by two distinct receptors, i.e., the GABA_A and the GABA_B receptors (13,21). It is known that GABA receptors in the NTS modulate the arterial baroreflex (12,15,18,22,23). Injections of GABA_A or GABA_B receptor agonists into the NTS of anesthetized rats increase mean arterial pressure (MAP), an effect that seems to be a consequence of the blockade of the sympatho-inhibitory component of the baroreflex (14,15,22). Besides the increase in MAP, injections of GABA_A (muscimol) and GABA_B (baclofen) receptor agonists into the NTS reduce baroreflex bradycardia in awake rats (18). Injections of

muscimol, but not of baclofen, into the NTS also inhibit the bradycardia of the chemoreflex (17). This effect is blocked by previous injection of bicuculline, indicating that the inhibitory effect of muscimol on the parasympathetic component of the chemoreflex is effectively mediated by GABA_A receptors.

Although previous studies have shown the role of GABA receptors on baro- and chemoreflex neurotransmission in the NTS of anesthetized and awake rats, the effects of GABA receptor activation in the NTS on the autonomic components of the Bezold-Jarisch reflex (*iv* 5-HT injections) in awake rats have not been studied before. We chose this model because it provides an effective way to assess some of the cardiovascular effects of neurotransmitters in the NTS without the inconvenience of blunting pathways with anesthetic agents. Therefore, in the present study we investigated the effects of bilateral injections of muscimol or baclofen into the NTS on the bradycardic and hypotensive responses elicited by injections of 5-HT in awake rats. In order to verify whether the hypotension was dependent on the bradycardia, the Bezold-Jarisch reflex was also tested before and after *iv* injection of atropine methyl bromide.

Material and Methods

Brain surgery

All experiments were performed on adult male Holtzman rats weighing 290-320 g. Four days before the experiments, rats under 2% tribromoethanol anesthesia were placed in a stereotaxic apparatus (Stoelting Instruments, Wood Dale, IL, USA) and implanted with bilateral guide cannulas in the direction of the lateral aspect of the NTS according to the coordinates of the Paxinos and Watson atlas (24). Through a hole in the skull bone (5 mm in diameter), 15-mm long stainless steel cannulas (23 gauge) were introduced

perpendicularly using the following coordinates: 14 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.9 mm below the skull surface. The tip of each guide cannula was placed in the cerebellum 1.0 mm above the dorsal surface of the brain stem. The guide cannulas were fixed to the skull with methacrylate and watch screws and fitted with a 30-gauge metal obturator until the day of the experiments.

Measurement of arterial pressure and heart rate

One day before the experiments, under 2% tribromoethanol anesthesia (1 ml/100 g, *ip*; Aldrich Chemical Company, Inc., Milwaukee, WI, USA), a catheter (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery to measure pulsatile 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. The next day, the arterial catheter was connected to a Statham Gould (Valley View, OH, USA) pressure transducer (P23 Db) coupled to a polygraph (Narcotrace 80, Narco Bio-Systems, Austin, TX, USA) to record pulsatile arterial pressure, MAP and HR under conscious freely moving conditions.

Effect of GABA agonists on the cardiovascular responses to *iv* 5-HT

Three groups of rats were used to test the effects of bilateral injections of saline (50 nl, N = 5), muscimol (50 pmol/50 nl, N = 8) and baclofen (12.5 pmol/50 nl, N = 7) into the NTS on the peak bradycardic and hypotensive responses to *iv* 5-HT injections. After a control recording of MAP and HR for 15 min, a functional identification of the injection site was performed with injections of L-glutamate (2 nmol/50 nl), that produces pres-

or and bradycardic responses when injected into the NTS (7,25,26). The control response was tested by *iv* injections of 5-HT (2 µg/rat) 15 min after functional identification of the NTS. The injections of 5-HT were repeated 5, 15 and 60 min after bilateral injections of saline (volume control), muscimol or baclofen into the NTS, in accordance with previous studies (17,18) carried out to evaluate the effects of GABA agonists on the baro- and chemoreflex at the same times as used in the present study.

We used doses of muscimol and baclofen effective in the activation of both subtypes of GABA receptors but causing no important visual changes in ventilation. In preliminary experiments (17) we observed that the doses of 25 and 50 pmol/50 nl muscimol produced a similar increase in baseline MAP when compared with their respective control, and also that neither dose produced visual changes in the ventilatory pattern. Muscimol (50 pmol/50 nl) reduced bradycardia of the baro- and chemoreflex (17,18). For these reasons we used the dose of 50 pmol/50 nl muscimol to evaluate the effect of a GABA_A agonist on the autonomic components of the Bezold-Jarisch reflex. With respect to baclofen, we observed that the dose of 12.5 pmol/50 nl produced a larger increase in baseline MAP than the dose of 6.25 pmol/50 nl compared to their respective baseline control and reduced the gain of baroreflex bradycardia (18) but not of chemoreflex bradycardia (17). The visual observation of the chest movements during the injection of baclofen doses higher than 12.5 pmol/50 nl into the NTS showed that baclofen produced significant changes in ventilation. For this reason we used the dose of 12.5 pmol/50 nl baclofen to evaluate the effect of the GABA_B agonist in response to *iv* 5-HT injections.

In the present study, we also evaluated the cardiovascular changes produced by 5-HT in awake rats before (control) and 10 min after cholinergic blockade with *iv* injections

of atropine methyl bromide (1 mg/kg body weight).

Histology

At the end of the experiments, 50 nl 2% Evans blue was injected into the same sites for histological analysis. The animals were then submitted to intracardiac perfusion with saline followed by 10% formalin under pentobarbital sodium (80 mg/kg, *ip*) anesthesia. The brains were removed, fixed in 10% formalin, frozen, cut into 50- μ m serial coronal sections, stained with cresyl violet, and analyzed by light microscopy. Only the rats in which the sites of injections were located bilaterally in the NTS (0.5 mm lateral to the midline) were used for data analysis.

Statistical analysis

Data are reported as means \pm SEM. One-way ANOVA followed by the Tukey test was used to assess the statistical significance of the effects of bilateral injections of GABA agonists into the NTS on the cardiovascular effects of *iv* 5-HT injections. None of the rats in which the injections of GABA agonists were located outside the NTS showed important changes in basal MAP and HR or in the bradycardic response to *iv* 5-HT injections and these animals were excluded from analysis. The peak bradycardic and hypotensive responses to 5-HT prior to and after *iv* atropine methyl bromide were compared by a paired *t*-test. Differences were considered to be significant at $P < 0.05$.

Chemicals

Muscimol hydrobromide, (\pm)-baclofen, and 5-HT were from Research Biochemical International (Natick, MA, USA) and atropine methyl bromide was from Sigma Chemical Co. (St. Louis, MO, USA). All injections into the NTS were performed in a volume of 50 nl. The solutions were freshly

dissolved in 0.9% saline. The pH of all solutions injected into the NTS was adjusted to a value between 7.2 and 7.4.

Results

Histology

Only results for rats with bilateral injections into the NTS were considered for analysis. Figure 1 shows the sites of bilateral injections into the NTS.

Effects of GABA agonists into the NTS on the cardiovascular responses to *iv* 5-HT

MAP increased 5 and 15 min after bilateral injections of muscimol or baclofen into the NTS, with no change in baseline HR (Figure 2). The bradycardic and hypotensive responses to 5-HT injections (2 μ g/rat, *iv*) were significantly reduced 5 and 15 min after bilateral injections of muscimol, but not of baclofen, into the NTS (Figure 3). The responses to 5-HT returned to control values 60 min after muscimol into the NTS.

Effect of atropine methyl bromide on the cardiovascular changes induced by *iv* 5-HT

Figure 4 shows that 5-HT injections in awake rats produced bradycardia and hypotensive responses. The cholinergic blockade with *iv* atropine methyl bromide almost abolished the bradycardic and hypotensive responses to 5-HT injections.

Discussion

The present results show that the activation of GABA_A receptors with bilateral injections of muscimol into the NTS, differently from the activation of GABA_B receptors with baclofen, reduced the bradycardic and hypotensive responses to the Bezold-Jarisch reflex (*iv* 5-HT injections). Although previous studies have shown that bradycar-

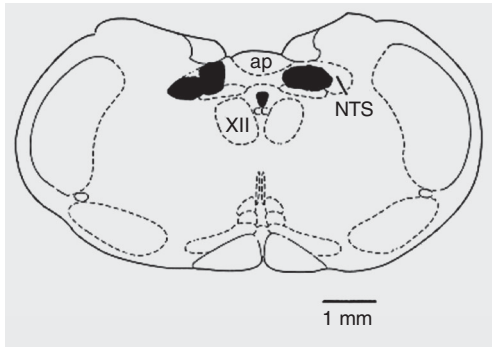


Figure 1. Schematic representation of a coronal section of a rat brain stem showing the sites of bilateral injections in the nucleus tractus solitarius (NTS). ap = area postrema; XII = hypoglossal nucleus; cc = central canal.

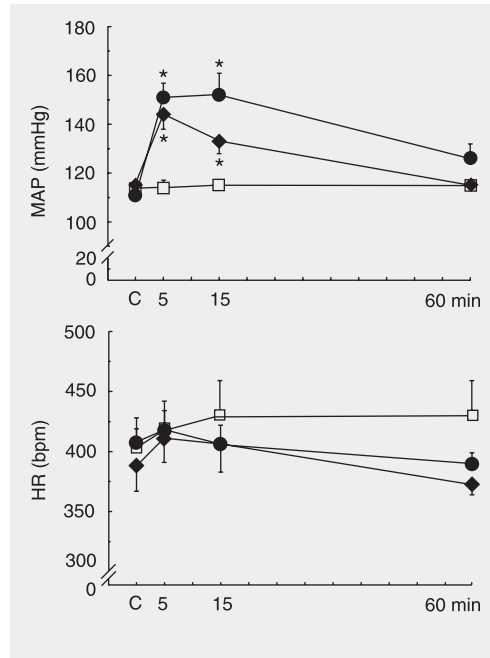


Figure 2. Mean arterial pressure (MAP, top) and heart rate (HR, bottom) before (control, C) and 5, 15, and 60 min after bilateral injections of vehicle (0.9% saline, N = 5; squares), muscimol (50 pmol/50 nl, N = 8; lozenges) or baclofen (12.5 pmol/50 nl, N = 7; circles) into the NTS. *P < 0.05 compared to the respective control (ANOVA followed by the Tukey test).

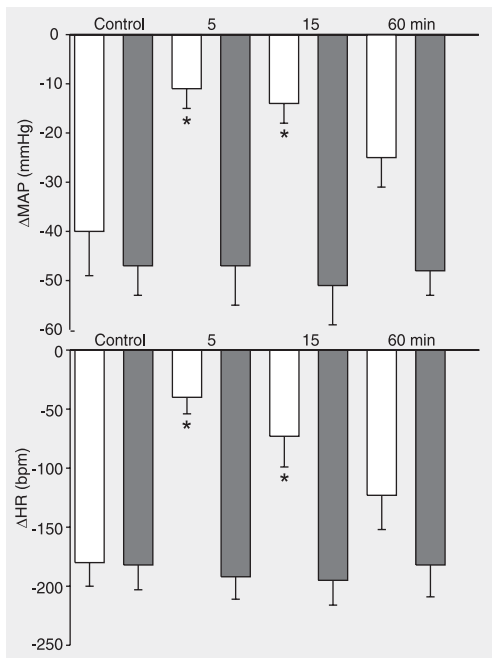


Figure 3. Changes in mean arterial pressure (Δ MAP, top) and heart rate (Δ HR, bottom) in response to serotonin injection (5-HT, 2 μ g/rat, *iv*) before (control response) and 5, 15, and 60 min after bilateral injections of muscimol (50 pmol/50 nl, N = 8; open bars) or baclofen (12.5 pmol/50 nl, N = 7; filled bars) into the NTS. *P < 0.05 compared to the respective control (ANOVA followed by the Tukey test).

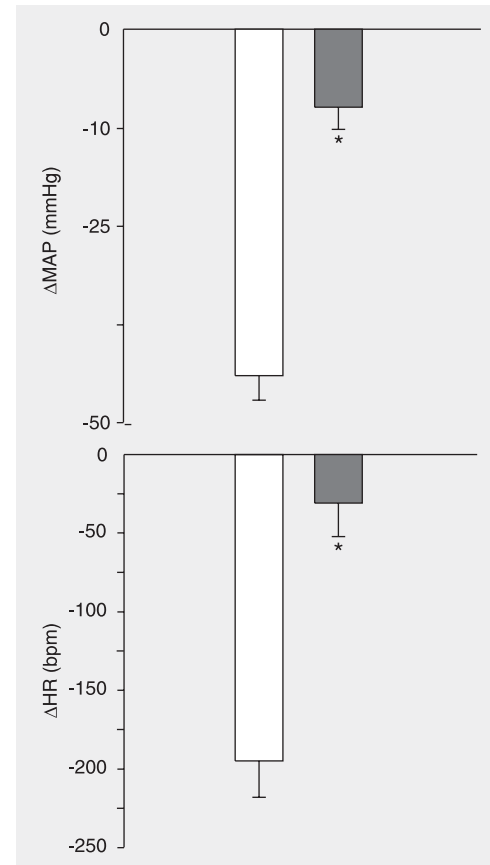


Figure 4. Changes in mean arterial pressure (Δ MAP, top) and heart rate (Δ HR, bottom) produced by *iv* injections of 5-HT (2 μ g/rat) before (control response, open bars) and 10 min after cholinergic blockade with *iv* injections of atropine methyl bromide (1 mg/kg, N = 8; filled bars). Data are reported as means \pm SEM. *P < 0.05 compared to the respective control (paired *t*-test).

dia of the baro- and chemoreflex is inhibited by muscimol injected into the NTS (12,15,17,18,23,27,28), the present study is the first to show effects of GABA_A receptor activation in the NTS on the bradycardia in response to 5-HT injections in awake rats. Injections of muscimol and baclofen into the NTS also increased MAP, as previously shown (14,15,18,22). According to previous studies (17), prior treatment with local injections of bicuculline blocks the effects of muscimol into the NTS at the same doses as used in the present study. Taken together, the present and previous results suggest that activation of GABA_A receptors in the NTS, independently of the changes in baseline MAP or HR, is involved in the inhibitory neuromodulation of the cardiac parasympathetic component of different cardiovascular reflexes tested (baroreflex, chemoreflex and Bezold-Jarisch reflex). We have shown (18) that MAP increased by injections of muscimol and baclofen into the NTS was back to baseline after infusion of sodium nitroprusside and in these conditions we observed that muscimol, but not baclofen, into the NTS reduced the gain of the baroreflex. It is necessary to consider that the conclusions of the present study are limited to the effects of the lateral NTS, the site of injections of muscimol and baclofen. Further studies, including injections of these drugs into the medial NTS, are required because previous studies have suggested the existence of neurons involved in the signaling of the Bezold-Jarisch reflex in different subregions of the NTS (3,5,6,29).

In view of the suggested localization of GABA_A and GABA_B receptors in neurons of the NTS (16,30,31) and the results of different studies that have investigated the effects of GABA_A and GABA_B receptor activation in the NTS on different cardiovascular reflexes (17,18, and the present study), it is possible to suggest that GABA_B receptors are present in the pre- and postsynaptic neurons of arterial baroreceptors. This is based

on the fact that GABA_B receptors in the NTS modulate the bradycardia occurring in response to baroreflex activation (18) but not the bradycardia occurring in response to chemoreflex (17) or Bezold-Jarisch reflex activation (present results), while GABA_A receptors in the NTS are localized in the postsynaptic neurons that activate the parasympathetic component of all cardiovascular reflexes tested thus far.

It has been reported that bilateral injections of baclofen into the NTS of urethane-anesthetized rats produced no effect on baseline MAP (32). In addition, baclofen reduced the bradycardic but not the hypotensive response to Bezold-Jarisch reflex activation. We showed here that bilateral injections of muscimol, but not of baclofen, into the NTS at doses that produced no visual changes in the ventilatory pattern reduced the bradycardic and hypotensive responses to *iv* injection of 5-HT. The opposite results obtained in the two studies may be related to differences in the methodology used, i.e.: a) the present study was performed in awake rats, b) the injection volume and doses of GABA_B agonist were different ((±)-baclofen at the dose of 12.5 pmol/50 nl in the present study and (-)-baclofen at the dose of 60 pmol/110 nl in the study of Seifert and Trippenbach (32)), and c) the sites of baclofen injections into the NTS were not necessarily the same (in the present study the centers of injections were located ~0.2 mm rostral to the calamus scriptorius and 0.5 mm lateral to the midline).

Confirming and extending literature data (33), we observed that the peripheral blockade of muscarinic receptors with atropine methyl bromide almost abolished both bradycardia and hypotension in response to *iv* injections of 5-HT, indicating that in awake rats the fall in MAP was secondary to the intense reduction in HR. In contrast, other studies (9,34,35) have shown that *iv* serotonin or intra-atrial phenylbiguanide injections in anesthetized rats elicited not only the

well-known bradycardic and hypotensive responses, but also inhibition of lumbar sympathetic nerve discharge with resulting vasodilatation and inhibition of sympatho-excitatory barosensitive neurons, suggesting the existence of a sympathetic component of the cardiopulmonary reflex. An explanation for the effects of atropine is that atropine interferes with muscarinic transmission not only in the central arch of the cardiopulmonary reflex but also in the myocardial muscarinic transmission which regulates HR changes, and that this additive effect eventually produces a marked inhibition of 5-HT-elicited bradycardia (36). It is not the aim of the present study to argue about the relative contribution of parasympathetic vs sympathetic components of the reflex in awake rats, but we showed that the activation of the Bezold-Jarisch reflex produces bradycardia and hypotension, which at the level of the NTS is reduced by GABA_A but not by GABA_B receptor agonists. However, the hypothesis about the role of both GABA receptors in processing the sympatho-inhibitory component of the cardiopulmonary reflex at the level of the NTS requires further investigation. The effects of the stimulation of GABA_A receptors with muscimol into the NTS on baseline MAP (increase) and on the parasympathetic component of the cardiopulmonary reflex (reduction) are similar to those reported for the stimulation of presynaptic serotonergic 5-HT₃ receptors with chlorophenylbiguanide or 2-methylserotonin (9,11). Pretreatment with bicuculline blocked the inhibitory effect of the 5-HT₃ agonist on the parasympathetic component of the cardiovascular reflexes, suggesting that GABA_A receptors play a key role in this inhibitory neuromodulation (9,20). In addition, pretreatment with bicuculline had no effect on the increase in baseline MAP produced by injections of 5-HT₃ agonists into the NTS

and on the pressor response to chemoreflex activation (20). The increase in baseline MAP produced by bilateral injections of muscimol into the NTS may be due to the activation of GABA_A receptors located postsynaptically on neurons involved in the sympatho-inhibitory projection of the baroreflex. The effect of baclofen on baseline MAP may be due to the activation of GABA_B receptors presynaptically located in the terminals of the baroreceptor afferent fibers in the NTS that may inhibit the release of the excitatory amino acid L-glutamate, the putative neurotransmitter involved in the parasympathetic and the sympatho-inhibitory components of the baroreflex in the NTS (7,37).

The present study and previous ones (7, 9-11,20,33,38) clearly demonstrate that GABA_A and 5-HT₃ receptor activation and NMDA receptor blockade inside the NTS always inhibit the parasympathetic component of the baro- and chemoreflex and Bezold-Jarisch reflex. The involvement of the same mechanisms in the control of the sympathetic component of the cardiovascular reflexes has not been fully defined.

GABA_A receptor activation within the lateral NTS reduces the bradycardia and hypotension occurring in response to *iv* 5-HT injections in awake rats. These data, together with those of others (17,18,39) suggest that within the NTS the GABAergic mechanisms may modulate the parasympathetic component of the Bezold-Jarisch reflex similarly to the modulation already described for the arterial baro- and chemoreflex.

Acknowledgments

We thank Reginaldo C. Queiróz for histological technical assistance, Sílvia Fógliã for expert technical assistance, Silvana A.D. Malavolta for secretarial assistance, and Ana L.V. de Oliveira for animal care.

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