Pressor response to chemoreflex activation before and after microinjection of glycine into the NTS of awake rats

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Pimentel, Franklin F., Leni G. H. Bonagamba, and Benedito H. Machado. Pressor response to chemoreflex activation before and after microinjection of glycine into the NTS of awake rats. Am J Physiol Regul Integr Comp Physiol 284: R1000–R1009, 2003. First published December 5, 2002; 10.1152/ajpregu.00310.2002.—Microinjection of glycine into the rostral (bilateral) and caudal (midline) commissural nucleus of the solitary tract (NTS) using three guide cannulas implanted in the direction of these sites produced an increase in mean arterial pressure (MAP) and abolished the pressor response to chemoreflex activation [potassium cyanide (n = 7)]. Strychnine, a glycine receptor antagonist, attenuated the increase in MAP, and in this new experimental condition (n = 5) the pressor response to chemoreflex activation was not altered. Considering that the effect of glycine on the attenuation of the pressor response to chemoreflex activation could be secondary to the increase in baseline MAP, in a third group of rats (n = 5) sodium nitroprusside infusion (intravenous) after microinjections of glycine into the NTS normalizes MAP. In this case, the pressor response to chemoreflex activation was similar to the control. These data show that glycine when microinjected bilaterally into the lateral commissural NTS as well as into the medial commissural NTS plays no major inhibitory role in the processing of the neurotransmission of the sympathoexcitatory component of the chemoreflex.

glycine receptors; autonomic regulation; sympathetic activity; cardiovascular regulation

THERE IS ANATOMIC and functional evidence that the first synapse of the arterial chemoreceptor afferents occurs at the nucleus of the solitary tract (NTS) level (4, 8, 13, 20, 22, 27). Activation of the chemoreflex with potassium cyanide (KCN; intravenous) produces pressor and bradycardic responses, which are originated by independent activation of the sympathetic and parasympathetic autonomic components (9, 10, 12). In previous studies from our laboratory, we verified that the parasympathetic component of the chemoreflex was blocked in a dose-dependent manner by microinjection of D,L-Z-amino-5-phosphonovaleric acid, an N-methyl-d-aspartate (NMDA) receptor antagonist, into the NTS (12), whereas the pressor response was not affected by this antagonist and only partially reduced by 6,7-dinitroquinoxaline-2,3-dione, a non-NMDA receptor antago-

METHODS

Male Wistar rats weighing 290–310 g were used in the present study. Four days before the experiments, rats under 2.5% tribromethanol anesthesia (1 ml/100 g ip; Aldrich Chemical, Milwaukee, WI) were placed in a stereotaxic apparatus (David Kopf, Tujunga, CA), and the technique described by Michelin and Bonagamba (19) was adapted to implant three guide cannulas, two of them implanted in the direction of the rostral aspect of the commissural NTS (0.5 mm lateral to midline and 0.5 mm rostral to the calamus scriptorium) and one implanted in the direction of the caudal aspect of the commissural NTS (on the midline and 0.5 mm caudal to calamus scriptorium) for microinjection of L-glutamate or strychnine. The guide cannulas were implanted in accordance with the coordinates of the atlas of Paxinos and Watson (21). Additional anesthesia was provided when the rat reacted to frequent toe pinching during stereotaxic surgery. To implant each guide cannula, we made a small window in the skull and introduced a 15-mm-long stainless steel guide cannula (22-gauge) perpendicularly through the window at the following coordinates: 14.0 mm (NTS rostral) or 14.5 mm (NTS caudal) caudal to the bregma, 0.5 mm (NTS rostral) or 0.0 mm (NTS caudal) lateral to the midline, and

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The guide cannulas were 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 to PE-10 tubing to a 1-μl syringe (Hamilton, Reno, NV). After removal of the occluder, the needle for microinjection of drugs into the NTS was carefully inserted into the guide cannula, and manual injection was initiated 30 s later. The first microinjection was initially performed on one side, the needle was withdrawn and repositioned on the contralateral side, and then a second microinjection was performed and the same procedure was repeated for the third cannula. Therefore, the time interval for microinjections into the three sites of the NTS was ~1.5 min and the volume of each microinjection was 50 nl. Glycine (50 nmol/50 nl) and strychnine (150 pmol/50 nl) were freshly diluted in saline, and sodium bicarbonate was added to the saline to adjust the pH to the 7.4 range. The solutions of glycine and strychnine were used based on previous studies by Wu et al. (25) performed on the NTS of cats. At the end of each experiment, Evan’s blue dye (2%, 50 nl) was microinjected for histological identification of the sites of microinjection and the animals were then submitted to intracardiac perfusion with 0.9% saline followed by 10% buffered formalin while they were under ether anesthesia. The brains were removed and stored in buffered formalin for 2 days, and serial coronal sections (15-μm thickness) were cut and stained with the Nissl method. Only the rats in which the sites of microinjections were located in the rostral (bilateral) and caudal (midline) commissural NTS were considered for data analysis. On average, 30% of the rats implanted with the three guide cannulas in the different experimental protocols presented positive histology, i.e., the injections were centered in the appropriate site in the NTS.

One day before the experiments, while the rats were 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 to measure pulsatile arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR). A second catheter was inserted into the femoral vein for systemic administration of KCN. Both catheters were tunneled subcutaneously and exteriorized through the back of the neck to be connected to the pressure transducer under conscious freely moving conditions on the subsequent day. PAP and MAP were measured with a pressure transducer (model CDX III, Cobe Laboratories, Lakewood, CO) connected to a polygraph (NarcoTrace 80, Narco Bio-Systems, Austin, TX). HR was quantified with a Narco Biomedical Coupler (model 7302) and recorded on the same polygraph. In a protocol involving the intravenous infusion of sodium nitroprusside (3 μg·50 μl⁻¹·min⁻¹) to normalize the MAP after microinjection of glycine into the NTS, a third catheter was inserted into the contralateral femoral vein and on the day of the experiment it was connected to a syringe mounted on an infusion pump (Ati-Orion Sage model 361, Boston, MA). All experimental approaches to the rats were performed in accordance with the “Guiding Principles for Research Involving Animals and Human Beings” established by the American Physiological Society.

The chemoreflex was activated by intravenous injection of KCN (40 μg/rat; Merck, Darmstadt, Germany) as described in previous studies by Franchini and Krieger (9, 10) and from our laboratory (11, 12). The magnitude of the changes in MAP and HR in response to chemoreflex activation was quantified at the peak of the responses. In the different experimental protocols, the chemoreflex was activated before and 2, 10, and 20 min after 1) microinjections of saline (vehicle) and glycine into the NTS, 2) microinjections of strychnine and glycine into the NTS, and 3) microinjections of saline and glycine into the NTS, combined with the infusion of sodium nitroprusside to normalize MAP. A previous study from our laboratory (2) documented that the microinjection of saline (vehicle) into the NTS produced no effect on the cardiovascular responses to chemoreflex activation.

All data are expressed as means ± SE. The results were analyzed by one-way ANOVA followed by the Tukey posttest (P < 0.05).

RESULTS

Chemoreflex activation before and after microinjection of saline and glycine into the NTS. Figure 1 shows tracings of one rat representative of the group in which chemoreflex activation was performed before and 2, 10, and 20 min after microinjection of glycine (50 nmol/50 nl) into the NTS. Microinjection of glycine into three sites of the NTS after microinjection of saline (vehicle) into the same sites produced a significant increase in baseline MAP (108 ± 3 vs. 157 ± 7 mmHg, P < 0.001) and no significant changes in baseline HR. The pressor response to chemoreflex activation 2 min after glycine was almost abolished and the bradycardic response was clearly reduced. At 10 and 20 min after glycine, the baseline MAP as well as the pressor and bradycardic responses presented a tendency to return to the control values. Figure 3A summarizes the data for a group of seven rats and shows that microinjection of glycine into the NTS produced a significant increase in baseline MAP (108 ± 3 vs. 157 ± 7 mmHg, P < 0.001) and no significant changes in HR. Figure 4A summarizes the changes in MAP and HR in response to chemoreflex activation before and after microinjection of glycine into the NTS, and these data show that 2 min after glycine the pressor response was almost abolished and the bradycardic response was significantly reduced. Ten minutes later the pressor response was back to the control levels, whereas the bradycardic response remained significantly reduced and was back to control levels 20 min later.

Chemoreflex activation before and after microinjection of strychnine and glycine into the NTS. Figure 2 shows the tracings of one rat representative of the group in which chemoreflex activation was performed before and 2, 10, and 20 min after microinjection of glycine into the NTS. Microinjection of glycine (50 nmol/50 nl) into three sites of the NTS after microinjection of strychnine (150 pmol/50 nl) into the same sites produced a mild increase in MAP and no major changes in baseline HR. The pressor and bradycardic responses to chemoreflex activation 2 min after strychnine and glycine into the NTS did not differ significantly from control. At 10 and 20 min after glycine and strychnine, baseline MAP as well as the pressor and bradycardic responses were also similar to control values. Figure 3B summarizes data for a group of five rats and shows that microinjection of glycine into the NTS after strychnine also produced a significant increase in
baseline MAP (99 ± 4 vs. 137 ± 12 mmHg, \( P < 0.05 \)). However, this increase in MAP was significantly smaller than that observed in the corresponding control group, which received microinjections of glycine into the NTS after saline (106 ± 3 vs. 156 ± 7 mmHg, \( P < 0.001 \)). Figure 4B summarizes the changes in MAP and HR in response to chemoreflex activation before and after microinjection of strychnine and glycine into

Fig. 1. Traces of 1 rat showing the changes in heart rate (HR), pulsatile arterial pressure (PAP), and mean arterial pressure (MAP) in response to chemoreflex activation with potassium cyanide (KCN) before (control) and 2, 10, and 20 min after sequential microinjections of saline (vehicle) and glycine (50 nmol/50 nl) into the rostral (bilateral) and caudal commissural nucleus of the solitary tract (NTS). bpm, Beats/min.

Fig. 2. Traces of 1 rat showing the changes in HR, PAP, and MAP in response to chemoreflex activation with KCN before (control) and 2, 10, and 20 min after sequential microinjections of strychnine (150 pmol/50 nl) and glycine (50 nmol/50 nl) into the rostral (bilateral) and caudal commissural NTS.
the NTS, and these data show that 2 min after glycine the pressor and bradycardic responses did not differ significantly from control values.

Chemoreflex activation before and after microinjection of saline and glycine into the NTS combined with sodium nitroprusside infusion. Figure 5 shows tracings of one rat in which chemoreflex activation was performed before and 2, 10, and 20 min after microinjection of glycine (50 nmol/50 nl) into the NTS combined with sodium nitroprusside infusion. Microinjection of glycine into three sites of the NTS after microinjection of saline (vehicle) into the same sites produced a marked increase in MAP and a mild increase in baseline HR. One minute after microinjection of glycine into the NTS, infusion of sodium nitroprusside was initiated to normalize the MAP, and when MAP was back to control levels, the chemoreflex was activated. In the case illustrated in Fig. 5, the pressor response to chemoreflex activation was similar to the control response, whereas the bradycardic response was reduced, although on average (see Fig. 7) the changes in HR were not significantly different compared with the control. The tracings in Fig. 5 also show that at 10 and 20 min after glycine when the baseline MAP was naturally back to control levels, the pressor and bradycardic responses to chemoreflex activation presented a tendency to return to control values. Figure 6 summarizes data for the group (n = 5) and shows that 1 min after microinjection of saline plus glycine into the NTS a significant increase in baseline MAP occurred (113 ± 4 vs. 166 ± 7 mmHg, P < 0.001) and 2 min later, i.e., during sodium nitroprusside infusion, MAP was back to control levels (116 ± 6 mmHg). Figure 6 also shows that 1 or 2 min after saline plus glycine combined with sodium nitroprusside infusion HR was not different from control. Figure 7 summarizes the changes in MAP and HR in response to chemoreflex activation before and after microinjection of saline and glycine into the NTS, and these data show that 2 min after microinjection of glycine combined with the sodium nitroprusside

Fig. 3. A: baseline MAP and HR before (control) and 2 min after sequential microinjections of saline (vehicle) and glycine (50 nmol/50 nl) into the rostral (bilateral) and caudal commissural NTS (n = 7). B: baseline MAP and HR before (control) and 2 min after sequential microinjections of strychnine (150 pmol/50 nl) and glycine (50 nmol/50 nl) into the rostral (bilateral) and caudal commissural NTS (n = 5). *Significantly different from control (P < 0.05).
infusion the pressor (57 ± 5 vs. 55 ± 5 mmHg) and bradycardic (−160 ± 108 vs. −138 ± 53 beats/min) responses to chemoreflex activation did not differ from the control values.

Misplacement of one or two guide cannulas for microinjections of glycine into the NTS. The analysis of the data of one group of rats (n = 9) in which the positive sites of microinjections into the NTS were restricted to one (lateral or medial commissural NTS) or two sites of microinjections (one in the medial and one in the lateral commissural NTS or two in the lateral aspects of the commissural NTS) showed that the increase in baseline MAP (from 106 ± 3 to 124 ± 7 mmHg) was significantly smaller than that verified when glycine was microinjected into three sites of the NTS [Fig. 3A (from 108 ± 3 to 157 ± 7 mmHg, n = 7) and Fig. 6A (from 113 ± 4 to 166 ± 7 mmHg, n = 5)].

Histology of the sites of microinjection. Figure 8 presents two photomicrographs of transverse sections of the brain stem of one rat representative of the groups showing the sites of bilateral microinjections into the rostral commissural NTS (Fig. 8A) and of medial microinjection into the caudal commissural NTS (Fig. 8B).

DISCUSSION

The purpose of the present study was to explore the possible role of glycine as an inhibitory neuromodulator in the NTS on the autonomic responses to chemoreflex activation. This hypothesis was based on previous
studies using different experimental approaches, which documented that previous microinjection of glycine attenuated the cardiovascular responses to microinjection of L-glutamate into the NTS (16, 23) or the responses to aortic depressor nerve stimulation (14). Studies by Bennett et al. (1) also documented that glycine inhibits the evoked activity of cat NTS neurons activated by electrical stimulation of the aortic depressor nerve. Therefore, considering this evidence that glycine affects the neurotransmission of the baroreflex at the NTS level and also that in a series of previous experiments from our laboratory we were not able to block the sympathoexcitatory component of the chemoreflex using different antagonists at the NTS level, in the present study we evaluated the effect of glycine on the pressor and bradycardic responses to chemoreflex activation in awake rats.

Data of the present study indicate that glycine plays no major role in the processing of the neurotransmission of the chemoreflex but are in accordance with previous studies by Kubo and Kihara (14, 15) showing that this inhibitory amino acid may play an important role in the neuromodulation of the baroreflex at the NTS level. Glycine was microinjected simultaneously at three different sites in the commissural NTS (rostral aspect of the commissural NTS [bilaterally] and caudal aspect of the commissural NTS [midline]) for the microinjections to reach most of the subregions of the NTS involved in the neurotransmission of the chemoreflex at the NTS level. When glycine (50 nmol/50 nl) was microinjected into these sites, we observed a marked and significant increase in baseline MAP, which was back to the control level 10 min later. In this case, we observed that the pressor response to chemoreflex activation 2 min after glycine was almost abolished (Figs. 1 and 4A) and was back to control level 10 min later when baseline MAP was also back to the control level. A preliminary conclusion from this experiment would indicate that glycine really blocked the pressor response of the chemoreflex. However, the magnitude of
the pressor response to chemoreflex activation could be blunted by the large increase in the baseline MAP as a consequence of an increase in sympathetic outflow, probably due to the inhibition of the sympathoinhibitory pathways to the baroreflex arch at the brain stem level.

To evaluate the mechanisms involved in the increased baseline MAP and the consequent blockade of the pressor response produced by microinjection of glycine into the NTS, we used strychnine to block the site on which the agonist would be acting. The microinjections of strychnine into the NTS produced no significant changes in baseline MAP, indicating that strychnine-sensitive glycine receptors play no major role in the tonic maintenance of basal autonomic activity on the cardiovascular system. In the sequence, glycine still produced an increase in the baseline MAP (Fig. 2), which was significantly smaller than that produced by glycine after saline (Fig. 3). In this case, the pressor response to chemoreflex activation tended to be reduced (Fig. 2) but was not statistically different compared with the control responses (Fig. 4B). Although strychnine was not enough to completely block the increase in baseline MAP, probably due to the fact that the dose used was not enough to block all the receptors, it was possible to verify that the blockade of the pressor response to chemoreflex activation observed in the absence of strychnine was probably related to the large increase in baseline MAP.

With respect to the possibility that the changes in baseline MAP observed after microinjections of glycine

![Fig. 6. Baseline MAP (A) and HR (B) of a group of rats (n = 5) before (control) and at the first minute after sequential microinjections of saline (vehicle) and glycine (50 nmol/50 nl) into the rostral (bilateral) and caudal commissural NTS and at the second minute after saline and glycine combined with sodium nitroprusside infusion (3 μg·50 μl⁻¹·min⁻¹). *Significantly different from control; +significantly different from 1 min (P < 0.05).](image)

![Fig. 7. Changes in the pressor (ΔMAP; A) and bradycardic (ΔHR; B) responses to chemoreflex activation (KCN, 40 μg/rat iv) before (control) and 2, 10, and 20 min after sequential microinjections of saline (vehicle) and glycine into the commissural NTS combined with intravenous infusion of sodium nitroprusside (n = 5).](image)
into the NTS could be the main factor in the blockade of the pressor response to chemoreflex activation, we conceived an experimental protocol to bring MAP back to normal level after microinjections of glycine. In this case we used intravenous infusion of sodium nitroprusside for periods as long as 2 to 3 min. During the normalization of MAP, the chemoreflex was activated and these data (Fig. 5) clearly indicated that the magnitude of the pressor response to chemoreflex activation in this experimental condition was similar to control, as summarized in Fig. 7. Therefore, these data allow us to conclude that the blockade of the pressor response to chemoreflex observed after microinjections of glycine into the NTS was secondary to the large increase in baseline MAP and not related to a direct effect on the neurons involved in the sympathoexcitatory pathways of the chemoreflex at the NTS level. These data also show that the pressor response to chemoreflex activation was preserved and suggest that in the first protocol the pressor response to chemoreflex was probably masked by an increased baseline vascular tonus due to the increase in the sympathetic activity probably produced by the inhibitory effect of glycine on the sympathoinhibitory component of the baroreflex. In this case, we may suggest that the magnitude of the pressor response to chemoreflex activation in the control corresponded to the maximal contractile effect produced by activation of the sympathetic activity on the vascular beds by the chemoreflex and that after glycine the vascular contractility was increased due to the increase in sympathetic outflow. Under these circumstances, even though the activation of the chemoreflex may produce a similar increase in sympathetic activity, it will not result in additional vasoconstriction due to the physical limitation of the contractility of the vascular smooth muscle cells. This possibility is supported by data from the current study in our laboratory in which the chemoreflex was activated before and during phenylephrine infusion to produce an increase in the baseline MAP in the range of 40 mmHg. In this case, we verified that the pressor response to chemoreflex activation was almost abolished.

Fig. 8. Photomicrographs of the brain stem of 1 rat representative of the groups showing the sites of microinjections into the rostral (A) and caudal (B) aspects of the commissural NTS, indicated by the arrows. AP, area postrema; CC, central canal.
during phenylephrine infusion. The fact that in the present study the magnitude of the pressor response to chemoreflex activation was back to control levels when we normalized the MAP with sodium nitroprusside infusion is additional evidence that the baseline vascular tone may play a key role in the magnitude of the pressor response.

In a previous study from our laboratory, we verified that previous microinjections of glycine into the NTS produced a dose-dependent blockade of the pressor response to microinjection of l-glutamate into the same site (16). At that time, we considered the possibility that l-glutamate into the NTS of awake rats could be activating the chemoreflex pathways. In subsequent studies, we verified that although microinjection of l-glutamate into the NTS and chemoreflex activation in awake rats produce similar patterns of cardiovascular responses, the neural pathways and the mechanisms involved in the generation of the pressor responses do not seem to be the same (17, 18). In addition, we were also unable to block the pressor response of the chemoreflex when we performed the following microinjections into the NTS: selective and nonselective antagonists of excitatory amino acids (11, 12), an antagonist of substance P receptors (26), an adenosine receptor antagonist (7), agonists of serotonin receptors (3), and agonists of GABA receptors (2). Therefore, the present study indicating that glycine also produced no effect on the processing of the sympathoexcitatory component of the chemoreflex at the NTS level adds new and relevant information about the possibility that the sympathoexcitatory pathways of the chemoreflex are not under an inhibitory neuromodulation, at least in the NTS.

These data also indicate that microinjection of glycine into three sites of the commissural NTS produced a significant increase in baseline MAP, indicating that this inhibitory amino acid may play an important role in the modulation of the sympathoinhibitory component of the baroreflex at the NTS level, as previously documented by Kubo and Kihara (14, 15) in anesthetized rats. It is important to note that the larger increase in baseline MAP [from 106 ± 3 to 156 ± 7 mmHg (Fig. 3A) or from 113 ± 4 to 166 ± 7 mmHg (Fig. 6A)] was observed in groups of rats that received microinjections of glycine into three sites of the NTS, whereas in the group that received microinjections into one or two sites the magnitude of the increase in MAP (from 106 ± 3 to 124 ± 7 mmHg, n = 9) was significantly smaller indicating that the effect of glycine on baseline MAP is proportional to the area of the NTS reached by the spreading of the volume microinjected. With respect to the mechanism involved in the increase in baseline MAP produced by glycine, we may speculate that it was due to the activation of glycnergic receptors sensitive to strychnine postsynaptically located. This hypothesis is supported by studies investigating the presence of postsynaptic strychnine-sensitive glycine receptors in several areas of the brain including midbrain, brain stem, and spinal cord (5, 6, 24). In functional studies by Talman and Robertson (23) and Kubo and Kihara (15), it was also observed that the cardiovascular responses to microinjection of glycine into the NTS of anesthetized rats were also blocked by strychnine. The effect of glycine on the bradycardic response to chemoreflex activation was probably due to its effect on strychnine-sensitive glycine receptors and not on its specific site in the NMDA receptors, because in a series of experiments from our laboratory, we verified that the bradycardic responses to the activation of the cardiovascular reflexes were blocked in a dose-dependent manner by an NMDA receptor antagonist while in the present study strychnine blocked the inhibitory effect of glycine on the bradycardic responses to chemoreflex activation (Fig. 4B). It is important to note that the bradycardic response to chemoreflex activation was significantly reduced after glycine and reversed by strychnine, suggesting that glycine may play an inhibitory role in strychnine-sensitive glycine receptors located in neurons of the NTS involved in the parasympathetic component of the chemoreflex. However, we cannot rule out the possibility that the reduction of the bradycardic response to chemoreflex activation observed after microinjections of glycine into the NTS probably was related to the large increase in the baseline MAP, considering that the absence of tachycardia after glycine may be related to the activation of the baroreflex due to the large increase in MAP. In this case, the new baseline parasympathetic component to the heart may be overactive, possibly explaining the attenuation of the bradycardic response to chemoreflex activation.

Data from the present study indicate that glycine when microinjected bilaterally into the lateral aspect of the commissural NTS as well as into the medial aspect of the commissural NTS plays no major inhibitory role in the processing of the neurotransmission of the sympathoexcitatory component of the chemoreflex. However, additional studies involving multiple sites of microinjection into the different subregions of the NTS are required before we can completely rule out the involvement of glycine in the inhibitory neuromodulation of the sympathoexcitatory component of the chemoreflex at the NTS level.

**Perspectives**

Data from the present study showing that the inhibitory amino acid glycine plays no modulatory role in the sympathoexcitatory component of the chemoreflex at the NTS level, taken together with previous experiments from our laboratory indicating that this sympathoexcitatory response was also not blocked by microinjection of antagonists of excitatory amino acid receptors (11) or by microinjection of GABA agonists (2) into the NTS, open new and interesting perspectives about the identification of the putative neurotransmitter of the sympathoexcitatory component of the chemoreflex in the NTS and about the possibility that the pathways involved in this autonomic response are not under inhibitory neuromodulation of glycnergic or GABAergic mechanisms, at least in the NTS.
Considering that the chemoreflex is activated essentially under critical circumstances such as hypoxia, we may suggest that, during the risk situation, the pathways involved in the sympathoexcitatory component of this reflex may not be under any inhibitory neuro-modulation to allow an appropriate cardiovascular response. In case of unusual chronic activation of the sympathoexcitatory component of the chemoreflex pathways, including an overexcitation of the sympathetic activity, it is plausible to consider the possibility that this condition may contribute to the development of some autonomic dysfunction such as arterial hypertension. To validate this hypothesis, a series of additional experimental approaches is required, especially with the use of the experimental model of chronic intermittent hypoxia in rats. Among these approaches we must consider 1) the possible involvement of ATP and purinergic receptors in the processing of the sympathoexcitatory component of the chemoreflex at the NTS level, 2) the involvement of neurons in the paraventricular nucleus of the hypothalamus and in the parabrachial nucleus in the generation and/or modulation of this sympathoexcitatory response, and 3) the use of the experimental model of chronic intermittent hypoxia to allow a full expression of excitatory and inhibitory neurotransmitters in different areas of the brain involved in the complex modulation of the sympathoexcitatory response to chemoreflex activation.

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