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Short communication

Microinjection of NMDA antagonist into the NTS of conscious rats blocks the Bezold–Jarisch reflex

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Abstract

The purpose of the present study was to evaluate whether or not cardiovagal excitatory and sympatho-inhibitory pathways of the Bezold–Jarisch reflex at the NTS level were mediated by NMDA receptors. The Bezold–Jarisch reflex was activated by intravenous (i.v.) injection of serotonin in conscious rats before and after microinjection of phosphonovaleric acid (AP-5), a selective NMDA antagonist, into the NTS. The Bezold–Jarisch reflex was also activated before and after methyl-atropine (i.v.) in order to evaluate if the changes in mean arterial pressure were dependent on the bradycardic response. The data showed that AP-5 into the NTS produced a dose-dependent reduction in both bradycardic and hypotensive responses to activation of the Bezold–Jarisch reflex. Methyl-atropine also blocked the bradycardic and hypotensive responses to Bezold–Jarisch reflex activation. The data show that in conscious rats the cardiovagal component of the Bezold–Jarisch reflex plays a major role in the cardiovascular changes produced by the activation of this reflex and suggest that the neurotransmission of the cardiovagal component of the Bezold–Jarisch reflex is mediated by NMDA receptors.

Keywords: C-fiber; Cardiopulmonary receptor; Amino acid receptor; Phosphonovaleric acid; Bradycardia; Sympatho-inhibition

The physiological role of the cardiopulmonary receptors in the regulation of arterial pressure is not completely understood. The activation of these receptors with serotonin or phenylbiguanide produces hypotension, bradycardia and apnea, which are characterized as the Bezold–Jarisch reflex [3,8,14]. The chemical activation of cardiopulmonary afferent C-fibers endings with phenylbiguanide or serotonin (5-HT) is considered a valid index for the evaluation of the cardiopulmonary reflex [1,3,14,16,19]. The projections of the afferent C-fibers of these receptors to the central nervous system have their first synapse at the level of the NTS [7]. The functional evidence for such synapses was provided by several studies [2,16,18] which show that the microinjection of kynurenic acid, a nonselective antagonist of excitatory amino acid (EAA) receptors, completely blocked the Bezold–Jarisch reflex in conscious and anesthetized rats.

The aims of the present study were (a) to evaluate the role of NMDA receptors in the NTS in the neurotransmission of the cardiovagal excitatory and sympatho-inhibitory components of the Bezold–Jarisch reflex, and (b) to verify whether or not the fall in arterial pressure in response to the activation of the Bezold–Jarisch reflex is dependent on the bradycardic response. Considering that anesthesia may have a distorting effect on neurotransmission at the NTS level [10], all the experiments were performed on conscious freely moving rats.

Male Wistar rats weighing 250–270 g were used in the present study. Four days before to the experiments, rats under pentobarbital sodium (40 mg/kg, i.p.) anesthesia were placed in a stereotaxic apparatus (David Kopf Instruments, USA) and implanted with bilateral guide cannulas in the direction of the commissural NTS, according to the coordinates of Paxinos and Watson [12] and the technique of Michelini and Bonagamba [11]. The procedures for microinjections into the NTS are described elsewhere [4,6,10].

One day before the experiments, under ether anesthesia, the rats had a catheter inserted into the femoral artery for measurement of pulsatile arterial pressure (PAP) and heart rate (HR) and a second catheter inserted into the femoral vein for 5-HT (Sigma, St. Louis, MO) injection. Both catheters were tunneled and exteriorized through the back

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of the neck to be connected to the pressure transducer under conscious freely moving conditions. PAP and mean arterial pressure (MAP) were measured with a pressure transducer (COBE CDX III, COBE Laboratories, Inc., Lakewood, CO) connected to a Narcotrace 80 Physiological Recorder (NARCO Bio-Systems, Austin, TX) and the HR was quantified by a NARCO Biotachometer Coupler (Model 7302).

The activation of the Bezold–Jarisch reflex was performed by intravenous (i.v.) injection of 5-HT at the dose of 20 μg/kg, which produces in conscious rats important cardiovascular responses [1]. The injection of 5-HT was performed before and 10 and 40 min after bilateral microinjection of AP-5 (RBI, Natick, MA) into the NTS.

AP-5 microinjected into the NTS was diluted in saline (pH 7.0) and the microinjections were performed in a volume of 100 nl. Three different doses of AP-5 (0.1, 0.5 and 2.0 nmol/100 nl) were microinjected into the NTS of three different groups of rats [first ($n = 6$), second ($n = 6$) and third group ($n = 6$)]. The rats of each group received only one dose of AP-5. The activation of the Bezold–Jarisch reflex was also performed before and 10 min after bilateral microinjection of saline as a volume control and no significant changes in hypotensive and bradycardic responses were observed.

After the experiments under conscious conditions, 100 nl of Evans blue (2%) was microinjected into the same sites and under ether anesthesia the brain was removed for histological preparation (Nissl). Only rats with the microinjection sites located in the commissural NTS were considered for data analysis.

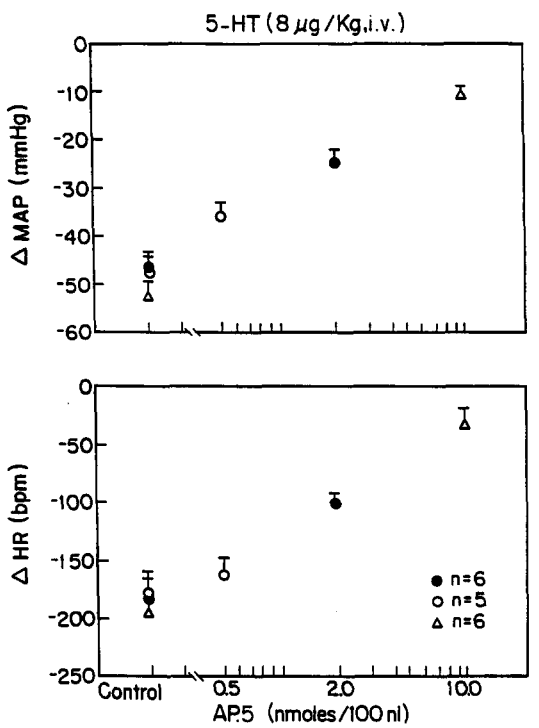

Fig. 1. Changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) produced by serotonin (5-HT, 8 μg/kg, i.v.) before and after bilateral microinjection of AP-5 into the NTS of 3 groups of rats. [AP-5: 0.5 (○), 2.0 (●) and 10.0 nmol/100 nl (△)].

Statistical analysis for comparison of the mean values of cardiovascular responses to Bezold–Jarisch reflex activation before and 10 min after AP-5 into the NTS was performed by the Student t-test and statistical significance was set at the 0.05 level.

Fig. 1 summarizes the effect of AP-5 on the changes in MAP and HR produced by activation of the Bezold–Jarisch reflex. In the first group, AP-5 (0.5 nmol/100 nl) produced no significant changes when we compared the hypotensive (-48 ± 6 vs. -36 ± 3 mmHg) and bradycardic (-178 ± 20 vs. -159 ± 13 b.p.m.) responses to Bezold–Jarisch reflex activation before and after AP-5 microinjection. In the second group AP-5 (2 nmol/100 nl) produced a significant reduction in both hypotensive (-47 ± 3 vs. -25 ± 4 mmHg) and bradycardic responses (-183 ± 16 vs. -99 ± 10 b.p.m.) and in the third group AP-5 also produced a significant reduction in the hypotensive (-53 ± 5 vs. -11 ± 3 mmHg) and bradycardic responses (-195 ± 28 vs. -32 ± 15 b.p.m.).

Fig. 2 is a photomicrograph of a coronal section of the brainstem from one representative rat in which the microinjection sites were located bilaterally in the lateral portion of the commissural NTS.

In the protocol related to the cardiovascular changes produced by 5-HT before and after injection of methyl-atropine (i.v.) we observed in a specific group of 8 rats that the cholinergic blockade almost abolished the bradycardic (-156 ± 15 vs. -14 ± 7 b.p.m.) and hypotensive responses (-44 ± 6 vs. -1 ± 6 mmHg) to the activation of the Bezold–Jarisch reflex.

Despite activation of different sensory mechanisms, the cardiovascular responses produced by the Bezold–Jarisch reflex and the baroreflex are essentially the same, i.e., sympatho-inhibition (hypotension) and parasympatho-excitation (bradycardia). Several lines of evidence have shown that the two reflexes share neural pathways in the brainstem [17]. For both reflexes, the neural circuits for sympatho-inhibition include excitatory amino acid receptors in the NTS and CVLM, and GABAergic projections from the CVLM to the RVLM as well as excitatory projections from the NTS to the nucleus ambiguus, which seems to be glutamatergic [5,9,15–19]. However, the subtypes of excitatory amino acid receptors involved in the neurotransmission of baro-, chemo- and cardiopulmonary-receptors afferents at the NTS level have not yet been determined. In a recent study from our laboratory [6] we observed that the bilateral microinjection of AP-5 into the NTS of conscious rats produced a dose-dependent blockade of the cardiovagal component and no changes in the sympatho-excitatory component of the chemoreflex. These previous studies indicated that the cardiovagal component of the chemoreflex is mediated by NMDA receptors in the NTS, while the pressor response to the same stimulus seems to be mediated by non-NMDA receptors. In function of these find-

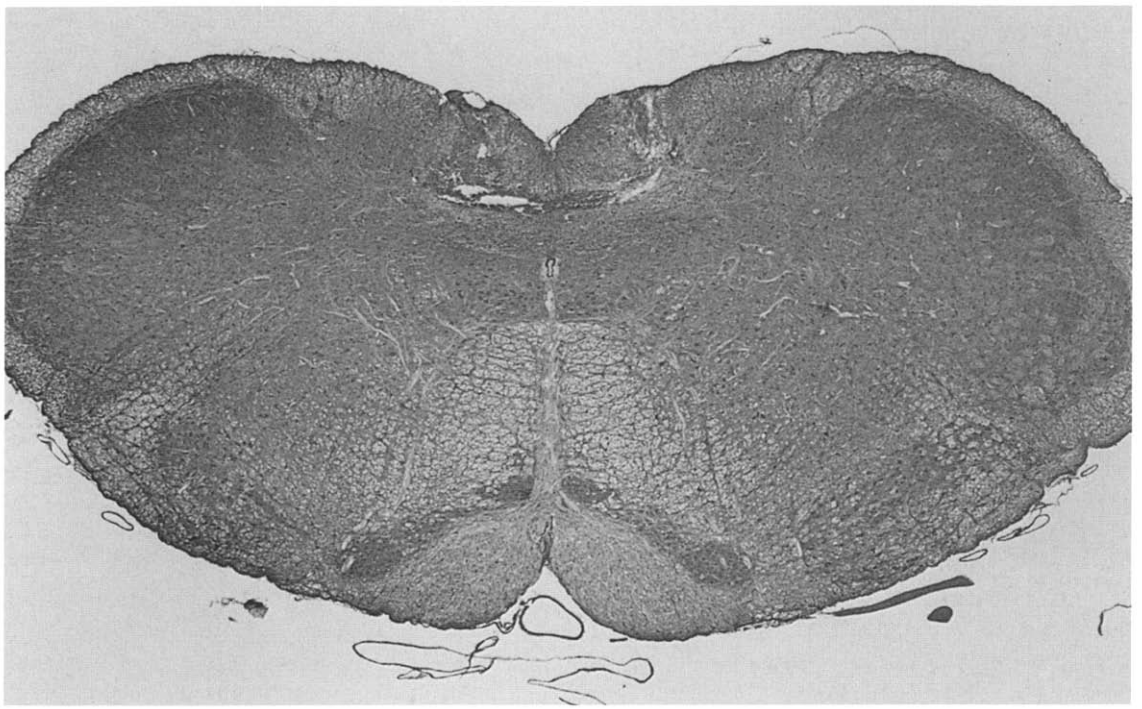

Fig. 2. Photomicrograph of a coronal section of the brain stem showing a typical bilateral microinjection sites in the NTS commissural.

ings we hypothesized that NMDA and non-NMDA receptors may play a specific role not only in the processing of the chemoreflex afferents in the NTS but also in the autonomic processing of the baroreflex and the Bezold–Jarisch reflex afferents in the NTS.

In the present study we observed that selective antagonism of NMDA receptors with AP-5 produced a dose-dependent blockade of both the bradycardic and hypotensive responses. These data may suggest that both cardiovagal excitation and sympatho-inhibition components of the reflex are mediated by the same subtype of EAA receptors. However, an important question associated with the autonomic responses to the activation of the Bezold–Jarisch reflex is related to the controversy about whether or not the activation of this reflex produces sympatho-inhibition in addition to intense cardiovagal excitation. Studies by Verberne and Guyenet [18] and Sevoz et al. [13] performed in anesthetized rats have shown that activation of the Bezold–Jarisch reflex with phenylbiguanide produces a sharp reduction in sympathetic nerve activity which does not seem to be enough to produce a significant fall in MAP, considering that an intense bradycardia was also observed. In the present study, considering that AP-5 blocked both bradycardia and hypotension, we evaluated in conscious rats whether or not the cholinergic blockade with methyl-atropine affects the fall in arterial pressure. The data show that both responses were completely abolished by methyl-atropine, indicating that the fall in arterial pressure was essentially due to an intense reduction in HR, which could momentarily reduce cardiac output and consequently arterial pressure.

The findings related to the blockade of bradycardia and hypotension by methyl-atropine are in accordance with previous studies by Willete et al. [18], which also showed that atropine blocked both bradycardic and hypotensive responses. However, in experiments performed in our laboratory (data not shown) with rats under urethane anesthesia, methyl-atropine abolished the bradycardic responses but did not block hypotensive response to Bezold–Jarisch reflex activation. This difference in the autonomic response to the activation of the Bezold–Jarisch reflex between conscious and anesthetized animals indicates that anesthesia produces an important change in the processing of this afferent system in the different synapses involved in this neural pathway.

The data obtained with AP-5 indicate that both bradycardia and hypotension are blocked by AP-5, suggesting that hypotension is also driven by the excitation of the same cardiovagal pathways in the NTS. Therefore, considering that both hypotension and bradycardia were blocked by AP-5 microinjected into the NTS and that methyl-atropine blocked the hypotension in addition to the bradycardia, we suggest that in conscious rats the activation of the Bezold–Jarisch reflex mainly produces an excitation of the cardiovagal component, which at the level of the NTS is mediated by the NMDA receptors.

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