Antagonism of adenosine A1 receptors in the NTS does not affect the chemoreflex in awake rats

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De Paula, Patrícia M., and Benedito H. Machado. Antagonism of adenosine A1 receptors in the NTS does not affect the chemoreflex in awake rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R2072–R2078, 2001.—The possible involvement of adenosine A1 receptors in neurotransmission of the sympathoexcitatory component of the chemoreflex in the nucleus tractus solitarii (NTS) of awake rats was evaluated. Unilateral microinjection of increasing doses of adenosine (0.01, 0.06, 0.12, 1.25, 2.5, and 5.0 nmol/50 nl) into the lateral aspect of the commissural NTS produced a long-lasting increase in baseline mean arterial pressure (MAP) and no changes in baseline heart rate (HR). Microinjection of adenosine at 1.25 nmol/50 nl (ED50) into the NTS (n = 9) produced a significant increase in baseline MAP (119 ± 3, 122 ± 4, and 117 ± 4 mmHg at 30 s, 1 min, and 2 min, respectively) compared with control (102 ± 3 mmHg) but no significant changes after previous microinjection of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A1 receptor antagonist (107 ± 3, 107 ± 3, and 106 ± 3 mmHg at 30 s, 1 min, and 2 min, respectively) compared with control (102 ± 3 mmHg). Microinjection of adenosine before and after DPCPX into the same site of the lateral commissural NTS produced no changes in baseline HR. In another group of rats (n = 8), microinjection of DPCPX (0.285 nmol/50 nl) into lateral and midline aspects of the commissural NTS produced no significant changes in pressor (+46 ± 4 vs. +47 ± 2 mmHg) or bradycardic responses (−216 ± 9 vs. −226 ± 12 beats/min) to chemoreflex activation with intravenous potassium cyanide compared with control responses. These data show that microinjection of adenosine into the NTS produced a small and long-lasting pressor response by activating A1 receptors and that blockade of these receptors produced no changes in cardiovascular responses to chemoreflex activation. We conclude that adenosine A1 receptors are not involved in processing of the chemoreflex afferents at the NTS level.

8-cyclopentyl-1,3-dipropylxanthine; purinergic receptors; sympathoexcitation; cardiovascular reflexes

ACTIVATION OF CAROTID CHEMORECEPTORS by intravenous injection of potassium cyanide (KCN) produces increases in arterial pressure and bradycardia (9, 10). In a previous study, Haibara et al. (13) showed that the bradycardic response to chemoreflex activation was mediated by N-methyl-D-aspartate (NMDA) receptors, because bilateral microinjection of dl-2-amino-5-phosphonovaleric acid (AP-5), a selective NMDA receptor antagonist, into the lateral aspect of the commissural nucleus tractus solitarii (NTS) blocked the bradycardic response in a dose-dependent manner and produced no effect on the pressor response to chemoreflex activation (sympathoexcitatory component). In another study, Haibara et al. (12) documented that bilateral microinjection of 6,7-dinitroquinoxaline-2,3-dione (DNQX, a selective non-NMDA receptor antagonist) or kynurenic acid (a nonselective ionotropic receptor antagonist) into the NTS produced only a partial blockade of the pressor response to chemoreflex activation, suggesting that the neurotransmission of the sympathoexcitatory component of the chemoreflex involves neurotransmitters other than L-glutamate.

In a series of recent studies, adenosine was considered a nonpeptide neurotransmitter or a neuromodulator in the central nervous system (2, 3, 5, 11, 12, 23, 24, 26, 27), and its concentration increased in different areas of the brain (16, 19, 34) and in cerebrospinal fluid (CSF) during hypoxia (4). There is also evidence that microinjection of adenosine into different areas of the brain produces cardiovascular responses (2, 30–32). Considering that the role of adenosine and adenosine receptors in the processing of the sympathoexcitatory component of the chemoreflex has not been previously studied, particularly in awake rats, in the present study we evaluated the cardiovascular effects of microinjection of adenosine into the NTS as well as the effect of the blockade of adenosine A1 receptors on the pressor response to chemoreflex activation in awake rats.

METHODS

Four days before the experiments, male Wistar rats (290–310 g body wt) were anesthetized with 2.5% tribromoethanol (1 ml/100 g ip; Aldrich Chemical, Milwaukee, WI) and placed in a stereotaxic apparatus (David Kopf, Tujunga, CA). The technique described by Michelini and Bonagamba (21) was adapted to implant guide cannulas in the following experimental protocols: 1) bilateral guide cannulas in the direction of the lateral NTS (0.5 mm lateral to midline and −0.5 mm...
rostral to calamus scriptorius) for injection of adenosine and the adenosine A₁ receptor antagonist and 2) three guide cannulas, two implanted in the direction of the lateral NTS (0.5 mm lateral to midline and ~0.5 mm rostral to calamus scriptorium) and one implanted in the direction of the medial NTS (0.0 mm lateral to midline and at the calamus scriptorium level) for injection of the adenosine A₁ receptor antagonist. The guide cannulas were implanted in accordance with the coordinates of the atlas of Paxinos and Watson (25). 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 a 15-mm-long stainless steel guide cannula (22 gauge) was introduced perpendicularly through the window at the following coordinates: 14.0 mm (lateral aspect of NTS) or 14.5 mm (medial aspect of NTS) caudal to the bregma, 0.5 mm (lateral aspect of NTS) or 0.0 mm (medial aspect of NTS) lateral to the midline, and 7.8 mm below the skull surface at the bregma (lateral and medial aspects of NTS). The tip of each guide cannula was positioned 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 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). 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 the 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. At the end of each experiment, Evans blue dye (2%, 50 nl) was microinjected for histological identification of the sites of microinjection, and later the animals were 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 thick) were cut and stained by the Nissl method. Only the rats in which the site of microinjection was located in the lateral aspect of the commissural NTS (adenosine protocol) or in the lateral and medial aspects of the commissural NTS (chemoreflex protocol) were considered for data analysis. On average, 30% of the rats implanted with the guide cannulas in the different experimental protocols presented positive histology; i.e., the injections were centered in the appropriate site in the NTS.

Adenosine was diluted in artificial CSF containing (in mM) 3 KCl, 0.6 MgCl₂, 2 CaCl₂, 132 NaCl, 24 NaHCO₃, and 4 dextrose, while the antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was dissolved in artificial CSF containing 2.5% DMSO (Sigma, St. Louis, MO), because it was not soluble in water. The solutions were freshly dissolved, and sodium bicarbonate was added to adjust pH to 7.0–7.4.

Initially, one specific group of rats was used to construct a dose-response curve to microinjection of adenosine into the NTS. Different doses of adenosine (0.01, 0.06, 0.12, 1.25, 2.5, and 5.0 nmol/50 nl) were microinjected into the NTS in a random sequence, and the changes in mean arterial pressure (MAP) and heart rate (HR) were evaluated at 30 s and 1, 2, 5, and 10 min after microinjection of each dose of adenosine into the NTS. At the end of the experimental protocol, the same volume of artificial CSF was microinjected into the NTS as a volume control. The dose of adenosine corresponding to the ED₅₀ was used to determine the effective dose of the antagonist DPCPX to be used in the experimental protocol.

Fig. 1. Changes in mean arterial pressure (MAP) and heart rate (HR) in response to microinjection of increasing doses of adenosine into the nucleus tractus solitarii (NTS) of awake rats. bpm, beats/min.

Fig. 2. Traces of 1 rat, representative of the group, showing HR, pulsatile arterial pressure (PAP), and MAP before and 30 s and 1, 2, 5, and 10 min after unilateral microinjection of adenosine (0.12 nmol/50 nl) into the lateral aspect of the commissural NTS.
involving the activation of the chemoreflex before and after bilateral microinjection of this antagonist into the NTS.

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 pulsatile arterial pressure (PAP), MAP, and 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 (Narco Trace 80, Narco Bio-Systems, Austin, TX). HR was quantified with a biotachometer coupler (model 7302, Narco) and recorded with the same polygraph.

The chemoreflex was activated by intravenous injection of KCN (40 mg/rat; Merck, Darmstadt, Germany) in accordance with the original studies by Franchini and Krieger (9, 10) and previous experiments from our laboratory (12, 13).

Values are means ± SE. Results were analyzed by one-way ANOVA, and the differences between individual means were determined by Student’s t-test, with the level of significance set at 0.05 in all analyses.

RESULTS

Dose-response curve to microinjection of adenosine into the NTS. Figure 1A shows the dose-response pressor response to microinjection of adenosine into the NTS. The dose of 0.12 nmol/50 nl produced an increase in MAP corresponding to ~50% of the maximal pressor response, and this dose (ED₅₀) was used in the next protocol to determine the effective dose of the antagonist to be used in the protocol involving the activation of the chemoreflex. Figure 1B shows that the changes in HR in response to microinjection of adenosine into the NTS also followed a dose-response pattern. However, the pattern of the changes in HR were different from the changes in arterial pressure: low doses produced a bradycardic response, while high doses produced a tachycardic response.

Fig. 3. Changes in HR, PAP, and MAP in response to unilateral microinjection of adenosine (0.12 nmol/50 nl) before and 1 and 30 min after unilateral microinjection of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.285 nmol/50 nl) into the lateral aspect of the commissural NTS of 1 rat, representative of the group.

Fig. 4. MAP and HR in response to unilateral microinjection of adenosine (0.12 nmol/50 nl) before and 1 min after unilateral microinjection of DPCPX (0.285 nmol/50 nl) into the lateral aspect of the commissural NTS (n = 9). *Significantly different from adenosine (ADN) control (P < 0.05).
**Effect of adenosine on MAP and HR.** Figure 2 is a typical trace of one rat representative of the group showing the changes in MAP and HR in response to microinjection into the NTS of 0.12 nmol/50 nl of adenosine, which corresponds to the ED\textsubscript{50}. Unilateral microinjection of this dose of adenosine into the lateral aspect of the commissural NTS produced a long-lasting pressor response and no significant changes in HR. Microinjection of adenosine into the NTS produced a pressor response lasting ~2 min, and 10 min later the MAP returned to the control level.

**Blockade of A\textsubscript{1} purinergic receptor with DPCPX.** Figure 3 is a typical trace of one rat representative of the group showing the effect of previous microinjection of DPCPX (0.285 nmol/50 nl) on the pressor response to adenosine (0.12 nmol/50 nl) microinjected into the lateral NTS. Figure 3A shows the cardiovascular responses to control injection of adenosine into the NTS. The pressor response to unilateral microinjection of ADN was blocked 1 min after unilateral microinjection of DPCPX (Fig. 3B) and returned to the control level 30 min later (Fig. 3C), showing the reversibility of the blockade. The data summarized in Fig. 4 indicate that DPCPX blocked the pressor response to microinjection of adenosine into the NTS. In addition, unilateral microinjection of vehicle (2.5% DMSO) into the lateral aspect of the commissural NTS produced no changes in the pressor response to microinjection of adenosine into the NTS.

**Effect of microinjection of DPCPX into the lateral and medial NTS on the cardiovascular responses to chemoreflex activation.** Figure 5 shows traces of one rat, representative of the group, in which microinjection of DPCPX (0.285 nmol/50 nl) into the lateral and medial NTS produced no changes in the pressor or bradycardic responses to chemoreflex activation. The data of the group summarized in Fig. 6 show that DPCPX microinjected into the lateral and medial NTS produced no significant changes in the cardiovascular response to chemoreflex activation.

**Histology of the sites of microinjections.** Figure 7 is a photomicrograph of a transverse section of the brain stem of one rat, representative of the group, showing the sites of microinjections into the lateral and medial aspects of the commissural NTS.

**DISCUSSION**

In addition to affecting the ventilatory adjustments, activation of the arterial chemoreceptors is also important in cardiovascular regulation (20), and the neurotransmission of the chemoreflex afferents in the NTS has been studied by several laboratories (12, 13, 17, 18, 22, 33). Different studies have shown that the activation of carotid chemoreceptors with KCN produces pressor and bradycardic responses (9, 10, 12, 13). Studies by Haibara et al. (13) showed that the bradycardic response to chemoreflex activation was blocked in a dose-dependent manner by AP-5, an NMDA receptor antagonist, whereas the pressor response was not affected. In another study, Haibara et al. (12) showed that different ionotropic (kynurenic acid and DNQX) or metabotropic (α-methyl-4-carboxyphenylglycine) receptor antagonists were not able to block the pressor response of the chemoreflex, suggesting that excitatory amino acid receptors may not be involved in the pro-

![Fig. 5. Changes in HR, PAP, and MAP in response to intravenous injection of potassium cyanide (KCN, 40 μg·0.1 ml\textsuperscript{-1}·rat\textsuperscript{-1}) before and 1, 15, 30, and 60 min after microinjection of DPCPX (0.285 nmol/50 nl) into the lateral and medial aspect of the commissural NTS of 1 rat, representative of the group.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00430.2001)
cessing of neurotransmission of the sympathoexcitatory component of the chemoreflex at the NTS level. On the basis of these findings by Haibara et al. (12, 13) and several other studies indicating an important role for adenosine and adenosine receptors in neurotransmission/neuromodulation in the central nervous system (26, 27), in the present study, we evaluated the possible role of adenosine and adenosine $A_1$ receptors in the neurotransmission/neuromodulation of the chemoreflex in the NTS of awake rats.

The data show that unilateral microinjection of adenosine (0.12 nmol/50 nl) into the lateral commissural NTS produced a long-lasting pressor response and no significant changes in HR. The pressor response to unilateral microinjection of adenosine was almost blocked 1 min after previous unilateral microinjection of DPCPX, and 30 min later it returned to control level, showing the reversibility of the blockade. These data related to the cardiovascular responses to microinjection of adenosine into the NTS are consistent with the finding of St. Lambert et al. (28, 29) indicating a high density of adenosine $A_1$ receptors in the NTS. Furthermore, the effect of DPCPX in blocking the cardiovascular responses to microinjection of adenosine into the NTS is also consistent with previous studies indicating that this antagonist has a high selectivity for adenosine $A_1$ receptor (14, 15).

The data of the present study, obtained in awake rats, are in accordance with studies by Abdel-Rahman and Mao (1), in which microinjection of adenosine into different subregions of the NTS in awake rats produced a pressor response, which was antagonized by DPCPX. Studies by Barraco and Phillis (3) also showed in anesthetized rats that microinjection of the adenosine $A_1$ receptor agonist $N^6$-cyclopentyladenosine into the NTS produced a pressor response that was blocked by DPCPX. However, studies by Tseng et al. (32), also performed on anesthetized rats, showed that microinjection of adenosine into the NTS produced hypotensive and bradycardic responses. These different cardiovascular responses to microinjection of adenosine into the NTS of anesthetized rats may be related to the anesthetic used as well as the level of anesthesia. The specific site of adenosine microinjection into the NTS and the selective activation of adenosine receptor subtypes (3) may also contribute to explaining the different responses to microinjection of adenosine observed in anesthetized rats.

The pressor response to microinjection of adenosine into the lateral aspect of the commissural NTS in awake rats produced a significant increase in baseline MAP (~15 mmHg) lasting for >5 min. This pattern of response differed from the fast and large increase in...
MAP produced by activation of the chemoreflex, and this profile seems to be typical of the responses to microinjections of neuromodulators and not of neurotransmitters (26, 27). Therefore, the profile of the cardiovascular responses to microinjection of adenosine into the NTS in the present study was the primary evidence that adenosine may not be the neurotransmitter of the chemoreflex at the NTS level.

Different studies have shown that the first synapse of the afferent projections of the carotid chemoreceptor afferents occurs in the lateral commissural NTS (8, 22). However, studies by Chitravanshi et al. (6, 7) showed that chemoreceptor afferents project mainly to the midline portion of the commissural NTS, at the calamus scriptorius level. Therefore, to evaluate the possible role of adenosine A1 receptors in the neurotransmission/neuromodulation of the pressor response to chemoreflex, we microinjected DPCPX simultaneously into the different subregions (lateral and medial) of the commissural NTS.

The data of the present study show that microinjection of DPCPX into the lateral and medial aspects of the NTS produced no significant changes in the pressor (sympathoexcitatory component) or bradycardic (cardiovagal component) responses to chemoreflex activation. Therefore, these data indicate that adenosine A1 receptors are not involved in the neuromodulation/neurotransmission of this reflex at the NTS level. It is important to note that microinjection of DPCPX into the lateral and medial commissural NTS had no effect on baseline MAP, suggesting that adenosine A1 receptors also play no major role in the tonic regulation of arterial pressure.

Although adenosine A1 receptors play no major role in the neurotransmission of the chemoreflex in the NTS, the effect of adenosine microinjection on baseline MAP is an important aspect that remains to be understood. The possibility that adenosine plays a role as a neuromodulator of the baroreflex has been studied by Mosqueda-Garcia et al. (23), and their data showed that microinjection of the adenosine A1 receptor antagonist into the NTS of anesthetized rats inhibited the baroreflex bradycardia, indicating that the adenosine A1 receptor may have a neuromodulatory role in the cardiovagal component of the baroreflex. However, the role of adenosine and its different receptor subtypes in the processing of the baroreflex in the commissural NTS of awake rats is an important matter that requires further investigation.

In conclusion, the present data show that blockade of the adenosine A1 receptors in the NTS produced no effect on the sympathoexcitatory component (pressor response) of the chemoreflex, indicating that this subtype of purinergic receptors is not involved in the neurotransmission of the chemoreflex at the NTS level.

Perspectives

The neurotransmission of the sympathoexcitatory component of the chemoreflex is an important aspect of the autonomic processing of the cardiovascular reflexes in the NTS, which we are exploring in our laboratory in awake rats. In previous studies we were not able to block the pressor response to chemoreflex activation with microinjections into the NTS of antagonists of the excitatory amino acid receptors (kynurenic acid, DNQX, AP-5, and α-methyl-4-carboxyphenylglycine) or an antagonist of substance P (neurokinin-1 receptor antagonist). The data of the present study showing that DPCPX produced no effect on the cardiovascular responses to chemoreflex activation indicate that adenosine A1 receptors also play no major role in the neurotransmission of the sympathoexcitatory component of the chemoreflex at the NTS level. The involvement of other subtypes of adenosine (A2 and A3) or ATP (P2) receptors in the neurotransmission of the sympathoexcitatory component of the chemoreflex in the NTS remains to be further explored. In the present study we also verified that microinjection of adenosine into the NTS produced an increase in baseline MAP, suggesting that adenosine may be involved in the modulation of the sympathoinhibitory component of the baroreflex. Therefore, a possible neuromodulatory role of adenosine A1 receptors in the NTS in the gain of the baroreflex of awake rats is another important aspect that also requires further investigation. Additional studies on purinergic receptors in the neurotransmission/neuromodulation of the autonomic processing of the cardiovascular reflexes at the NTS level will provide new knowledge about its physiological relevance as well as its possible physiopathological implication in abnormalities such as hypertension.

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