Cardiovascular responses to microinjection of ATP into the nucleus tractus solitarii of awake rats

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De Paula, Patrícia M., Vagner R. Antunes, Leni G. H. Bonagamba, and Benedito H. Machado. Cardiovascular responses to microinjection of ATP into the nucleus tractus solitarii of awake rats. Am J Physiol Regul Integr Comp Physiol 287: R1164–R1171, 2004. First published July 1, 2004; doi:10.1152/ajpregu.00722.2003.—Microinjection of increasing doses of ATP (0.31, 0.62, 1.25, and 2.5 nmol/50 nl) into the nucleus tractus solitarii (NTS) produced a dose-dependent pressor response. Prazosin abolished the pressor response and produced no change in the bradycardic response to ATP. Microinjection of pyridoxal phosphate-6-azophenyl-2’,4’-disulfonic acid (0.25 nmol/50 nl), a nonselective P2 receptor antagonist into the NTS, reduced the bradycardic response but had no effect on the pressor response to microinjection of ATP (1.25 nmol/50 nl) into the NTS. Microinjection of suramin (2 nmol/50 nl), another nonselective P2 receptor antagonist, had no effect on the pressor and bradycardic responses to microinjection of ATP (1.25 nmol/50 nl) into the NTS. Antagonism of A1 receptors of adenosine with 1,3-dipropyl-8-cyclopentylxanthine also produced no changes in the cardiovascular responses to microinjection of ATP into the NTS. The involvement of excitatory amino acid (EAA) receptors in the pressor and bradycardic responses to microinjection of ATP into the NTS was also evaluated. Microinjection of kynurenic acid, a nonselective EAA receptor antagonist (10 nmol/50 nl), into the NTS reduced the bradycardic response and had no effect on the pressor response to microinjection of ATP into the NTS. The data show that 1) microinjection of ATP into the NTS of awake rats produced pressor and bradycardic responses by independent mechanisms, 2) the activation of parasympathetic component may involve an interaction of P2 and EAA receptors in the NTS, and 3) the sympathoexcitatory response to microinjection of ATP into the NTS was not affected by the blockade of P2, A1, or EAA receptors.

THE CONCEPT THAT ATP may act as a nonadrenergic noncholinergic neurotransmitter on peripheral nerves was established by Burnstock (4), and several recent studies indicate that ATP and adenosine can act as neurotransmitters or neuromodulators in the central nervous system, including areas involved in cardiovascular regulation, such as the nucleus tractus solitarii (NTS) (1–3, 7, 8, 13, 19, 20, 24, 26–28, 33). Immunohistochemical studies documented the presence of different subtypes of P2 receptors in the NTS of rats (11, 15, 34, 35). ATP interacts with two distinct families of receptors, P2X and P2Y, which differ in structure and signal transduction mechanism (9, 21). There is evidence indicating that the available P2 receptor antagonists such as pyridoxal phosphate-6-azophenyl-2’,4’-disulfonic acid (PPADS) and suramin produced no blockade of P2X4 and P2X6 receptor subtypes (25).

Studies performed in anesthetized rats documented that microinjection of ATP into the NTS produced a fall in arterial pressure and bradycardia, which were altered in different manner by suramin (8). These cardiovascular responses to ATP in anesthetized rats exhibited a typical pattern of fast transmitter similar to the responses to microinjection of L-glutamate into the NTS of anesthetized rats, i.e., a fall in arterial pressure and bradycardia (14, 32). Studies from our laboratory have shown that microinjection of L-glutamate into the NTS of conscious rats produced a dose-related pressor and bradycardic response, contrasting to the dose-related depressor response observed when the same rats were studied under urethane or chloralose anesthesia (16), indicating that anesthetics may affect the autonomic responses to microinjection of a putative neurotransmitter into the NTS. To avoid this potential problem, the present study was performed in awake rats, and the following aspects related to the purinergic transmission in the NTS were evaluated: 1) the effect of microinjection of increasing doses of ATP into the NTS on mean arterial pressure (MAP) and heart rate (HR), 2) the autonomic components involved in the cardiovascular responses to microinjection of ATP, and 3) the effect of previous microinjection of P2 receptor antagonists available (suramin or PPADS), A1 receptor antagonist [8-cyclopentyl-1,3-dipropylxanthine (DPCPX)], or excitatory amino acid (EAA) receptor antagonist (kynurenic acid) into the NTS on the cardiovascular responses to microinjection of ATP into the NTS.

METHODS

All experimental approaches were performed in accordance with the Guide for the Care and Use of Laboratory Animals and the Ethical Principles for Animal Experimentation established by the Brazilian Committee for Animal Experimentation and approved by the Animal Care and Ethics Committee of the School of Medicine of Ribeirão Preto, University of São Paulo.

Four days before the experiments, male Wistar rats weighing 290–310 g were anesthetized with tribromoethanol (250 mg/kg ip, Aldrich Chemical, Milwaukee, WI) and placed in a stereotaxic apparatus (David Kopf, Tujunga, CA). The technique described by Michelini and Bonagamba (17) was adapted to implant bilateral guide cannulae in the direction of the lateral aspect of the commissural NTS (0.5 mm lateral to the midline and ~0.5 mm rostral to the calamus scriptorium). The guide cannulae were implanted according to the coordinates of the atlas of Paxinos and Watson (23). Additional anesthesia was provided when the rat reacted to frequent toe pinching during stereotaxic surgery. Bilateral guide cannulae [a 15-mm-long stainless steel guide cannula (22-gauge)] were introduced perpendic-
ularly through a small window in the skull at the following coordinates: 14.5 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.8 mm below the skull surface at the bregma. The tip of each guide cannula was positioned in the cerebellum ~1.0 mm above the dorsal surface of the brain stem.

The guide cannulae 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 polyethylene 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. Manual injection was initiated 30 s later, and the volume microinjected in all experimental protocols was 50 nl. Guide cannulae were implanted bilaterally to increase the possibility that, at least in one side, the cannula was correctly positioned in the direction of the NTS, and only the first side responsive to microinjection was used for microinjections in all experimental protocols.

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. The catheter was 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 was measured with a pressure transducer (model CDX III, Cobe Laboratories, Lakewood, CO) connected to a polygraph (Narcotrace 80, Narco Bio-Systems, Austin, TX). MAP was derived from the PAP using a universal coupler (model 7189, Narco Bio-Systems), and HR was quantified from the PAP using a Biotachometer Coupler (model 7302, Narco Bio-Systems) and recorded in the same polygraph.

Five experimental protocols related to the microinjection of ATP into the NTS of awake rats were used in the present study. In the first protocol, the dose-related effects of microinjection of increasing doses of ATP (0.31, 0.62, 1.25, and 2.5 nmol/50 nl) into the NTS before and after microinjection of kynurenic acid (1.25 nmol/50 nl) was microinjected into the NTS before and after microinjection of PPADS (0.25 nmol/50 nl) or suramin (2 nmol/50 nl) in different groups of rats. In the fourth protocol, ATP was microinjected into the NTS before and after microinjection of kynurenic acid (10 nmol/50 nl) into the NTS. In the fifth protocol, ATP was microinjected into the NTS before and after microinjection of an effective dose of DPCPX, a selective A1 adenosine receptors antagonist (0.285 nmol/50 nl), in accordance with a previous study from our laboratory (7). The drugs used in these experiments (Sigma Chemical, St. Louis, MO) were ATP, prazosin, methyl-atropine, kynurenic acid, suramin, PPADS, and DPCPX. All drugs were freshly dissolved in saline (154 mM NaCl), and sodium bicarbonate was added to adjust the pH to 7.4.

At the end of each experiment, 50 nl of 2% Evans blue were microinjected into the same sites of the NTS for histological analysis. The animals were killed with an overdose of thiopental sodium (100 mg/kg iv) and submitted to intracardiac perfusion with saline (154 mM) followed by 10% buffered formalin. The brains were removed and stored in buffered formalin for 2 days, and serial coronal sections (15-µm thickness) were cut and stained by the Nissl method. Only the rats with the center of the microinjection site located in the lateral aspect of the commissural NTS were considered for data analysis. All data are expressed as means ± SE. The 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 P ≤ 0.05.

RESULTS

Dose-response curve to microinjection of ATP into the NTS.

Figure 1 presents typical tracings of four rats representative of their respective groups showing the changes in HR, PAP, and MAP in response to microinjection of increasing doses of ATP (0.31, 0.62, 1.25, and 2.5 nmol/50 nl) into the NTS. Unilateral microinjection of ATP into the commissural NTS produced a dose-dependent pressor response. In this range of doses, the magnitude of the bradycardic response does not follow a dose-related pattern. Figure 1 also shows that ATP produced a consistent initial hypotensive response, which was secondary to the intense bradycardic response, because intravenous injec-

![Fig. 1. Tracings of 4 rats, representative of their respective groups, showing the changes in heart rate (HR), pulsatile arterial pressure (PAP), and mean arterial pressure (MAP) in response to microinjection of increasing doses of ATP (0.31, 0.62, 1.25, and 2.5 nmol/50 nl) into the nucleus tractus solitarii (NTS) of awake rats. bpm, Beats/min.](http://ajpregu.physiology.org/)
tion of methyl atropine abolished the bradycardia and the initial hypotensive response to microinjection of ATP into the NTS (data not shown). The pressor and bradycardic responses are summarized in Fig. 2, and Fig. 2, top, shows that the pressor response to microinjection of ATP into the NTS is dose dependent. The dose of 1.25 nmol/50 nl produced an increase in MAP corresponding to approximately the half-maximal effective dose, and it was used in the subsequent protocols against P2, A1, and EAA receptor antagonists. Figure 2, bottom, summarizes the data related to HR and shows that the bradycardic response to microinjection of ATP into the NTS was at the maximal level with the lowest dose used (0.31 nmol/50 nl), indicating that this dose was already at the plateau of the dose-response curve.

Microinjection of the vehicle (50 nl, saline) into the NTS in a group of rats (n = 8) used in this experimental protocol produced a negligible effect on the baseline MAP (–4 ± 4 mmHg) and HR (–10 ± 13 beats/min).

Peripheral autonomic blockade with prazosin or methyl atropine. Figure 3A shows that the pressor response produced by microinjection of ATP (1.25 nmol/50 nl) into the NTS was replaced by a hypotensive response 5 min after prazosin (+37 ± 4 vs. –25 ± 12 mmHg), whereas Fig. 3C shows that the bradycardic response was not significantly altered after prazosin (–215 ± 23 vs. –291 ± 22 beats/min). Figure 3B shows that the pressor response to microinjection of ATP into the NTS 2 min after methyl atropine was not statistically different in relation to the control pressor response (51 ± 7 vs. 68 ± 5 mmHg). Figure 3D shows that microinjection of ATP into the NTS produced bradycardia, which was replaced by a tachycardic response 2 min after methyl atropine (–238 ± 21 vs. +53 ± 10 beats/min).

Blockade of P2 receptors with PPADS or suramin. Figure 4 presents typical tracings of one rat, representative of the group, showing that 2 min after microinjection of PPADS (0.25 nmol/50 nl) into the commissural NTS the bradycardic response to microinjection of ATP into the same site of the commissural NTS was significantly reduced and that after 10 min it was back to the control level. Previous microinjection of PPADS produced no major change in the pressor response to microinjection of ATP into the commissural NTS. The data of this experimental protocol are summarized in Fig. 5, and Fig. 5, bottom, shows that the bradycardic response was significantly reduced only on minute 2 after microinjection of PPADS (–224 ± 10 vs. –177 ± 10 beats/min), and Fig. 5, top, shows no significant change in the pressor response (25 ± 3 vs. 32 ± 6 mmHg) to microinjection of ATP into the same site of the commissural NTS.

With respect to suramin (2 nmol/50 nl), we verified that the pressor (19 ± 7 vs. 37 ± 5 mmHg) and bradycardic responses (–216 ± 23 vs. –224 ± 14 beats/min) to microinjection of ATP into the NTS of a specific group of rats (n = 7) were not significantly altered by previous microinjection of this P2 receptor antagonist into the same site in the NTS.

Blockade of A1 receptors of adenosine with DPCPX. In another specific group of rats (n = 4), the microinjection of ATP into the NTS was performed before and 2 and 10 min after local microinjection of DPCPX, an adenosine A1 receptor antagonist. The data show that pressor (42 ± 8 vs. 40 ± 7 mmHg) and bradycardic responses (–225 ± 25 vs. –222 ± 38 beats/min) to microinjection of ATP on minute 2 after local microinjection of DPCPX (0.285 nmol/50 nl) were not statistically different in relation to the control responses. At minute 10 after DPCPX, the pressor (42 ± 9 mmHg) and bradycardic responses (–237 ± 12 beats/min) to microinjection of ATP were also not different in relation to control.

Blockade of EAA receptors with kynurenic acid. Figure 6 presents typical tracings of one rat, representative of the group, showing the effect of previous microinjection of kynurenic acid (10 nmol/50 nl) on the cardiovascular responses to ATP (1.25 nmol/50 nl) microinjected into the commissural NTS. Microinjection of kynurenic acid into the commissural NTS almost blocked the bradycardic response and produced no major changes in the pressor response to microinjection of ATP. The effect of kynurenic acid was reversible considering that, 30 min later, the bradycardic response was back to the control level. The data of this experimental protocol are summarized in Fig. 7 and indicate that kynurenic acid microinjected into the commissural NTS produced no significant changes in the pressor responses (Fig. 7, top) and a significant reduction in the bradycardic response to microinjection of ATP into the commissural NTS on minute 2 (–64 ± 23 beats/min) and minute 10 (–83 ± 24 beats/min) compared with the control response (–234 ± 15 beats/min).

Histology. Figure 8A is a photomicrograph of a transverse section of the brain stem of one rat, representative of all groups studied, showing the site of unilateral microinjection into the lateral aspect of the commissural NTS. Figure 8B is a line drawing of a transverse section of the brain stem (~13.7 mm caudal to the bregma), modified from Paxinos and Watson (23), showing the center of microinjections into the lateral aspect of the commissural NTS (dark circles) of 8 representative rats from a group of 54 animals presenting positive histology, which were used in the five experimental protocols of the present study.
Fig. 3. Left: changes in MAP (A) and HR (C) in response to microinjection of ATP into the NTS before (control, closed bars) and 5 min after intravenous injection of prazosin (1 mg/kg iv; hatched bars; n = 7). Right: changes in MAP (B) and HR (D) in response to microinjection of ATP into the NTS before (control, closed bars) and 2 min after intravenous injection of methyl atropine (2 mg/kg iv; hatched bars; n = 6). *Significantly different from control (P ≤ 0.05).

Fig. 4. Tracing of 1 rat, representative of the group, showing the changes in HR, PAP, and MAP in response to unilateral microinjection of ATP (1.25 nmol/50 nl) before (control) and 2, 10, 30, and 60 min after microinjection of pyridoxal phosphate-6-azophenyl-2,4'-disulfonic acid (PPADS; 0.25 nmol/50 nl) into the commissural NTS.
DISCUSSION

The data of the present study shows that microinjection of ATP into the NTS of awake rats produced important cardiovascular responses. However, it is important to note that the endogenous release of ATP in the synapses of the NTS may not necessarily produce the same pattern of responses to microinjections, especially due to the relatively large volume of 50 nl used in this experimental approach, which may reach different subpopulations of neurons and interneurons related to a diverse neuronal network at the NTS level. In fact, there are several technical limitations for studying specific synaptic transmission in the central nervous system, especially in the NTS of whole animals in anesthetized or awake preparations.

The most important finding of the present study is related to the dose-dependent increase in MAP in a range from 10 to 40 mmHg in response to microinjection of ATP into the NTS of awake rats. In studies performed in anesthetized rats, the microinjection of ATP or the noncatabolizing analog α,β-methylene ATP into the NTS usually produced a depressor response (8, 27, 28). It is conceivable that anesthetics play a critical role that may explain the difference in terms of the pattern of the cardiovascular response to microinjections of a putative neurotransmitter in the NTS. In a previous study from our laboratory, we verified that microinjection of L-glutamate into the NTS of awake rats produced an increase in arterial pressure, whereas the microinjection of the same dose into the NTS of the same rat, under anesthesia, produced a depressor response (16). The observed increase in the MAP in response to microinjection of ATP into the NTS of awake rats strongly suggest that this nucleotide and P2 receptors may play important roles in the processing of the sympathoexcitatory responses at the NTS level.

The increase in the arterial pressure in response to microinjection of ATP into the NTS was really due to sympathoexcitation because the intravenous injection of prazosin, a α1-adrenoceptor antagonist, abolished the pressor response. In fact, microinjection of ATP after prazosin produced an important hypotensive response, which appears to be essentially dependent on the remaining intense bradycardic response. In the case of the blockade of the bradycardic response with methyl atropine, we verified that microinjection of ATP produced an increase in HR (Fig. 3D) and a tendency of additional increase in MAP (Fig. 3B). Despite a predominant cardiac parasympathetic excitatory effect produced by ATP, it is remarkable that this nucleotide microinjected into the NTS also produced an increase in the sympathetic activity to the heart, observed only after the blockade of the parasympathetic component.

In the present study, we verified that the pressor response to microinjection of ATP into the NTS was not blocked by PPADS or suramin. These data from the experimental protocols using different P2 receptor antagonists raise doubts about the selectivity and the efficacy of these compounds. Although we had used a relatively large concentration of these antagonists, compared with other studies performed in anesthetized rats (8) or in the working heart-brain stem preparation (22), we were not able to affect the pressor response to microinjection of ATP.

Considering that ATP activates P2 receptors, we must consider other mechanisms than those explored in the present study.
study to understand why the blockade of these receptors were not effective in reducing the pressor response to microinjection of ATP into the NTS. There is evidence indicating that PPADS and suramin, the available P2 receptor antagonists used in the present study, are not effective in the blockade of P2X4 and P2X6 receptor subtypes (25). Therefore, further studies using different approaches such as 1) a more selective antagonists of P2 receptors subtypes, 2) antibodies to these specific receptors subtypes, and 3) ATP antisense should be a requirement in subsequent studies for our understanding of the effective role of P2 receptors in the sympathoexcitatory response to microinjection of ATP into the NTS.

Studies performed in anesthetized or in vitro preparations (6, 12, 18, 31) indicated that the responses to application of ATP into neural tissues were mediated by adenosine. In a study by Kato and Shigetomi (12) performed in brain stem slices, an inhibitory effect of ATP was shown on the NTS neurons, which was not reproduced by α,β-methylene ATP, a noncatabolizing ATP analog, and also was not affected by PPADS. In fact, Kato and Shigetomi (12) were able to block this inhibitory effect of ATP with adenosine A1 receptor antagonists 8-cyclopentyltheophylline or DPCPX, indicating that this inhibitory effect of ATP was mediated by adenosine A1 receptors. For this reason, in the present study, we also explored the possibility that the adenosine resulting from ATP catabolization may be acting on A1 receptors and producing the observed pressor response. However, the data shows that the A1 receptor antagonism with DPCPX, in a dose that was effective in blocking the effect of microinjection of adenosine into the NTS (7), produced no effect on the cardiovascular responses to microinjection of ATP, ruling out the possible involvement of A1 receptors in the pressor response to microinjection of ATP into the NTS of awake rats.

In a study performed in anesthetized rats, Ergene et al. (8) documented that microinjection of ATP into the NTS produced a depressor response. In these experiments, suramin produced a diverse pattern of changes in the depressor response to ATP: 1) it transformed the depressor response in a transient increase in pressure; 2) it produced an augmentation of the depressor response, or 3) it completely blocked the depressor response to ATP. Therefore, suramin may affect the P2 receptors in the NTS in a different manner, probably due to the fact that it may also affect P2Y receptors and also because some of the P2X receptors, such as P2X4 and P2X6, are not blocked by suramin (25). Ergene et al. (8) also documented that suramin was effective in blocking the effect of α,β-methylene ATP, a noncatabolizing analog of ATP. Therefore, it is plausible that the effects of different compounds resulting from the catabolization of ATP are not necessarily blocked by suramin.

In the present study, performed in awake rats, the data indicates that PPADS was effective because it produced a significant reduction in the bradycardic but not in the pressor response to microinjection of ATP. To explain the absence of the blockade of the pressor response, we may also consider the possibility that the pressor effect of ATP after suramin or PPADS is related to the activation of a different neurotransmitter system, considering that ATP may act as a cotransmitter with different neurotransmitters (5). In this case, the EAA L-glutamate is the natural candidate for this cotransmission, and considering the possibility that ATP may also release L-glutamate and EAA receptors. We verified that the antagonism of the EAA receptors blocked the bradycardic response but did not affect the pressor response to microinjection to ATP, suggesting that the sympathoexcitatory response induced by ATP in the NTS does not involve L-glutamate and EAA receptors. These data suggest that the cardiovascular responses to microinjection of ATP into the NTS are mediated by two different mechanisms: 1) a glutamatergic mechanism for the bradycardia (parasympathetic com-

![Fig. 7. ΔMAP (top) and ΔHR (bottom) in response to unilateral microinjection of ATP (1.25 nmol/50 nl) into the NTS before (control) and 2, 10, 30, and 60 min after unilateral microinjection of kynurenic acid (10 nmol/50 nl) into the commissural NTS (n = 11). *Significantly different from ATP control (P ≤ 0.05).](image)

![Fig. 8. A: photomicrograph of a transverse section of the brain stem showing a unilateral microinjection site in the lateral aspect of the commissural NTS. Arrow shows the center of microinjection at the level of the area postrema (X30). AP, area postrema; CC, central canal. B: line drawing of a transverse section of the brain stem (~13.7 mm caudal to the bregma, adapted from Paxinos and Watson (23) showing the centers of microinjections in the commissural NTS (dark circles: 3 on the right and 5 on the left side) of 8 representative rats of the group of 16 animals used in the protocol of the dose-response curve to microinjection of ATP into the NTS. The site of the center of the microinjection into the NTS of the remaining 46 rats with positive histology used in the 5 different experimental protocols were located in the same subregion of the NTS. 10, dorsal motor nucleus of the vagus; 12, hypoglossal nucleus.](image)

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ponent) and 2) an unknown mechanism for the pressor response (sympathetic component). In a recent study by Scislo and O’Leary (29), it was documented that the blockade of ionotropic glutamatergic receptors abolished the fast but not the slow component of the response to activation of P2 receptors by α,β-methylene ATP. These findings indicate a complex interaction of P2 with glutamatergic ionotropic receptors at the NTS level, which requires further investigation.

The bradycardic response to microinjection of ATP into the NTS was not due to the activation of the baroreflex, because the α1-adrenoceptor antagonism with prazosin blocked the pressor response to ATP but did not affect the bradycardic response, which was abolished only after muscarinic receptors blockade with methyl atropine, indicating again that microinjection of ATP into the NTS trigger two independent autonomic pathways, i.e., a parasympathetic pathway related to the bradycardic response and a sympathetic pathway related to the pressor response. The pattern of the bradycardic response to microinjection of ATP into the NTS was not dose dependent, suggesting that the magnitude of the bradycardic responses was already at the plateau of the dose-response curve even with the lowest dose used. This possibility suggests a different sensitivity of the autonomic components activated by ATP in the NTS and also that the parasympathetic component is more sensitive to ATP than the sympathetic component. We may also suggest an interaction of ATP and L-glutamate on EAA receptors, considering that PPADS, a P2 receptor antagonist, or kynurenic acid, a nonselective EAA receptor antagonist, significantly reduced the bradycardic response to microinjection of ATP into the NTS. In this direction, there is evidence that ATP acting on P2X receptors at presynaptic level in the dorsal root ganglion neurons results in the increase of glutamate release (10) and also that activation of presynaptic P2X receptors with α,β-methylene ATP in the NTS triggered Ca2+-dependent glutamate release (30). In addition, recent studies by Paton et al. (22) using an anaesthetized decerebrate working heart-brain stem preparation showed that bilateral microinjection of suramin or PPADS into the commissural NTS produced a significant reduction in the bradycardic responses to chemoreflex activation, suggesting that ATP and P2 receptors are important in the processing of the parasympathetic component of the chemoreflex at the NTS level. Whether the bradycardic response to microinjection of ATP into the NTS is due to a presynaptic release of L-glutamate is an important matter for further investigation.

We conclude that microinjection of ATP into the NTS of awake rats produced two independent cardiovascular responses, i.e., pressor and bradycardic responses. The bradycardic response involves P2 and EAA receptors, whereas the pressor response was not affected by the blockade of P2 receptors of ATP or A1 receptors of adenosine. The interaction of P2 and EAA mechanisms in the processing of the parasympathetic component at the NTS level is still a matter for further investigation. A new series of functional experiments using more selective antagonists for each subtype of P2 receptors are required to elucidate the specific mechanisms involved in the pressor response to microinjection of ATP into the NTS of awake rats.

**Perspectives.** The pressor and bradycardic responses to microinjection of ATP into the NTS open a very interesting possibility about the involvement of this purine in the processing of the cardiovascular reflexes at this level of the central nervous system. The most attractive possibility to be explored in further studies is related to the neurotransmission of the sympathoexcitatory component of the chemoreflex, considering that the pattern of the pressor response to microinjection of ATP into the NTS is similar to that observed in response to chemoreflex activation with potassium cyanide. However, the data of the present study clearly indicate that for a better pharmacological evaluation of the involvement of ATP and P2 receptors in the processing of the sympathoexcitatory component of the chemoreflex, at the NTS level, it will be necessary to use the selective antagonists for the different P2 receptors subtypes. The use of P2 receptor antibodies and/or ATP antisense will be also useful for a precise evaluation of the purinergic neurotransmission at the NTS level. Another important aspect that also requires further investigation is related to the interaction of glutamatergic and purinergic mechanisms in the neurotransmission of the cardiovascular reflexes at the NTS, especially on the bradycardic components of the baroreflex, chemoreflex, and cardiopulmonary reflex. The evaluation of the possible interaction of respiratory and sympathetic mechanisms related to the complex pattern of responses to microinjection of ATP into the NTS is another important subject to be investigated. The complete evaluation of the role of purinergic neurotransmission in the processing of the sympathoexcitatory component of the chemoreflex at the NTS level, for example, may bring an important contribution to the understanding of the mechanisms underlying the sympathetic overactivity observed in pathophysiological situations such as hypertension, obstructive sleep apnea, and heart failure.

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