Adenosine Modulates α_2 -Adrenergic Receptors within Specific Subnuclei of the Nucleus Tractus Solitarius in Normotensive and Spontaneously Hypertensive Rats

Daniel C. CARRETTIERO¹⁾, Renato S. ALMEIDA¹⁾, and Debora R. FIOR-CHADI¹⁾

Adenosine is known to modulate neuronal activity within the nucleus tractus solitarius (NTS). The modulatory effect of adenosine A_1 receptors (A_{1R}) on α_2 -adrenoceptors (Adr_{2R}) was evaluated using quantitative radioautography within NTS subnuclei and using neuronal culture of normotensive (WKY) and spontaneously hypertensive rats (SHR). Radioautography was used in a saturation experiment to measure Adr_{2R} binding parameters (B_{max} , K_d) in the presence of 3 different concentrations of N^6 -cyclopentyladenosine (CPA), an A_{1R} agonist. Neuronal culture confirmed our radioautographic results. [3H]RX821002, an Adr_{2R} antagonist, was used as a ligand for both approaches. The dorsomedial/dorsolateral subnucleus of WKY showed an increase in B_{max} values (21%) induced by 10 nmol/L of CPA. However, the subpostremal subnucleus showed a decrease in K_d values (24%) induced by 10 nmol/L of CPA. SHR showed the same pattern of changes as WKY within the same subnuclei; however, the modulatory effect of CPA was induced by 1 nmol/L (increased B_{max} , 17%; decreased K_d , 26%). Cell culture confirmed these results, because 10^{-5} and 10^{-7} mol/L of CPA promoted an increase in [3H]RX821002 binding of WKY (53%) and SHR cells (48%), respectively. DPCPX, an A_{1R} antagonist, was used to block the modulatory effect promoted by CPA with respect to Adr_{2R} binding. In conclusion, our study shows for the first time an interaction between A_{1R} that increases the binding of Adr_{2R} within specific subnuclei of the NTS. This may be important in understanding the complex autonomic response induced by adenosine within the NTS. In addition, changes in interactions between receptors might be relevant to understanding the development of hypertension. (*Hypertens Res* 2008; 31: 2177–2186)

Key Words: adenosine A_1 receptor, α_2 -adrenoceptor, nucleus tractus solitarius, hypertension, modulation

Introduction

Adenosine is an endogenous nucleoside that generally acts as a neuromodulator in the central nervous system (CNS). Its actions are mediated by adenosine receptors, four of which have been cloned and pharmacologically characterized: A_1 , A_{2A} , A_{2B} , and A_3 (1). Among the four subtypes, A_1 and A_{2A} are associated with behavioral effects that are exhibited in ani-

mals treated with adenosine analogs (2). Numerous studies have shown substantial evidence that adenosine modulates cardiovascular control at the level of the nucleus tractus solitarius (NTS) (3–6). However, the exact mechanism of this modulation is not well understood.

The NTS is the main nucleus responsible for integrating different signals from other brain nuclei or organs in order to originate a specific and orchestrated regional autonomic response. This nucleus contains the greatest density of adeno-

From the ¹⁾Department of Physiology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil.

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Address for Reprints: Debora R. Fior-Chadi, Ph.D., Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, 321, 05508–900 São Paulo, SP, Brasil. E-mail: drfchadi@usp.br

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sine uptake sites in the CNS (7). Adenosine is released within the NTS, primarily by the baroreceptor afferent and hypothalamic defense areas (8). Barraco and Phillis (4) have reported that stimulation of adenosine A₁ and A_{2A} receptors in subpostremal NTS evokes pressor and depressor responses, respectively. Such experiments also increase or decrease the heart rate and other regional sympathetic activity (9, 10). Scislo and O'Leary (6) have shown that in approximately 30% of cases a biphasic or depressor response may be elicited by stimulation of the adenosine A₁ receptor.

This finding suggests that adenosine A₁ receptors may regulate the pressor response in a certain way within the context of more complex NTS processing. Indeed, studies from our laboratory (11) have reported that adenosine A₁ receptors are heterogeneously distributed in three subnuclei located longitudinally from the rostral to the caudal NTS, namely the dorsolateral/dorsomedial, medial/intermediate, and subpostremal NTS subnuclei. This might partially explain the complex responses promoted by adenosine A₁ receptors within the NTS circuitry, although the biphasic or depressor responses are not yet understood.

Changing receptor function with the stimulation of other receptor, namely receptor crosstalk, has been extensively studied for noradrenergic axon terminals (12). The noradrenergic neurons of the CNS are endowed with presynaptic receptors by which noradrenaline release is inhibited by noradrenaline itself (*via* α_2 -autoreceptor) and by other neurotransmitters (*via* heteroreceptors) like adenosine A₁ receptor (13, 14). The interaction between receptors has also been studied and characterized in other systems, including those that feature the well-known adenosine A₂/dopamine D₂ interaction (15–17).

The noradrenergic system is well characterized in the NTS (18). Stimulation of α_2 -adrenoceptor within this nucleus promotes a hypotensive response (19). Adenosine A₁ receptors have been specially reported to modulate the α_2 -adrenoceptors in the hippocampus and spinal cord (13, 14). Both types of receptors are abundant in the NTS and their activation leads to cardiovascular changes (13, 14, 20). In this context, we could postulate that adenosine A₁ receptor activation might intensify the hypotensive response promoted by noradrenaline action within the NTS, thereby explaining the biphasic or depressor responses observed by Scislo and O'Leary (6). Taken together, this may indicate a possible interaction between adenosine A₁ receptors and α_2 -adrenoceptors within the NTS. This possibility may, in turn, be relevant for central cardiovascular control and therapeutic treatments.

The medulla oblongata, the region where the NTS is located, is the only brain area that shows significantly lower levels of noradrenaline and a reduced number and affinity of α_2 -adrenoceptors in spontaneously hypertensive rat (SHR) compared with the normotensive Wistar Kyoto (WKY) rat. This is true even as early as 4 weeks after birth, when hypertension is not yet apparent. This result suggests that certain mechanisms in the NTS α_2 -adrenergic transmission system

may be altered and could trigger the development of hypertension in SHR (21). Functional defects have been reported in purinergic neurotransmission related to the development of arterial hypertension in SHR (22). Long-term treatment with 1,3-dipropyl-8-*p*-sulfophenylxanthine (DPSPX), a non-selective antagonist of the adenosine receptor, causes a hypertensive state in rats. In addition, exogenous adenosine interferes differently with the noradrenergic neurotransmission in SHR compared with WKY (23); thus, the cardiovascular regulation of arterial pressure in SHR within the NTS is dependent on NTS noradrenergic mechanisms and on purinergic neurotransmission.

In light of this, we speculate that adenosine might interact with the noradrenergic system within the NTS in order to modulate certain particular autonomic responses and that it might be relevant in the development of hypertension. The aim of the present study is to provide evidence for the existence of an α_2 -adrenergic/adenosine A₁ interaction within the NTS of normotensive and hypertensive rats.

Methods

Animals

Adult male WKY rats and SHR from the Institute of Biosciences, University of São Paulo (São Paulo, Brazil), weighing 180–230 g were housed in individual cages under a regular light-dark cycle (lights on at 7:00 AM and off at 7:00 PM) in temperature- and humidity-controlled rooms. The animals received food and water *ad libitum* and were used for radioautographic studies. For dorsomedial brainstem cell culture, we used 1-d-old neonatal SHR and WKY rats (P₁) from the Butanta Institute, São Paulo, Brazil. All procedures and protocols were in accordance with our Institutional Guidelines for Animal Experimentation.

Quantitative Receptor Radioautography

The procedure for quantitative receptor radioautography has been described elsewhere (16). Coronal sections (20 μ m thick) of the medulla oblongata were made in a Leica cryostat (CM3050) at bregma levels from –14.30 to –13.30 mm, consistent with the atlas proposed by Paxinos and Watson (24), and subsequently thaw-mounted on gelatin-coated slides for radioautography. We studied the *in vitro* NTS-based modulatory effect of *N*⁶-cyclopentyladenosine (CPA) (Sigma, St. Louis, USA), an adenosine A₁ receptor agonist, on the characteristics of [³H]RX821002 (specific activity 62 Ci/mmol, Amersham) binding, an α_2 -adrenoceptor antagonist. We used saturation experiments, which were performed using 10 different concentrations of [³H]RX821002 (0.5–20 nmol/L) in the presence or absence of 3 different concentrations of CPA (1, 10, 30 nmol/L). A total of 10 μ mol/L of phentolamine (Sigma), an α_2 -adrenoceptor antagonist, was used for non-specific binding and 300 nmol/L of DPCPX (Sigma), an ade-

Fig. 1. Representative radioautographic images illustrating [^3H]RX821002 binding within the nucleus tractus solitarius (NTS). *A*: Section of the medulla oblongata showing dense radioactive binding of α_2 -adrenoceptors by [^3H]RX821002 within the NTS (-13.80 mm from bregma). *B*: Image of the rostral NTS section (-13.30 mm from bregma) showing the three subnuclei analyzed within the NTS (separated by a continuous line, 4): 1, dorsomedial/dorsolateral, 2, subpostremal, and 3, medial/intermediate. *C*: Image of the caudal (-14.30 mm from bregma) showing the three subnuclei analyzed. Weak radioactive labeling was observed in the dorsal motor nucleus of the vagus nerve (X in B, C) and hypoglossal nucleus (XII in B, C). 4V, 4th ventricle.

nosine A_1 receptor antagonist, was used to block the modulatory effect promoted by CPA. The solvent used for DPCPX was ethanol. The effect of ethanol was also evaluated as an experimental control.

Forty adjacent sections from bregma levels -14.30 to -13.30 mm were obtained and separated in both the control (absence of CPA) and the treated groups (1, 10, and 30 nmol/L of CPA). All groups were equally distributed among the rostral-caudal sections for saturation experiments in a single rat for a total of six rats ($n=6$). The same procedure was performed to establish nonspecific binding using phentolamine.

Slide-mounted brain sections were pre-incubated with Tris-HCl buffer (170 mmol/L, pH 7.4) containing 0.2 U/mL of adenosine deaminase (ADA) (Sigma) at room temperature for 15 min to remove endogenous adenosine. The sections were then pre-incubated in a buffer of the same composition, with or without CPA (1, 10, or 30 nmol/L), for 15 min before the addition of [^3H]RX821002 (0.5–40 nmol/L) in a phosphate buffer of 0.01 mol/L, pH 7.4, containing 50 nmol/L KCl and

10 nmol/L of MgCl_2 for 60 min at 37°C . Following incubation, the slices were washed twice in phosphate buffer for 5 min each, rinsed twice in distilled water (0 – 4°C) and dried in a stream of cold air. The sections were then exposed to a tritium-sensitive film ([^3H]Hyperfilm; Amersham) for 7 weeks.

The autoradiograms were analyzed as previously described by Ferrari *et al.* (25). Mean gray values were determined by measurements of three subnuclei (dorsomedial/dorsolateral, medial/intermediate and subpostremal) within the NTS (Fig. 1) using a square as a sampling field (0.06 mm^2), as previously described by Carrettiero and Fior-Chadi (11, 26). A curve calculated from prefabricated [^3H]-labeled polymer strip for eight known activities (Microscale; Amersham) was used as a standard.

Dorsomedial Brainstem Cell Culture and [^3H]RX821002 Binding Assay

The procedure for brainstem cell culture has been described

elsewhere (27). The modulatory effect of 8 different concentrations of CPA (10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} mol/L) (Sigma), an adenosine A_1 receptor agonist, on [3 H]RX821002 binding (specific activity 62 Ci/mmol, Amersham), an α_2 -adrenoceptor antagonist, was evaluated using dorsomedial brainstem cell culture with binding assays. We used 3 nmol/L of [3 H]RX821002, a concentration that had previously been shown to be consistent with the K_d value in the context of saturation experiments.

The cell culture was washed (3×5 min) using Krebs Ringer bicarbonate buffer (KRGB), pH 7.4 (NaCl 120 mmol/L, KCl 5 mmol/L, CaCl_2 2.5 mmol/L, KH_2PO_4 1.2 mmol/L, MgSO_4 1.2 mmol/L, NaHCO_3 25 mmol/L, and glucose 13 mmol/L) containing 0.2 U/mL of adenosine deaminase (ADA; Sigma) at room temperature to remove endogenous adenosine. Thereafter, the cells were incubated in the same buffer with or without different concentrations of CPA (from 10^{-9} to 10^{-4} mol/L) for 15 min at 4°C . The [3 H]RX821002 was added (3 nmol/L) and the cells were incubated for 1 h at 4°C . A total of 10 $\mu\text{mol/L}$ of phentolamine (Sigma), an α_2 -adrenoceptor antagonist, was added with [3 H]RX821002 for non-specific binding. A total of 300 nmol/L of DPCPX (Sigma), an adenosine A_1 receptor antagonist, was added 15 min before CPA in order to block the modulatory effect of CPA on [3 H]RX821002 binding. The solvent used for DPCPX was ethanol. The effect of ethanol was also evaluated as an experimental control (data not shown). Ethanol had no effect on [3 H]RX821002 binding.

Following incubation, the cells were washed in the same KRGB cold buffer (3×3 min), resuspended in deionized cold distilled water (4°C), and immersed in scintillation cocktail (Ecolume; ICN Biomedicals, Aurora, USA). The radioactivity was measured (Packard TriCarb 2100TR; Downers Grove, USA). Protein concentration was determined using the method of Bradford (28) and the result was expressed in terms of fmol/mg of protein.

Statistical Analysis

Statistical analysis was performed using the repeated-measures ANOVA test, followed by Dunnett's test, to compare different treatments against the control group. For radioautographic studies, the values from the control group and the treated group were compared considering the binding parameters ($B_{\max}$ and K_d) obtained using the GRAPHPAD program (Intuitive Software for Science, San Diego, USA). $*p < 0.05$, $n = 6$.

Results

Dense radioactive labeling of α_2 -adrenoceptors by [3 H]RX821002 was well defined in the NTS at intermediate levels (Fig. 1A) (-13.80 mm from bregma), rostral levels (Fig. 1B) (-13.30 mm from bregma) and caudal levels (Fig. 1C) (-14.30 mm from bregma). Within the NTS (continuous line 4 in Fig. 1B and C) we focused on three subnuclei,

namely the dorsomedial/dorsolateral (number 1 in Fig. 1B and C), subpostremal (number 2 in Fig. 1B and C) and medial/intermediate (number 3 in Fig. 1B and C). The modulatory effect of CPA on α_2 -adrenoceptors was analyzed in these subnuclei. Weak radioactive labeling was observed in the dorsal motor nucleus of the vagus nerve (X in Fig. 1B and C) and in the hypoglossus nucleus (XII in Fig. 1B and C).

Adenosine A_1 Receptors Modulate α_2 -Adrenoceptors in Normotensive Rats (WKY)

The dorsomedial/dorsolateral subnucleus within the NTS showed an increase in $B_{\max}$ value of [3 H]RX821002 (21%) induced by 10 nmol/L of CPA compared with the control group without CPA (Fig. 2A, dorsomedial/dorsolateral). No changes were observed in the K_d value using 10 nmol/L of CPA at this subnucleus. A total of 1 and 30 nmol/L of CPA also exhibited no effect on either the $B_{\max}$ or the K_d values of [3 H]RX821002 at this subnucleus. The medial/intermediate subnucleus showed no changes in either the $B_{\max}$ or the K_d of [3 H]RX821002 at any concentration of CPA (1, 10, or 30 nmol/L) (Fig. 2A, medial/intermediate). In contrast, the subpostremal subnucleus showed a decrease in K_d value of [3 H]RX821002 (24%) induced by 10 nmol/L of CPA compared with the CPA-free control group (Fig. 2A, subpostremal). No changes were observed in $B_{\max}$ value with 10 nmol/L of CPA at this subnucleus. A total of 1 and 30 nmol/L of CPA also exhibited no effect on either $B_{\max}$ or K_d values of [3 H]RX821002 at this subnucleus.

The adenosine A_1 receptor antagonist, DPCPX, was used to block the modulatory effect of CPA on [3 H]RX821002 binding (Fig. 2A, dorsolateral/dorsomedial, $B_{\max}$ and subpostremal, K_d). DPCPX alone did not induce any change in [3 H]RX821002 binding affinity.

Representative [3 H]RX821002 saturation curves from the control group (absence of CPA) and the treated group (presence of 1 and 10 nmol/L of CPA) are illustrated in Fig. 2B. Saturation curves illustrating the effect of 30 nmol/L of CPA were not calculated because this concentration of CPA had no effect on either the $B_{\max}$ or the K_d values of [3 H]RX821002 at any subnuclei analyzed. The adenosine A_1 receptor antagonist DPCPX alone and the solvent used (data not shown) had no effect on the binding parameters of [3 H]RX821002 (Fig. 2A, dorsomedial/dorsolateral, subpostremal). DPCPX also blocked the modulatory effect promoted by 10 nmol/L of CPA on α_2 -adrenoceptors (Fig. 2A).

Adenosine A_1 Receptors Are More Sensitive in Modulating α_2 -Adrenoceptors in Spontaneously Hypertensive Rats (SHR)

The dorsomedial/dorsolateral subnucleus within the NTS also showed an increase in $B_{\max}$ values of [3 H]RX821002 (18%); however, this increase in $B_{\max}$ was induced by 1 nmol/L of CPA instead of 10 nmol/L, as observed in WKY, when

Fig. 2. Effect of CPA (1, 10, and 30 nmol/L) on [3 H]RX821002 binding in dorsomedial/dorsolateral, subpostremal and medial/intermediate subnuclei within the NTS of Wistar Kyoto rats (WKY). **A:** Dorsomedial/dorsolateral subnuclei showed an increased B_{max} and subpostremal subnuclei showed a decreased K_d of α_2 -adrenoceptors with respect to [3 H]RX821002 induced by 10 nmol/L of CPA. The B_{max} and the K_d values are shown as means $\pm$ SEM, $n = 6$. A total of 300 nmol/L of DPCPX was used as an antagonist and was applied to the sections 15 min before CPA. Statistical analyses included repeated-measures ANOVA, followed by Dunnett's test. * $p < 0.05$ vs. the control group. **B:** Representative saturation curves showing an increased B_{max} value in dorsolateral/dorsomedial subnuclei treated with 10 nmol/L of CPA (●) and a decreased K_d value in subpostremal subnuclei also treated with 10 nmol/L of CPA (●) compared with the control group (▲). The 30 nmol/L curves are not shown because this concentration had no effect on any subnuclei for any concentration of CPA. A total of 10 μ mol/L of phentolamine was used as a non-specific binding agent. Statistical analysis is shown in A. Inset: Scatchard plots (same data) showing K_d and B_{max} values. M: mol/L.

Fig. 3. Effect of CPA (1, 10, and 30 nmol/L) on the characteristics of [3 H]RX821002 binding in subnuclei dorsomedial/dorsolateral, subpostremal and medial/intermediate subnuclei of spontaneously hypertensive rats (SHR). **A:** Dorsomedial/dorsolateral subnuclei showed an increased B_{max} and subpostremal subnuclei showed a decreased K_d of α_2 -adrenoceptors by [3 H]RX821002 induced by 1 nmol/L of CPA. The B_{max} and K_d values are shown as means $\pm$ SEM, $n = 6$. A total of 300 nmol/L of DPCPX was used as an antagonist, applied to the sections 15 min before CPA. Statistical analysis included repeated-measures ANOVA, followed by Dunnett's test. * $p < 0.05$ vs. the control group. **B:** Representative saturation curves showing an increased B_{max} value in dorsolateral/dorsomedial subnuclei treated with 1 nmol/L of CPA (■) and a decreased K_d value in subpostremal subnuclei also treated with 1 nmol/L of CPA (■) compared with the control group (▲). The 30 nmol/L curves were not shown because this concentration had no effect on any subnuclei regardless of CPA concentration. A total of 10 μ mol/L of phentolamine was used as a non-specific binding agent. Statistical analysis is shown in A. Inset: Scatchard plots (same data) showing K_d and B_{max} values. M: mol/L.

Fig. 4. Different modulatory effects of CPA, an adenosine A_1 agonist, on [3 H]RX821002 binding, an α_2 -adrenoceptor antagonist, in dorsomedial brainstem cell cultures from Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). **A:** α_2 -Adrenoceptors binding showed a significant increase in the presence of 10^{-7} and 10^{-5} mol/L of CPA in dorsomedial brainstem cell cultures from SHR and WKY rats, respectively. **B:** DPCPX, an adenosine A_1 receptor antagonist, blocks the effect of CPA on α_2 -adrenoceptor binding of the dorsomedial brainstem cell culture from SHR and WKY rats. DPCPX alone did not change the [3 H]RX821002 binding. The values are shown as mean $\pm$ SEM, $n = 6$. Three hundred nmol/L of DPCPX was administered to the cell culture 15 min before CPA. Statistical analysis included the repeated-measures ANOVA followed by Dunnett's test. * $p < 0.05$ vs. the control.

compared with the CPA-free control group (Fig. 3A, dorso-medial/dorsolateral). No changes were observed in the K_d value of [3 H]RX821002 in this subnucleus using 1 nmol/L of CPA. A total of 10 and 30 nmol/L of CPA had no effect on either the B_{max} or the K_d value of [3 H]RX821002 in the dorso-medial/dorsolateral subnucleus.

The medial/intermediate subnucleus showed no changes in either the B_{max} or the K_d values of [3 H]RX821002 at any concentration of CPA (1, 10, or 30 nmol/L) (Fig. 3A, medial/intermediate). In contrast, the subpostremal subnucleus showed a decrease in the K_d value of [3 H]RX821002 (26%) induced by 1 nmol/L of CPA compared with the control

group (Fig. 3A, subpostremal). No changes were observed in B_{max} value using 1 nmol/L of CPA at this subnucleus. Similarly, 10 and 30 nmol/L of CPA had no effect on either the B_{max} or the K_d values of [3 H]RX821002 at this subnucleus.

The adenosine A_1 receptor antagonist, DPCPX, was used to block the modulatory effect of CPA on [3 H]RX821002 binding (Fig. 3A, dorsolateral/dorsomedial, B_{max} and subpostremal, K_d). DPCPX alone did not induce any change in [3 H]RX821002 binding.

Representative [3 H]RX821002 saturation curves for the control group (absence of CPA) and the treated group (presence of 1 and 10 nmol/L of CPA) are illustrated in Fig. 3B.

Saturation curves illustrating the effect of 30 nmol/L were not showed because this concentration of CPA had no effect on either the $B_{\max}$ or the K_d values of [^3H]RX821002 in any of the subnuclei analyzed. The DPCPX alone or solvent alone (data not shown) had no effect on the binding affinity of [^3H]RX821002 (Fig. 3A, dorsomedial/dorsolateral, subpostremal). DPCPX also blocked the modulatory effect of 1 nmol/L of CPA on α_2 -adrenoceptors (Fig. 3A).

Modulatory Effect of Adenosine A_1 Receptors on α_2 -Adrenoceptors in Neuronal Culture from the Dorsomedial Brainstem of Normotensive and Spontaneously Hypertensive Rats

Dorsomedial brainstem cell culture from WKY rats showed an increase in [^3H]RX821002 binding in the presence of 10^{-5} mol/L of CPA (53%) (Fig. 4A). Dorsomedial brainstem cell culture of SHR rats also showed an increase in [^3H]RX821002 binding (48%), but this was effected by 10^{-7} mol/L of CPA instead of the 10^{-5} mol/L observed in cell cultures taken from WKY rats (Fig. 4A). The use of 300 nmol/L of DPCPX, an adenosine A_1 antagonist, completely blocked the CPA-induced modulatory effects on [^3H]RX821002 binding affinity in neuronal cultures from WKY and SHR (Fig. 4B).

Discussion

Our study offers evidence for the existence of a complex interaction between adenosine A_1 receptors and α_2 -adrenoceptors within the NTS of Wistar-Kyoto normotensive (WKY) and spontaneously hypertensive rats (SHR). Sections from the medulla oblongata of WKY and SHR showed that CPA, an adenosine A_1 agonist, induces an increase in the $B_{\max}$ and a decrease in the K_d values of [^3H]RX821002 binding in the dorsomedial/dorsolateral and subpostremal subnuclei of the NTS, respectively. These CPA-induced changes are altered in hypertensive rats. Our results suggest that a very well-organized network of modulation might impact different subnuclei within the NTS in order to elicit appropriate autonomic responses. This finding was confirmed by experiments with neuronal cell cultures from normotensive and hypertensive rats.

Adenosine exhibits a differential action within the NTS. Microinjection of adenosine into the caudal NTS of rats elicits a potent dose-related decrease in blood pressure and heart rate (5, 20). However, at more rostral sites, adenosine administration exerts a pressor effect (20). In agreement with this fact, previous research from our laboratory and others have reported the existence of separated populations of adenosine receptors within the NTS showing increased concentrations of adenosine A_1 receptors in the rostral region compared with the caudal zone (3, 11). The results suggested separate populations of adenosine receptors and also indicated a heterogeneous distribution of adenosine A_1 receptors within the NTS.

Researchers reported increased receptor concentrations in the dorsomedial/dorsolateral and subpostremal subnuclei (11). We argue that the changes in binding parameters of α_2 -adrenoceptor observed in our study in these specific subnuclei provide strong evidence for an interaction between adenosine A_1 receptors and α_2 -adrenoceptors.

Barraco and Phillis (4) have reported that stimulating adenosine A_1 receptors in the subpostremal NTS can evoke a pressor response. This action also increases the heart rate and impacts other regional sympathetic control activity (9, 10). Therefore, although the pressor responses to stimulation of the NTS adenosine A_1 receptor were clearly dominant, biphasic or depressor responses were also observed in approximately 30% of cases (6). In addition, White *et al.* have shown a hypotensive effect of adenosine A_1 receptor stimulation within the NTS (29). This seemingly contradictory effect is consistent with our results, which show that the adenosine A_1 analog, CPA, increases the $B_{\max}$ (increased number of receptor) or decreases the K_d (increased affinity of receptor) values of [^3H]RX821002 binding. This, in turn, may increase the depressor response mediated by noradrenaline within the NTS in the context of the α_2 -adrenoceptor. Thus, adenosine A_1 analogues might exert a depressor effect or an attenuated pressure response within the NTS, depending exclusively on the subregion stimulated, its concentration and the presence of noradrenaline.

It is interesting to note that stimulation of the NTS A_{2a} receptor evokes preferential hindlimb vasodilatation in a uniform manner (30). In contrast, NTS adenosine A_1 receptor stimulation elicits β -adrenergic vasodilatation in the hindlimb vascular bed and simultaneously triggers neural and humoral vasoconstriction, leading to marked variability in hemodynamic response (6). This means that stimulation of adenosine A_1 receptors within the NTS can elicit different autonomic responses in different regions of the body in order to maintain homeostasis. Thus, we would suggest that adenosine A_1 receptors may regulate the pressure response in a particular way at different subnuclei within the orchestrated processing of the NTS neurons. This might explain why CPA induced an increase in the $B_{\max}$ in one subnucleus and a decrease in the K_d values of [^3H]RX821002 binding in other subnuclei of the NTS.

The medial/intermediate subnucleus did not show any changes in the binding parameters of [^3H]RX821002 at any CPA concentrations. This observation is consistent with our previous study that showed a low concentration of adenosine A_1 receptors in this subnucleus (11). Higher concentrations of CPA (30 nmol/L) did not induce any changes in the binding parameters of [^3H]RX821002 at any of the three subnuclei analyzed.

The major finding from our study was that CPA in the nanomolar range of 1 nmol/L for SHR and 10 nmol/L for WKY significantly changed the number and the affinity of α_2 -adrenoceptors at the dorsomedial/dorsolateral and the subpostremal subnuclei, respectively. However, the CPA-

induced effect disappeared with higher CPA concentrations. We also observed a pattern of dose-response using neuronal cultures from normotensive and hypertensive rats (Fig. 4). This type of U-shape or inversely U-shape dose-response curve has been observed for other receptor-receptor interactions such as α_2 -adrenoceptor/neuropeptide Y (31), angiotensin II/ α_2 -adrenoceptors (15, 16), and bradykinin/ α_2 -adrenoceptor (32). Our results could be due to a desensitization phenomenon, to a depletion of a factor necessary for the interaction to take place, or even because of a gradual dose-dependent conformational change. The interaction between the adenosine receptor and other receptors has also been reported; examples include adenosine A_{2A} /dopamine D_2 and adenosine A_1 /dopamine D_1 (17, 33).

Our finding that SHR and WKY rats showed similar changes of comparable intensity was surprising because other researchers have reported increased concentrations of adenosine A_1 receptors within the NTS of SHR compared with WKY rats (11, 22). High numbers of adenosine A_1 receptors within the NTS of SHR rats would, in theory, allow for higher activation rates of this receptor and a concomitant increase in the number of α_2 -adrenoceptors, thereby increasing the depressor response mediated by noradrenaline. Consequently, adenosine A_1 receptor stimulation would promote an attenuated pressor response within the NTS of SHR, as demonstrated by Tseng *et al.* (34). Accordingly, although our results did not show a greater increase in the number of α_2 -adrenoceptors in SHR compared with WKY mediated by the adenosine A_1 analog, we did find that the α_2 -adrenoceptor system is responsive to a lower dose of adenosine A_1 analogs in SHR than in WKY rats. This means that adenosine A_1 analogs are more potent in modulating α_2 -adrenoceptor system within the NTS of hypertensive than normotensive rats. We conclude that the SHR α_2 -adrenoceptor system may promote an increased hypotensive output in the presence of adenosine A_1 agonist compared with WKY rats in certain specific subnuclei. It is important to note that this is a small increase in hypotensive response in the context of the total hypertensive output, because blood pressure in SHR is higher than normal. This hypothesis agrees with a recent functional study, which suggested that the SHR rats exhibit attenuated pressor, tachycardic and neuronal responses mediated by adenosine compared with WKY rats (34).

One explanation for the modulatory effect of adenosine on α_2 -adrenoceptors could be linked to a compensatory mechanism, given that α_2 -adrenoceptor concentration is decreased in SHR animals. We speculate that this modulatory effect may prevent a higher increase in blood pressure in these animals.

The same pattern of the result obtained from analyzing tissue sections was also observed in cell culture from the brainstems of SHR and WKY animals. Brainstem cells from SHR animals were also more responsive than those of WKY (Fig. 4). This result not only confirms our tissue sectioning data but also highlights a difference in the neurotransmission path-

ways of SHR and WKY very early in life. To our knowledge, this result is the first to report such modulation in cell cultures taken from newborn rats. This is particularly interesting because hemodynamic parameters are not altered at this age. Differences in blood pressure between the strains appear around 2 months of age (35). Strain-dependent changes before 2 months of age might be particularly relevant to the development of high blood pressure. Indeed, several studies show adenosine A_1 receptor alterations at the early stages of SHR development (22, 26, 36). In addition, our group has found that the subpostremal subnucleus seems to be particularly important for the development of hypertension in the SHR, because changes in adenosine A_1 receptor binding between strains (SHR-WKY) were observed in this subnucleus in 15-d-old rats (26). Our data suggest that an altered neurotransmission, in this case an interaction between A_1/α_2 -adrenoceptor, might be causally related to the development of hypertension.

It is important to recognize that α_2 -adrenoceptor concentrations are decreased during early stages of development (21). The sensitization of α_2 -adrenoceptors by the A_1 agonist in SHR cell cultures from newborns compared with WKY might suggest that the interaction between A_1/α_2 occurs early in life. This mechanism might be important in regulating blood pressure in SHR animals during development.

These results highlight for the first time the nature of adenosine-related modulatory effects within specific subnuclei of the NTS neuronal circuitry. These effects may, in turn, play a role in promoting a complex autonomic response. In conclusion, our work offers a valuable contribution to understanding the action of a potent endogenous modulator such as adenosine in changing the nature of α_2 -adrenoceptors. Our results emphasize the importance of a careful analysis of adenosine activity sites within the NTS. Phenomena such as the interaction between adenosine A_1 receptors and α_2 -adrenoceptors suggest promising new approaches for drug development in the context of blood pressure disorders.

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