Brainstem Adenosine A₁ Receptor Signaling Masks Phosphorylated Extracellular Signal-Regulated Kinase 1/2-Dependent Hypotensive Action of Clonidine in Conscious Normotensive Rats

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ABSTRACT

Central adenosine A₁ and A_{2A} receptors mediate pressor and depressor responses, respectively. The adenosine subtype A_{2A} receptor (A_{2A}R)-evoked enhancement of phosphorylated extracellular signal-regulated kinase (pERK) 1/2 production in the rostral ventrolateral medulla (RVLM), a major neuroanatomical target for clonidine, contributes to clonidine-evoked hypotension, which is evident in conscious aortic barodenervated (ABD) but not in conscious sham-operated (SO) normotensive rats. We conducted pharmacological and cellular studies to test the hypothesis that the adenosine A_{2A}R-mediated (pERK1/2-dependent) hypotensive action of clonidine is not expressed in SO rats because it is counterbalanced by fully functional central adenosine subtype A₁ receptor (A₁R) signaling. We first demonstrated an inverse relationship between A₁R expression in RVLM and clonidine-evoked hypotension in ABD and SO rats.

The functional (pharmacological) relevance of the reduced expression of RVLM A₁R in ABD rats was verified by the smaller dose-dependent pressor responses elicited by the selective A₁R agonist N⁶-cyclopentyladenosine in ABD versus SO rats. It is important that after selective blockade of central A₁R with 8-cyclopentyl-1,3-dipropylxanthine in conscious SO rats, clonidine lowered blood pressure and significantly increased neuronal pERK1/2 in the RVLM. In contrast, central A₁R blockade had no influence on the hypotensive response or the increase in RVLM pERK1/2 elicited by clonidine in ABD rats. These findings support the hypothesis that central adenosine A₁R signaling opposes the adenosine A_{2A}R-mediated (pERK1/2-dependent) hypotensive response and yield insight into a cellular mechanism that explains the absence of clonidine-evoked hypotension in conscious normotensive rats.

Clonidine-evoked hypotension is minimal or absent in conscious normotensive rats (Abdel-Rahman, 1992; Ricci et al., 1992; Medvedev et al., 1998) in contrast to animal preparations that exhibit baroreflex dysfunction such as anesthetized (Borkowski and Finch, 1979; Sannajust et al., 1992; Su et al., 2002; Sato et al., 2005) and hypertensive (Judy et al., 1976, 1979; Judy and Farrell, 1979; Prados et al., 1998) rats and after surgically induced baroreceptor dysfunction [aortic barodenervation (ABD)] (Abdel-Rahman, 1992; Nassar and Abdel-Rahman, 2006, 2008). However, the cellular mecha-

nisms that explain these findings remain unclear. Our findings link central adenosine A_{2A}R signaling in the brainstem to clonidine-evoked hypotension in conscious ABD rats (Nassar and Abdel-Rahman, 2008) and A₁R signaling to baroreceptor function in conscious normotensive rats. Compared with sham-operated (SO) rats, ABD rats exhibit up-regulation of the adenosine A_{2A}R in the RVLM, the major neuroanatomical target of clonidine (Nassar and Abdel-Rahman, 2008). On the other hand, adenosine A₁R knockdown in the brainstem, by antisense against A₁R, reduced baroreflex sensitivity in conscious normotensive rats (Mao et al., 1994) to a level comparable with that reported in ABD rats and SHR (Judy et al., 1976, 1979; Judy and Farrell, 1979; Abdel-Rahman, 1992; Prados et al., 1998). Whether attenuation of central A₁R signaling reveals clonidine-evoked hypotension in conscious normotensive rats has not been explored. Re-

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ABBREVIATIONS: ABD, aortic barodenervated rat; A_{2A}R, adenosine subtype A_{2A} receptor; A₁R, adenosine subtype A₁ receptor; SO, sham operated; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rat; pERK, phosphorylated extracellular signal-regulated kinase; i.c., intracisternal; CPA, N⁶-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; BP, blood pressure; HR, heart rate; aCSF, artificial cerebrospinal fluid; DMSO, dimethyl sulfoxide; MAP, mean arterial pressure; ANOVA, analysis of variance; ir, immunoreactive; CGS21680, 2-[4-[(2-carboxyethyl) phenyl] ethylamino]-5'-N-ethylcarboxamidoadenosine.

ported findings seem to suggest that the balance between central adenosine A₁R and A₂R signaling determines the blood pressure response elicited by clonidine. In support of this notion, clonidine elicits hypotension in the SHR, which exhibits reduced pressor responsiveness elicited by intranucleus tractus solitarius adenosine administration (Abdel-Rahman and Tao, 1996); the latter is mediated via adenosine A₁R activation (Scislo and O'Leary, 2002). By the same token, the presence of fully functional brainstem A₁R signaling in conscious normotensive rats might impede clonidine-evoked hypotension in this preparation.

In the present investigation, we tested the hypothesis that central adenosine A₁R signaling counterbalances the A_{2A}R (pERK1/2)-dependent hypotension and accounts for the lack of clonidine-evoked hypotension in conscious normotensive (SO) rats. As a first step, it was important to evaluate the expression and function of central adenosine A₁R in SO and ABD rats. The goal of these immunohistochemical and dose-response [intracisternal (i.c.) N⁶-cyclopentyladenosine (CPA)] studies was to establish whether the A₁R expression in the RVLM (major neuroanatomical target of clonidine) and its function are differentially regulated in rat preparations in which clonidine elicits reduction (ABD) or no change (SO) in blood pressure. Additional studies were conducted in conscious ABD and SO rats to more directly test the hypothesis that central adenosine A₁R signaling masks clonidine-evoked hypotension in conscious SO rats; in these studies, we investigated the effect of central A₁R blockade [8-cyclopentyl-1,3-dipropylxanthine (DPCPX)] on the blood pressure response elicited by centrally administered clonidine in SO and ABD rats. Finally, we investigated the possibility that enhanced pERK1/2 production in the RVLM, caused at least partly via the activation of the unopposed A_{2A}R, underlies the hypotensive action of clonidine seen in SO rats after central A₁R blockade.

Materials and Methods

Animals

A total of 62 male Sprague-Dawley rats (11–12 weeks old; Harlan, Indianapolis, IN) weighing 310 ± 10 g were used in the present study. All rats were housed in a room with controlled environment at a constant temperature of 23 ± 1°C, humidity of 50 ± 10%, and a 12-h light/dark cycle. Food (Prolab RMH300; Granville Milling, Creedmoor, NC) and water were available ad libitum. Surgical procedures and postoperative care were performed in accordance with and approved by the Institutional Animal Care and Use Committee and in accordance with the Institute of Laboratory Animal Resources (1996).

Aortic Barodenervation, Intracisternal Cannulation, and Intravascular Catheterization

These surgical procedures were performed as in our previous studies (Abdel-Rahman, 1992; Nassar and Abdel-Rahman, 2006, 2008). In brief, 5 days before starting the experiment, a stainless steel guide cannula was implanted into the cisterna magna under pentobarbital anesthesia (60 mg/kg i.p.). A stainless steel cannula (23G; Small Parts, Inc., Miramar, FL) was passed between the occipital bone and the cerebellum, after the head was placed in a David Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA), so that its tip protruded into the cisterna magna. The guide cannula was secured in place with small metal screws and dental acrylic cement (Durelon; Thompson Dental Supply, Raleigh, NC). The guide cannula was considered patent when spontaneous outflow of cerebrospinal fluid was observed and by gross post mortem histological

verification after injection of 5 µl of fast green dye (EM Scientific, Gibbstown, NJ). Catheters (polyethylene 50) were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of blood pressure and i.v. injections, respectively. Aortic barodenervation was accomplished by bilateral transection of the aortic depressor nerves after a midline incision in the cervical region. Finally, the catheters were tunneled subcutaneously and exteriorized at the back of the neck between the scapulae. The catheters were flushed with heparin in saline (200 U/ml) and plugged by stainless steel pins. Incisions were closed by surgical staples and swabbed with povidone-iodine solution. Each rat received an i.m. injection of 30,000 U of penicillin G benzathine and penicillin G procaine in aqueous suspension (Durapen) and an subcutaneous injection of buprenorphine hydrochloride (Buprenex; 30 µg/kg) and was housed in a separate cage. On the day of the experiment, the arterial catheter was connected to a Gould-Statham pressure transducer (Oxnard, CA), and blood pressure (BP) and heart rate (HR) were displayed on a polygraph (model 7D; Grass Instruments, Quincy, MA). In all groups, 30 min was allowed at the beginning of the experiment for stabilization of BP and HR at baseline before intracisternal drug or vehicle [aCSF or dimethyl sulfoxide (DMSO)] administration.

Immunohistochemistry

The procedure reported for light microscopy and our recent study (Nassar and Abdel-Rahman, 2008) were followed. Brains were fixed by transcardiac perfusion of 4% paraformaldehyde in Tris-buffered saline after a lethal dose of sodium pentobarbital. Brain sections containing RVLM (10–12; 16 µm each; −12.8 to approximately −11.8 mm) were collected in each well of a cell culture plate (12 wells; BD Biosciences, San Jose, CA) containing ice-cold Tris-buffered saline. The avidin-biotin complex method was used following the manufacturer's instruction (Vectastain ABC kits; Vector Laboratories, Burlingame, CA). For adenosine A₁R immunohistochemistry, sections were incubated with a rabbit A₁R primary antibody (Sigma-Aldrich, St. Louis, MO) (1:100). For pERK1/2 immunohistochemistry, brain sections were incubated with primary antibody (phospho-p44/42 mitogen-activated protein kinase, Thr202/Tyr204, E10) (Cell Signaling Technology Inc., Danvers, MA) (1:400). The dehydration and sealing procedures applied in our recent study (Nassar and Abdel-Rahman, 2008) were followed. The images were processed by Micropublisher (QImaging, Surrey, BC, Canada). For each protein, A₁ or pERK1/2, sections from treatment and control rats were simultaneously processed, and the immunoreactive neurons (brown stain) were identified in the RVLM and counted in at least six sections from each rat using the ImageJ program (<http://rsb.info.nih.gov/ij/>) and averaged. The group data (mean ± S.E.M.) were used for statistical analysis.

Protocols and Experimental Groups

The rats used in the study were allowed 5 days for recovery after intracisternal cannulation and ABD or sham operation as in our previous studies (Nassar and Abdel-Rahman, 2006, 2008).

Experiment 1: Central Adenosine A₁R Expression and Function in SO and ABD Rats. In the first part of this experiment, two groups of conscious SO and ABD rats ($n = 6$ each) were used to collect brains for immunohistochemical detection/quantification of the adenosine A₁R in the RVLM. However, before sacrifice, the rats in both groups received intracisternal aCSF so that they could serve as controls for pharmacological interventions employed below and in experiment 2. aCSF was administered intracisternally after stabilization of BP and HR. Two additional groups of SO and ABD ($n = 7$, each) received intracisternal injections of the selective adenosine A₁ receptor agonist CPA (10, 20, 40, and 80 ng) to permit construction of dose-pressor response curves. The doses of CPA were based on a pilot study. Time to recovery was allowed before the administration of a subsequent dose of CPA.

Experiment 2: Effect of Clonidine on BP and RVLM pERK1/2 of SO and ABD Rats in the Absence or Presence of Central Adenosine A₁R Blockade. After stabilization of BP and HR at baseline, clonidine (0.6 μ g i.c.) was administered 30 min after the selective adenosine A₁R antagonist, DPCPX (3.5 μ g i.c.; $n = 6$), in SO ($n = 6$) and ABD ($n = 7$) rats; the dose of DPCPX, although smaller than the reported intracerebroventricular dose (Stella et al., 1998), adequately blocked the pressor response elicited by CPA in a pilot study. DMSO (vehicle for DPCPX) was administered before clonidine in SO ($n = 5$) and ABD ($n = 6$) rats. The BP and HR were monitored for 45 min after clonidine administration. At the end of the experiment, the brains of the SO and ABD rats that received aCSF and served as controls (experiment 1) were processed along with the brains collected under this experiment for the measurement of RVLM pERK1/2 by immunohistochemistry.

Drugs

Clonidine hydrochloride, DMSO, and CPA were purchased from Sigma-Aldrich. DPCPX was purchased from RBI/Sigma (Natick, MA). Pentobarbital sodium was provided by Webster Veterinary supplies (Vortech Pharmaceutical Ltd., Dearborn, MI).

Statistical Analysis

Mean arterial pressure (MAP) was calculated as: diastolic pressure + one third (systolic pressure – diastolic pressure). Mean arterial pressure and heart rate are expressed as mean $\pm$ S.E.M. change from their respective baseline. The time course data were analyzed by repeated measures ANOVA using SPSS 13.0 statistical package for Windows (SPSS Inc., Chicago, IL) for differences in time and treatment trends followed by a one-way ANOVA to assess individual differences at different time points among different groups. Tukey's (equal variance) and Games Howell (unequal variance) tests were used for post hoc analysis. Contrasts based on the Student's *t* test and the ANOVA error terms were used to compare pretreatment-to-post-treatment values in each group. The pre- to post-treatment comparisons were made through the use of contrasts that compared the pre- to post-treatment values in each group. The contrasts essentially averaged the pretreatment values and compared them with the averaged post-treatment values using the repeated measures ANOVA error term. These contrasts examined whether there were drug-evoked changes from baseline. $P < 0.05$ was considered significant. The dose-response curves were analyzed by repeated measures ANOVA using the SPSS 13.0 statistical package for Windows for differences in treatment trends. Contrasts based on the Student's *t* test and the ANOVA error terms were used to compare differences at each dose level in the dose-response curve in each group using the error term of the within-subject effects. Student's *t* test (unpaired, two-tailed) was used for comparing the mean value of immunoreactive positive cell count data, and $P < 0.05$ was considered significant. A one-way ANOVA was used to evaluate the effect of DPCPX or aCSF pretreatment on the expression of pERK1/2 elicited by clonidine, compared with the baseline (aCSF alone) in ABD and SO rats. $P < 0.05$ was considered significant.

Results

Central Adenosine A₁R Expression and Function in SO and ABD Rats. Adenosine A₁R expression was measured in the RVLM of SO and ABD rats, which received intracisternal aCSF and served as controls for subsequent drug treatments. At the time of sacrifice, the ABD and SO rats used in this experiment exhibited similar baseline MAP and HR (Table 1). The RVLM of the ABD, compared with SO, rats exhibited significantly ($P < 0.05$) fewer adenosine A₁R immunoreactive (ir) neurons (Fig. 1). Representative examples of A₁R immunostaining in the RVLM of ABD and SO rats are also shown in Fig. 1. Dose-pressor response curves

TABLE 1

Baseline MAP and HR values obtained 30 min after stabilization and before the following intracisternal pretreatments

Data presented as mean $\pm$ S.E.M.. The number of rats in each group is shown in parentheses.

Treatment	MAP	HR
	mm Hg	bpm
Aortic barodenervated		
aCSF ($n = 12$)	116.0 $\pm$ 6.0	407 $\pm$ 11
CPA ($n = 7$)	113.0 $\pm$ 6.0	370 $\pm$ 11
DPCPX ($n = 6$) ^a	112.0 $\pm$ 5.0	425 $\pm$ 10
DMSO ($n = 5$) ^a	102.3 $\pm$ 3.0	395 $\pm$ 10
aCSF ($n = 6$) ^a	105.0 $\pm$ 5.0	400 $\pm$ 15
Sham-operated		
aCSF ($n = 10$)	110.0 $\pm$ 5.0	393 $\pm$ 13
CPA ($n = 7$)	102.0 $\pm$ 3.0	390 $\pm$ 20
DPCPX ($n = 7$) ^a	108.0 $\pm$ 4.0	402 $\pm$ 12
DMSO ($n = 6$) ^a	109.0 $\pm$ 4.0	403 $\pm$ 16
aCSF ($n = 3$) ^a	97.0 $\pm$ 2.0	385 $\pm$ 7

^a Values after pretreatment and preceding i.c. clonidine.

Fig. 1. Photomicrographs showing the immunohistochemical images depicting the A₁R ir neurons in SO (left) and ABD (right) rats ($n = 6$ each) in RVLM. Scale bar, 100 μ m. Bar graphs, number of immunoreactive neurons in RVLM. Values are mean $\pm$ S.E.M. *, $P < 0.05$, compared with SO values.

were constructed with the selective adenosine A₁R agonist CPA (10, 20, 40, and 80 ng i.c.). ABD and SO rats, which received intracisternal aCSF and employed in the A₁R expression study above, served as controls. Baseline MAP and HR, measured before CPA or aCSF administration, were similar (Table 1). aCSF caused no change in blood pressure in either ABD or SO rats (data not shown), whereas CPA elicited dose-related increases in blood pressure in SO and ABD rats (Fig. 2). However, despite reaching maximal response after the third dose (40 ng), the A₁R-mediated pressor responses were significantly ($P < 0.05$) attenuated in ABD, compared with SO, rats. The heart rate responses associated with the pressor responses in SO and ABD rats were not significantly different (Fig. 2).

Effect of Clonidine on BP and RVLM pERK1/2 in SO and ABD Rats in the Absence or Presence of Central Adenosine A₁R Blockade. This experiment was conducted to determine whether central A₁R signaling opposes the A_{2A}R-mediated (pERK1/2-dependent) hypotensive action of clonidine in conscious SO rats. Therefore, we investigated the effect of clonidine on blood pressure in the absence or pres-

Fig. 2. Dose-pressor (top) and bradycardic (bottom) response curves constructed with intracisternal CPA (10, 20, 40, 80 ng) in conscious SO and ABD rats. Values are mean $\pm$ S.E.M.; numbers in parentheses indicate number of rats in each group. *, $P < 0.05$, compared with corresponding SO values.

ence of the selective adenosine A_1 R (DPCPX) blocker in conscious SO and ABD rats. The baseline blood pressure of ABD and SO rats that received the drug or vehicle treatments was similar (Table 1). Neither DPCPX nor its vehicle DMSO caused any significant changes in baseline MAP or HR in SO or ABD rats (Table 1). In DMSO-pretreated SO rats, clonidine ($0.6 \mu\text{g}$ i.c.) had no effect on blood pressure (Fig. 3). However, after central adenosine A_1 R blockade (DPCPX) in SO rats, clonidine significantly ($P < 0.05$, one-way ANOVA) reduced blood pressure (Fig. 3). In contrast, in DMSO-pretreated ABD rats, clonidine ($0.6 \mu\text{g}$ i.c.) caused significant reduction in blood pressure (Fig. 3); importantly, central A_1 R blockade (DPCPX pretreatment) did not influence ($P > 0.05$, one-way ANOVA) clonidine-evoked reduction in blood pressure in ABD rats (Fig. 3). Figure 4 shows the pERK1/2 level in the RVLM of the SO and ABD rats that received clonidine in the absence or presence of DPCPX and in the RVLM of rats that received only aCSF and served as control (basal pERK1/2). In vehicle (DMSO)-pretreated SO rats, clonidine had no effect ($P > 0.05$) on RVLM pERK1/2 level compared with basal expression level (Fig. 4). On the other hand, in DPCPX-pretreated SO rats and along with the appearance of the hypotensive response, clonidine caused a significant ($P < 0.05$) increase in the RVLM pERK1/2 level compared with basal or clonidine treatment in DMSO-pretreated SO rats (Fig. 4). In vehicle (DMSO)-pretreated ABD rats, clonidine significantly ($P < 0.05$) enhanced RVLM pERK1/2, compared with basal level (Fig. 4), and this response was not affected by DPCPX pretreatment (Fig. 4).

Discussion

Endogenous adenosine is implicated in the hypotensive action of clonidine (Nassar and Abdel-Rahman, 2006). In a more recent study, we demonstrated the dependence of clonidine-evoked hypotension on the A_{2A} R-mediated increases in pERK1/2 expression in the RVLM in ABD rats (Nassar and Abdel-Rahman, 2008). In the present study, we

Fig. 3. Time course of changes in MAP and HR after intracisternal clonidine ($0.6 \mu\text{g}$) in conscious SO (left) and ABD (right) rats. Arrow, clonidine administration 30 min after intracisternal administration of the adenosine A_1 R-selective antagonist DPCPX ($3.5 \mu\text{g}$) or its vehicle, DMSO, in SO rats (A and B) and ABD rats (C and D). Values are mean $\pm$ S.E.M. Number of rats in each group is shown in parentheses. *, $P < 0.05$, comparing the effects of DPCPX with DMSO pretreatment on clonidine-evoked responses. Repeated measures ANOVA error term showed that postclonidine values were significantly different from pretreatment values in the groups that received clonidine after DPCPX, but not DMSO, in SO rats. Postclonidine values were significantly different from the pretreatment value in the group that received clonidine after DMSO or DPCPX in ABD rats (i.e., DPCPX had no effect on clonidine-evoked hypotension in ABD rats).

tested the hypothesis that this cellular mechanism is offset by concurrent adenosine A_1 R signaling in conscious SO rats in which clonidine fails to change blood pressure. The most important findings of the present study are: 1) adenosine A_1 R expression in the RVLM is inversely related to clonidine-evoked hypotension because the latter was evident in ABD rats, which exhibited reduced RVLM A_1 R expression compared with SO rats; 2) dose-related pressor responses elicited by central A_1 R receptor activation (CPA) were significantly attenuated in ABD compared with SO rats, which lends functional support to the immunohistochemical data (A_1 R expression in the RVLM); 3) in SO rats, clonidine did not influence RVLM pERK1/2 or blood pressure; 4) after blockade of central A_1 R (DPCPX) in SO rats, clonidine significantly reduced blood pressure and increased RVLM pERK1/2; and 5) in contrast, central adenosine A_1 R blockade had no influence on RVLM pERK1/2 or blood pressure responses elicited by clonidine in ABD rats. These findings suggest that central adenosine A_1 R signaling downplays neuronal signaling that culminates in increased RVLM pERK1/2, a cellular response that underlies, at least in part, the hypotensive response elicited by clonidine. Furthermore, the findings yield insight into cellular mechanisms that might explain the absence of the hypotensive action of clonidine in conscious normotensive rats.

The present findings demonstrate that surgical barodenervation (ABD), which leads to baroreflex dysfunction (Abdel-Rahman, 1992), was associated with a significant reduc-

Fig. 4. Photomicrographs depicting the immunohistochemical images of pERK1/2 ir neurons in the RVLM under baseline conditions (left) and in response to clonidine (0.6 μ g i.c.) administered after aCSF (vehicle; middle) or the selective adenosine A₁R antagonist DPCPX (3.5 μ g i.c.; right) in SO and ABD rats. Scale bar, 100 μ m. Bar graphs, mean $\pm$ S.E.M. ($n = 3-5$) using one-way ANOVA. *, $P < 0.05$ compared with baseline level; #, $P < 0.05$ compared with aCSF pretreatment.

tion in adenosine A₁R expression in the RVLM. To establish relevance of this cellular response, we administered graded doses of the selective A₁R agonist CPA, which causes pressor response by activating the A₁R in the brainstem (Scislo and O'Leary, 2002). The immunohistochemical data (reduced A₁R in RVLM) and the associated attenuation of the CPA-evoked pressor responses in ABD, compared with SO rats, suggest reduced central A₁R signaling in ABD rats. These findings, which are in marked contrast with the enhanced A_{2A}R signaling in the RVLM in the same animal model (Nassar and Abdel-Rahman, 2008), demonstrate a shift in the balance of the central adenosine receptors in favor of the A_{2A}R in ABD rats. It is important that A_{2A}R signaling underlies clonidine-evoked hypotension, at least in part, via activation of pERK1/2-NOS signaling in the RVLM of ABD rats (Nassar and Abdel-Rahman, 2006, 2008). Therefore, the present findings inferred that reduced central A₁R signaling seems to contribute to clonidine-evoked hypotension in ABD rats. This finding, however, does not directly define the role of central A₁R signaling in clonidine-evoked hypotension because in ABD rats, the simultaneously enhanced A_{2A}R signaling seems to confound data interpretation. Therefore, it was important to delineate the role of central A₁R in blood pressure and neuronal (RVLM pERK1/2) responses elicited by clonidine.

We report a restraining influence of central adenosine A₁R signaling on the blood pressure response to clonidine because: 1) in the presence of fully functional central A₁R signaling in SO rats, clonidine failed to elicit hypotension, which fully agrees with reported findings including ours

(Abdel-Rahman, 1992; Ricci et al., 1992; Medvedev et al., 1998; Nassar and Abdel-Rahman, 2006); and 2) reduced A₁R signaling in the brainstem (reduced A₁R expression and CPA-evoked pressor responses) was associated with clonidine-evoked hypotension in ABD rats. Together, these findings seem to support the hypothesis that A₁R signaling opposes clonidine-evoked hypotension. As discussed above, the concomitantly enhanced A_{2A}R signaling in the RVLM of ABD rats (Nassar and Abdel-Rahman, 2008) makes it difficult to discern the role of A₁R signaling in clonidine-evoked hypotension. Although no study has directly investigated the role for central A₁R signaling in clonidine-evoked hypotension, our conclusion is strengthened by the findings that in the SHR, which exhibits attenuated pressor responses to intra-nucleus tractus solitarius adenosine (Abdel-Rahman and Tao, 1996), clonidine lowers blood pressure (Jastrzebski et al., 1995; Estato et al., 2004). It must be noted that the pressor response elicited by adenosine reflects A₁R signaling (Scislo and O'Leary, 2002). Therefore, A₁R signaling seems to hamper clonidine-evoked hypotension.

The mechanism by which A₁R signaling opposes clonidine-evoked hypotension is not fully understood. We focused on RVLM pERK1/2 because it is clearly implicated in the adenosine A_{2A}R-dependent hypotensive action of clonidine (Nassar and Abdel-Rahman, 2008), and clonidine-evoked hypotension is mediated, at least in part, via endogenous adenosine (Nassar and Abdel-Rahman, 2006). We show that in conscious SO rats, clonidine did not lower blood pressure, which agrees with reported findings (Bonham et al., 1984;

Abdel-Rahman, 1992; el-Mas et al., 1993, 1994) and had no effect on pERK1/2 expression in the RVLM (Figs. 3 and 4). In contrast, in ABD rats, clonidine enhanced RVLM pERK1/2 production and lowered blood pressure (Figs. 4 and 5); these findings agree with our recent findings, which suggest that these cellular and blood pressure responses elicited by clonidine are mediated by endogenously released adenosine via activation of central adenosine A_{2A} R (Nassar and Abdel-Rahman, 2006, 2008). We reasoned that, in conscious normotensive (SO) rats, after clonidine administration, the activation by endogenously released adenosine of the A_1 R might counterbalance the cellular and subsequent blood pressure responses triggered by A_{2A} R activation in the RVLM. To address this issue, we investigated the effects of centrally administered clonidine on RVLM pERK1/2 and blood pressure after blockade of central adenosine A_1 R.

The present study demonstrates, for the first time, that after central adenosine A_1 R blockade (DPCPX) in conscious normotensive rats, clonidine elicited a significant reduction in blood pressure and significantly increased pERK1/2 expression in the RVLM (Figs. 3 and 4). It is interesting that these cellular and blood pressure responses were similar to those elicited by clonidine in ABD rats in the present and reported (Abdel-Rahman, 1992; Nassar and Abdel-Rahman, 2006, 2008) studies. It is imperative to note that neither the cellular (enhanced RVLM pERK1/2) nor the hypotensive response elicited by clonidine in ABD rats was influenced by central A_1 R blockade. This pharmacological evidence lends support to the immunohistochemical and CPA data, which demonstrated reduced central A_1 R expression/signaling in ABD rats. Together, the present findings highlight the restraining role for brainstem A_1 R signaling against A_{2A} R signaling, which might explain the absence of clonidine-evoked hypotension in conscious normotensive rats. It is equally important that the findings further support a mechanistic role for pERK1/2 in mediating clonidine-evoked hypotension (Fig. 5).

The possibility must be considered that baroreflex dysfunction caused by central A_1 R blockade contributed to the hypotension caused by clonidine in conscious SO rats in the present study. Although baroreflex sensitivity was not measured in the present study, we and others have consistently shown that partial (aortic or carotid) or complete (sinoaortic) denervation causes reduction in baroreflex sensitivity and substantial enhancement of clonidine-evoked hypotension in conscious normotensive rats (Bonham et al., 1984; Abdel-Rahman, 1992; Ricci et al., 1992; Ricci and Taira, 1995, 1999; Medvedev et al., 1998). Furthermore, adenosine A_1 R knock-down in the brainstem, by antisense against A_1 R, reduced baroreflex sensitivity of conscious normotensive rats (Mao et al., 1994) to a level comparable with that reported in ABD and SHR rats (Judy et al., 1976, 1979; Judy and Farrell, 1979; Abdel-Rahman, 1992; Prados et al., 1998). Therefore, it is likely that a reduced BRS in ABD rats might reflect suppressed central adenosine A_1 R function and explain, at least in part, the enhanced hypotensive action of clonidine in this model.

In summary, the present study provides evidence for a restraining influence of central A_1 R against the A_{2A} R-mediated cellular events (enhanced pERK1/2-NOS signaling in the RVLM) that underlie clonidine-evoked hypotension. This conclusion, based on the model depicted in Fig. 5, is sup-

Fig. 5. Schematic presentation of the cellular (RVLM pERK1/2) and blood pressure responses elicited by manipulating central adenosine A_1 R and A_{2A} R signaling. This model is based on the present and our reported (Nassar and Abdel-Rahman, 2006, 2008) findings. Compared with SO, surgical ABD caused anatomical (brainstem) and functional up-regulation of A_{2A} R (large red circle) and the molecular targets for clonidine (I_1/α_{2A} , yellow and green circles, respectively) and down-regulation of A_1 R (blue circle). Direct (CGS21680) or indirect (clonidine) activation of central A_{2A} R enhanced pERK1/2 production in the RVLM, which causes NOS phosphorylation, increases NO production and eventually causes hypotension. Blockade of central A_{2A} R with SCH58261 or inhibition of NOS with N^G -nitro-L-arginine methyl ester (L-NAME) abrogated clonidine-evoked hypotension; however, only the former abrogated pERK1/2 expression. Blockade of central A_1 R (DPCPX) eliminates the restraining influence of central A_1 R signaling on clonidine (A_{2A} R dependent) enhancement of pERK1/2 production in RVLM and the subsequent hypotension in conscious normotensive (SO) rats. In the presence of attenuated central A_1 R signaling (shift of adenosine signaling toward the A_{2A} R) in ABD rats, blockade of central A_1 R has little impact on clonidine (A_{2A} R dependent) enhancement of pERK1/2 production in RVLM and the subsequent hypotension.

ported by the following findings. 1) In the presence of fully functional central adenosine A_1 R signaling in conscious normotensive (SO) rats, clonidine failed to influence RVLM pERK1/2 or blood pressure. 2) In ABD rats, which exhibit attenuated A_1 R expression (RVLM) and signaling, clonidine enhanced RVLM pERK1/2 production and lowered blood pressure. 3) After pharmacological blockade of central A_1 R signaling (DPCPX) in conscious normotensive (SO) rats, clonidine enhanced pERK1/2 production in RVLM and lowered blood pressure. Our conclusion is bolstered by the findings in ABD rats, which exhibited attenuated central A_1 R signaling, that central A_1 R blockade (DPCPX) had no impact on the cellular (enhancement of RVLM pERK1/2 production) or hypotensive response elicited by clonidine. Together, the findings yield insight into the role of central A_1 R in blood pressure regulation and explain, at least in part, the lack of clonidine-evoked hypotension in conscious normotensive rats.

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