Inhibition of Phenylephrine-Induced Cardiomyocyte Hypertrophy by Activation of Multiple Adenosine Receptor Subtypes

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ABSTRACT

Plasma adenosine levels are elevated in cardiovascular disease including hypertension and heart failure, and the nucleoside has been proposed to serve as an endogenous antimyocardial remodeling factor. We studied the modulation of phenylephrine-induced hypertrophy by adenosine receptor activation in isolated neonatal cultured ventricular myocytes. Phenylephrine (10 μM) increased cell size by 35% and significantly increased expression of atrial natriuretic peptide. These effects were reduced by the stable adenosine analog 2-chloroadenosine and were completely blocked by the adenosine A1 receptor agonist N6-cyclopentyladenosine (1 μM), the A2A receptor agonist 2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxamidoadenosine (100 nM), and the A3 receptor agonist N6-(3-iodobenzyl)adenosine-5’-methyluronamide (100 nM). The antihypertrophic effects of all three agonists were completely reversed by their respective antagonists. Phenylephrine significantly up-regulated expression of the immediate early gene c-fos especially within the first 30 min of phenylephrine treatment. These effects were almost completely inhibited by all adenosine receptor agonists. Although phenylephrine also induced early stimulation of both p38 mitogen-activated protein kinase and extracellular signal-regulated kinase, these responses were unaffected by adenosine agonists. The expression of the G-protein regulatory factors RGS2 and RGS4 were increased by nearly 3-fold by phenylephrine treatment although this was completely prevented by adenosine receptor agonists. These agents also blocked the ability of phenylephrine to up-regulate Na/H exchange isoform 1 (NHE1) expression in hypertrophied myocytes. Thus, our results demonstrate an antihypertrophic effect of adenosine acting via multiple receptor subtypes through a mechanism involving down-regulation of NHE1 expression. The ability to prevent regulators of G-protein signaling (RGS) up-regulation further suggests that adenosine receptor activation minimizes signaling which leads to hypertrophic responses.

Adenosine, a product of adenine nucleotide catabolism, has been demonstrated to exert numerous effects on the cardiovascular system. For example, the vasodilating property of adenosine is well established, and it has been proposed that adenosine is an important regulator of coronary vascular resistance particularly under hypoxic situations—a phenomenon termed the “Berne hypothesis”. Extensive evidence has been presented demonstrating a cardioprotective effect of adenosine, as well as adenosine analogs, against ischemic and reperfusion injury using a variety of in vitro and in vivo approaches (reviewed in Headrick et al., 2003) which may be due, at least in part, to inhibition of the deleterious effects of oxidative stress on the heart (Karmazyn and Cook, 1992).

There is emerging evidence that adenosine could represent an important regulator of the myocardial remodeling process in response to various stimuli. Plasma adenosine levels are elevated in experimental hypertension (Ohnishi et al., 1988; Yamada et al., 1992), and elevated plasma concentrations of the nucleoside have been demonstrated in patients with congestive heart failure irrespective of a causative factor (Funaya et al., 1997). With respect to the latter, the degree of elevation in plasma adenosine concentrations was dependent on the severity of heart failure according to the New York Heart Association (NYHA) classification with the greatest increases (more than 5-fold) observed in NYHA class IV patients (Funaya et al., 1997). A study from the same group

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ABBREVIATIONS: CPA, N6-cyclopentyladenosine; CGS 21680, 2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxamidoadenosine; IB-MECA, N6-(3-iodobenzyl)adenosine-5’-methyluronamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CSC, 9-(3-chlorostyryl) caffeine; MRS 1523, 3-propyl-6-ethyl-[5-ethyl(thio)carbonyl]-2-phenyl-4-propyl-3-pyridinecarboxylate; NHE1, sodium hydrogen exchanger isoform 1; ANP, atrial natriuretic peptide; bp, base pair(s); PCR, polymerase chain reaction; RGS, regulator of G-protein signaling; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase.

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also demonstrated that administration of nucleoside transport inhibitors diprydiamole and dilazep (which increase adenosine levels) for 6 months reduced the severity of heart failure with an improvement in both ejection fraction and maximal oxygen consumption with the beneficial effects reversing after drug discontinuation (Kitakaze et al., 1998). Elevated levels of adenosine in cardiovascular disease may therefore represent a protective mechanism; for example, elevated adenosine could counter the deleterious effects of sympathetic nervous stimulation by its well known direct vasodilating effect or by inhibition of neurotransmitter release. Adenosine could further exert anti hypertensive effects by blocking the renin angiotensin system (Ohnishi et al., 1988). In rats with pressure-overloaded hypertrophy, the adenosine uptake blocker diprydiamole reduced abnormal left ventricular chamber filling and preserved adrenergic responsiveness in pressure overload-induced hypertrophy (Chung et al., 1998).

Extensive studies on the potential direct effects of adenosine on the remodeling process have not been carried out particularly in terms of understanding mechanisms. In the present report, we used neonatal cultured rat ventricular myocytes to assess the effect of adenosine and more specifically, selective adenosine receptor agonists on hypertrophy induced by the α₁ receptor agonist phenylephrine.

Materials and Methods

Cell Cultures. Primary myocyte cultures were prepared from neonatal Harlan Sprague-Dawley rat heart ventricles as described previously (Karmazyn et al., 2003). Isolated primary myocytes were plated onto glass coverslips for physiologic studies or onto Primaria (Falcon, Cowley, UK) culture dishes or flasks for collection of cell extracts. Myocytes were maintained for 48 h in medium containing Dulbecco’s modified Eagle medium/Ham’s F-12 supplemented with 10% fetal bovine serum, 10 μg/ml insulin, 10 ng/ml selenium, 50 units/ml penicillin, 50 μg/ml streptomycin, 2 mg/ml bovine serum albumin, 5 μg/ml linoleic acid, 3 mM pyruvic acid, 0.1 mM minimum medium nonessential amino acids, 10% minimal essential medium minimum medium solution, 0.1 mM bromodeoxyuridine, 100 μM t-ascorbic acid, and 30 mM HEPES, pH 7.2.

Experimental Protocol. Cells were serum-starved 24 h prior to all experiments after which cells were treated for 24 h with 10 μM phenylephrine. To assess the effect of adenosine in modulating hypertrophic responses, we first determined the effect of the stable adenosine analog, 2Cl-adenosine (10 μM). The effect of phenylephrine was subsequently studied in the presence of the following adenosine analog, 2Cl-adenosine (10 μM), the A2A receptor agonist 2-phenylethylamino-5′-N6-methyl-N6-cyclopentyladenosine (CPA, 1 μM), and the A3 receptor agonist N6-(3-iodobenzyl)adenosine-5′-methyloxonamide (IB-MECA, 100 nM). Additionally, the effect of their corresponding antagonists 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 10 μM), 8-(3-chlorostyryl) caffeine (CSC, 10 μM), and 3-propyl-6-ethyl-5(ethylthio)carboxamidoadenosine (MRS 1523, 1 μM), respectively, was determined. The concentrations of drugs used in the present study reflect those who were found to exert maximal effects in initial experiments aimed at identifying optimal concentrations of each agent. To confirm selectivity of the effects of antagonist against their respective receptors, experiments were performed in which the effect of the specific receptor antagonist was also tested against agonists acting on either of the other two receptors. For all experiments, 2Cl-adenosine, adenosine receptor agonists, or the agonist-antagonist combination was added 10 min before administration of phenylephrine.

Measurement of Cell Surface Area. Cardiomyocyte surface area was determined for 50 randomly selected cells per experiment and averaged to provide an N value of one. Cells were visualized with a Leica inverted microscope equipped with a Polaroid digital camera using 20× magnification. Cell area was determined using Mocha software.

Determination of Gene Expression. Reverse transcription-polymerase chain reaction was used to analyze NHE1 and ANP mRNA expression. The NHE1 primers used were primer 1, 5′-TCTGTPGACCTGTTGAATGGA-3′ and primer 2, 5′-CTCACCTGAGCAAGGTGTGTA-3′ with a predicted product size of 210 bp and a competitor size of 292 bp. The forward and reverse primers for rat ANP were 5′-CTGTTAGACCCACTTGGAGGA-3′ and 5′-AAGCTGT-TGACGCTATTGC-3′, respectively, with a PCR product of 320 bp. Samples were then electrophoresed in 3% agarose gels containing ethidium bromide and quantified via computer densitometry. Glyceraldehyde-3-phosphate dehydrogenase was used as the reference gene for ANP expression, whereas for NHE1 expression the gene product was standardized using competitive PCR where NHE1 mRNA expression was compared with amplification of competitor fragments. For glyceraldehyde-3-phosphate dehydrogenase, the forward and reverse primers were 5′-AAAGGGCATCTGCGCTACA-3′ and 5′-CATGTTGTTGGGGCTGATGG-3′, respectively.

Real-time PCR was used to assess mRNA expression of c-Fos and RGS using a DNA Engine Opticon 2 system (MJ Research, Watertown, MA) according to the manufacturer’s instruction. The ribosomal 18S rRNA gene was used as an internal control. The following primer sequences were used: for RGS2, forward: 5′-AGCAAATATGGGCTGCT-TGCTGCAT-3′, reverse: 5′-GCTCTTGGTATTTTGCGGCTACA-3′; for RGS4, forward: 5′-GACTTCTGGATCAGCGTGTA-3′, reverse: 5′-CAGCTCGAGAATCTTGGC-3′; for c-Fos, forward: 5′-GCTTGGCT-GTCGAGGAGATTGC-3′; for 18S rRNA, forward: 5′-GTATCCGGTAAACCCACTT-3′, reverse: 5′-CCTACATGGTCATCAACCC-3′.

Western Blotting for p38 MAPK, ERK, and NHE1. The cells were plated at a concentration of 6 × 10³ cells/cm² dish. After washing with phosphate-buffered saline two times, the cells were scraped into 100 μl of lysis buffer (20 mM Tris, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 2 mM EDTA, 2 mM EGTA, 50 mM NaF, 200 μM Na₃VO₄, 10 mM NaF, 40 mM b-glycerophosphate, 10 μg/ml leupeptin, 1 μM peptatin A, 1 mM phenylmethylsulfonyl fluoride, and 1 μM calyculin A). The lysate was transferred to a 1.5-ml Eppendorf tube, homogenized, and centrifuged at 10,000g for 5 min at 4°C. The supernatant was transferred to a fresh tube. The protein concentration was assayed by the Bradford protein assay kit (Bio-Rad, Hercules, CA). Thirty micrograms of protein were loaded for 10% SDS-polyacrylamide gel electrophoresis and transferred to nylon membrane (Amersham Biosciences Inc., Piscataway, NJ). The membranes were blocked in 5% dry milk for 3 h, primary antibody for 2 h, secondary antibody for 1 h, and then detected by ECL reagent (Amersham Biosciences Inc.). Antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA) and used at 1:500, 1:1000, and 1:2000 dilutions for NHE1, p38 MAPK, and ERK, respectively.

Statistical Analysis. Results are given as means ± S.E.M. Statistical analyses were performed by using one-way analysis of variance. The post-test comparison was performed by the method of Bonferroni. Differences were considered significant when P < 0.05.

Results

Figure 1 demonstrates a representative example of cells exposed to phenylephrine in the presence or absence of adenosine agonists. The hypertrophic phenotype was evident in exposed to phenylephrine-treated cells but not in those treated with any of the adenosine agonists. Quantitative data are shown in Figs. 2–5. As shown in Fig. 2, 2Cl-adenosine, the nonhydrolyzable form of the nucleoside, blocked the phenylephrine-
induced hypertrophy as determined by cell size as well as ANP expression. We next examined the nature of adenosine receptor involvement in mediating the antihypertrophic effect of adenosine by determining the effects of specific receptor agonists. These agents included the A1 receptor agonist CPA, the A2A receptor CGS 21680 (CGS), or the A3 receptor agonist IB-MECA (IB). Original magnification = 20×.

![Fig. 1. Representative micrographs illustrating cardiomyocytes exposed for 24 h under control conditions or with phenylephrine alone (PE) or in the presence of 2 chloroadenosine (2Cl-Ad), the A1 receptor agonist CPA, the A2A receptor CGS 21680 (CGS), or the A3 receptor agonist IB-MECA (IB). Original magnification = 20×.](image)

Surprisingly, all three agonists equally inhibited the hypertrophic effect of phenylephrine. To further demonstrate receptor specificity in mediating the antihypertrophic effect of adenosine receptor agonists, we determined whether these effects could be reversed by their respective antagonists. As shown in Figs. 3–5, all adenosine receptor antagonists reversed the effect of their respective agonists against phenylephrine-induced hypertrophy. Moreover, none of the agonists on their own exerted any effects on basal cell area or ANP expression (Figs. 2–5). To confirm receptor antagonist selectivity against their specific receptor subtypes, CPA, the A2A receptor agonist CGS 21680, and the A3 receptor agonist IB-MECA. These results are summarized in Figs. 3–5. Although a slight attenuation of the antihypertrophic effect of CPA was observed with CSC, the results confirm the relative selectivity of adenosine receptor antagonists against their specific receptor subtypes.

![Fig. 2. The effect of 2Cl-adenosine (2Cl-Ade, 10 μM) on phenylephrine (PHE, 10 μM)-induced hypertrophy as determined by cell surface area and ANP expression. Bars indicate mean ± S.E. from 10 separate experiments. *: P < 0.05 from control; #: P < 0.05 from PHE alone. Bottom panel shows representative agarose gel for PCR products. All values were determined 24 h after phenylephrine treatment.](image)

![Fig. 3. The effect of the adenosine A1 agonist CPA (1 μM) in the absence or presence of the A1 antagonist DPCPX (10 μM) on phenylephrine (PHE, 10 μM)-induced hypertrophy as determined by cell surface area and ANP gene expression. Bars indicate mean ± S.E. from 10 separate experiments. *: P < 0.05 from control; #: P < 0.05 from PHE alone. Bottom panel shows representative agarose gel for PCR products. All values were determined 24 h after phenylephrine treatment.](image)
The ability of adenosine receptor agonists to activate MAP kinase was next determined by studying the early phosphorylation of p38 MAPK and p44/42 (ERK). Phenylephrine increased ERK phosphorylation with peak values attained by 5 and 15 min after drug addition (Fig. 6). However, there was generally no effect of any of the adenosine receptor agonists. Virtually identical results were observed with respect to p38 MAPK (Fig. 7). MAP kinase activities in cells treated for longer than 60 min were identical to basal values and unaffected by any treatment (not shown). Increased proto-oncogene expression has also been implicated in the development of cardiomyocyte hypertrophy, and we therefore determined mRNA expression of c-Fos after phenylephrine addition in the absence or presence of adenosine receptor agonists. As shown in Fig. 8, phenylephrine produced a rapid c-Fos induction during the initial 30 min of phenylephrine treatment, however, this was significantly attenuated by all adenosine receptor agonists.

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Discussion

We studied the effect of adenosine receptor modulators on the hypertrophic effect of the α₁ adrenoceptor agonist phenylephrine, an agent which has been used extensively to initiate hypertrophy in cultured myocytes. 2Cl-Adenosine, the nonselective adenosine receptor agonist that is not subjected to extensive hydrolysis by adenosine deaminase as is the parent nucleoside, effectively inhibited the hypertrophy produced by phenylephrine although the effect was generally less than that produced by individual receptor agonists alone. Although speculative, perhaps this reflects a nonspecific effect of the nucleoside or some degree of either hydrolysis or cellular uptake of 2Cl-adenosine by the nucleoside transporter thereby limiting its efficacy.

We then studied the effect of agonists acting on the A₁, A₂A, and A₃ adrenoceptors...
and A3 receptors on their ability to modulate the phenylephrine-induced hypertrophy. These receptor agonists were selected based on our ability to identify the abundant presence of these receptor subtypes using Western blotting, whereas only faint A2B receptor blots were seen (not shown). Moreover, the present lack of availability of highly selective A2B receptor ligands led us to concentrate our studies on the other three receptor subtypes. It was surprising that the antihypertrophic effects of adenosine receptor agonists were similarly observed with agents acting on the A1, A2A, and A3 receptors in relatively equal fashion. Nonetheless, despite the fact that these receptors are coupled to diverse cell signaling processes, activators of multiple adenosine receptor subtypes have also been shown to protect the ischemic and reperfused myocardium (Yao and Gross, 1993; Auchampach et al., 2003; Boucher et al., 2004). In the present study, the antihypertrophic effect of adenosine receptor agonists were similarly observed with agents acting on the A1, A2A, and A3 receptors in relatively equal fashion. Nonetheless, despite the fact that these receptors are coupled to diverse cell signaling processes, activators of multiple adenosine receptor subtypes have also been shown to protect the ischemic and reperfused myocardium (Yao and Gross, 1993; Auchampach et al., 2003; Boucher et al., 2004). In the present study, the antihypertrophic effect of adenosine agonists was associated with prevention of early up-regulation of the immediate early gene c-fos which is likely an important event during the development of early hypertrophy (Babu et al., 2000). The early activation of ERK and p38 MAPK, both of which are also likely important in the hypertrophic response (Zechner et al., 1997; Bueno and Molkentin, 2002), was unaffected by any of the adenosine receptor agonists.

In general, our study concurs with the recent report of Liao and coworkers who similarly demonstrated an antihypertrophic effect of 2Cl-adenosine in neonatal rat ventricular myocytes although in that study the effect was mimicked only by an A1 receptor agonist, whereas both an A2A and an A3 agonist were ineffective (Liao et al., 2003). The reasons for the differences in receptor-mediated effects are uncertain although the effects which we observed were unlikely to involve nonspecific effects of either the A2A or A3 agonists since their antihypertrophic effects, as well as those of the A1 agonist CPA, were prevented by their respective antagonists which on their own were devoid of direct effects. Moreover, at the concentrations used, the effects of these antagonists were restricted to their respective agonists suggesting a high degree of selectivity. We also used identical pharmacological tools to probe the adenosine system such that differences in agents used could not account for the differences in responses seen in the two studies although the concentration of phenylephrine in our study was 10-fold lower than that used by Liao et al. (2003) since in our hands this concentration produced maximum effect on hypertrophy. However, whether this accounts for the different responses seen in the two studies remains to be determined. It is interesting, however, to add that a recent study reported an antimitogenic effect of adenosine in cardiac fibroblasts which could further contribute to an antiremodeling role for the nucleoside (Dubey et al., 2001). However, in that study, these effects were found to be exclusively dependent on adenosine A2B receptor activation with no effect of either an A1 or an A2A receptor agonist (Dubey et al., 2001). Thus, the exact nature of the receptors mediating the antihypertrophic or antiremodeling effect of adenosine requires further studies.

Our study was also designed to explore the potential mechanisms underlying the antihypertrophic effect of adenosine. As previously suggested (Kitakaze and Hori, 2000), there are a number of potential mechanisms by which adenosine could exert salutary effects in heart failure such as attenuation of norepinephrine release or decreased endothelin, angiotensin II, or cytokine production. Moreover, collagen and protein synthesis by isolated cardiac fibroblasts is inhibited by adenosine suggesting that adenosine has an antifibrosis effect (Dubey et al., 2001). Lastly, it should be emphasized that in

![Fig. 6. Profiles of p44/42 and phospho p44/42 (P-p44/42) levels in cardiomyocytes after addition of phenylephrine (PHE, 10 μM) alone or in the presence of the A1 receptor agonist CPA (1 μM), the A2A receptor agonist CGS 21680 (CGS, 100 nM), and the A3 receptor agonist IB-MECA (IB, 100 nM). Bars indicate mean ± S.E. from 10 separate experiments with corresponding examples of Western blots for both p44/42 and P-p44/42. *P < 0.05 from control.](https://jpet.aspetjournals.org)
our study the antihypertrophic effect of adenosine or adenosine receptor agonists was restricted to determining their effects against phenylephrine, and it remains to be determined whether the effect we report with multiple adenosine receptor agonists can also be applied to other hypertrophic stimuli or to the in vivo situation.

We also examined NHE1 expression for a number of reasons. For example, there is now strong evidence that suggests an important role for this transporter in mediating cardiac hypertrophy (Cingolani, 1999; Karmazyn, 2001). Secondly, there is also evidence that adenosine inhibits /A1 adrenergic-mediated activation of NHE1 in cardiac cells through a mechanism mediated by A3 receptors (Avkiran and Yokoyama, 2000). Although the effect of A2A or A3 receptor activation on NHE1 activity in cardiac cells has not been reported, preliminary studies in our laboratory indicate that CGS 21680 and IB-MECA at concentrations effective in reducing hypertrophy are also effective in inhibiting NHE1 activity in cardiomyocytes subjected to intracellular acidosis by ammonium chloride pulsed. Thus, it appears that activation of multiple adenosine receptor subtypes could inhibit NHE1 activity. In the spontaneously hypertensive rat, treatment with an NHE1 inhibitor significantly attenuates myocardial hypertrophy and fibrosis (Camilone de Hurtado et al., 2002), whereas standard antihypertensive treatment with an angiotensin-converting enzyme inhibitor, a calcium channel, or AT1 receptor blocker normalizes up-regulated myocardial NHE1 activity (Alvarez et al., 2002). Taken together, the possibility that NHE1 is related to the antihypertrophic effect of adenosine receptor activation was considered. Our results did indeed demonstrate that the antihypertrophic effect of adenosine receptor activation was associated with completely blocked NHE1 up-regulation that occurred after phenylephrine administration in the absence of adenosine receptor agonists. Although this does not prove a cause and effect relationship, the results suggest that inhibition of NHE1 up-regulation may account, at least in part, for the antihypertrophic effect of adenosine receptor agonists.

Adenosine receptors have been initially classified primarily with respect to their ability to affect adenylate cyclase. For example, adenosine A1 and A3 receptor stimulation results in adenylate cyclase inhibition, whereas A2A receptor mediates adenylate cyclase activation (reviewed in Schulte and Fredholm, 2003). Thus, it is unlikely that the ability of adenosine receptor activation to modulate adenylate cyclase activity was a common mechanism for the antihypertrophic effect of adenosine receptor agonists. It is interesting that phenylephrine significantly increased both RGS2 and RGS4 expression that was completely prevented by adenosine receptor agonists. RGS proteins that act as GTPase-activating proteins thus limiting G-protein-dependent responses are up-regulated in a variety of cells and tissues by different stimuli, including the activation of Gq- and Gs-coupled receptors. Gq-mediated increases in RGS2 have been attributed to increased intracellular calcium, increased protein kinase C activity, or both (reviewed in Chidiac and Roy, 2003). Thus, the increase in RGS2 observed herein in response to the A3 adrenoceptor activation may be related to these effects, although this needs to be determined with further experiments. It is interesting that although
RGS proteins appear to be antihypertrophic, overexpression of RGS4 in transgenic mice has been shown to enhance mortality following left ventricular pressure overload produced by aortic coarctation possibly due to reduced cardiac compensation (Rogers et al., 1999). The mechanism(s) via which adenosine receptor agonists attenuate RGS protein up-regulation is, at present, unclear. We have previously demonstrated that adenosine A1 receptor activation inhibits phenylephrine-induced cardiomyocyte changes including the increase in the calcium transient; however, no effect was seen with either an A2A or A3 agonist (Hoque et al., 2000). Cardiomyocyte adenosine receptors are not known to decrease phospholipase C activity, and indeed, some adenosine receptor subtypes are linked to protein kinase C activation (Henry et al., 1996). Intriguingly, one study has shown RGS2 to be increased in human heart failure and that symptomatic relief resulting from using a left ventricular assist device was accompanied by a decrease in this protein back to levels observed in nonfailing hearts (Takeishi et al., 2000). It is interesting that the use of a left ventricular assist device has been shown to reverse left ventricular hypertrophy (Zafeiridis et al., 1998). Although extrapolation to the present study should be done cautiously, the finding may be analogous to the antihypertrophic effect of adenosine. Taken together with the present results, a logical scenario may be that RGS proteins are up-regulated to minimize deleterious signaling under hypertrophic conditions, returning to normal levels when such stresses are relieved, i.e., with adenosine receptor activation. However, the precise role of either RGS2 or RGS4 in mediating hypertrophic or antihypertrophic responses requires further studies particularly in view of, as already alluded to above, a potential deleterious effect of RGS protein up-regulation under certain conditions (Rogers et al., 1999). Indeed, an indepth study to determine the role of RGS proteins in general in mediating the antihypertrophic influence of adenosine would be important.

![Fig. 8. Profiles of c-Fos mRNA levels in cardiomyocytes after addition of phenylephrine (PHE, 10 μM) alone or in the presence of the A1 receptor agonist CPA (1 μM), the A2A receptor agonist CGS 21680 (CGS, 100 nM), and the A3 receptor agonist IB-MECA (100 nM). Data were obtained using real-time PCR. Bars indicate mean ± S.E. from eight separate experiments. *, P < 0.05 from respective values; +, significantly higher values from 5-min controls. All values were determined 24 h after phenylephrine treatment.](image1)

![Fig. 9. Effect of phenylephrine (PHE, 10 μM) on NHE1 gene expression in cardiac myocytes. Cells were treated with phenylephrine alone or in the presence of adenosine agonists in the presence or absence of their respective antagonists. Bars indicate mean ± S.E. from 10 separate experiments. *, P < 0.05 from control. All values were determined 24 h after phenylephrine treatment.](image2)

![Fig. 10. Profiles of RGS2 and RGS4 mRNA levels in cardiomyocytes after addition of phenylephrine (PHE, 10 μM) alone or in the presence of the A1 receptor agonist CPA (1 μM), the A2A receptor agonist CGS 21680 (CGS, 100 nM), the A3 receptor agonist IB-MECA (IB, 100 nM), or the adenosine analog 2Cl-Ade (10 μM). Data were obtained using real-time PCR. Bars indicate mean ± S.E. from eight separate experiments. *, P < 0.05 from control. All values were determined 24 h after phenylephrine treatment.](image3)

Although neonatal cardiomyocytes have been used exten-
sively to study the cellular and molecular basis for hypertrophic responses, nonetheless studies with these cells should be interpreted cautiously because of potential differences when compared with cardiomyocytes from adult animals. Our results add strength to the concept that endogenous adenosine serves as anti-hypertrophic factors especially under conditions where plasma levels of the nucleoside are elevated such as hypertension or heart failure.

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References


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