Adenosine A1 Receptor Activation Attenuates Cardiac Injury Produced by Hydrogen Peroxide

Morris Karmazyn and Michael A. Cook

Adenosine has been shown to protect the ischemic and reperfused myocardium. To examine whether the protective effect of the nucleoside is mediated by modulation of oxidative stress, isolated rat hearts were perfused for 30 minutes with 100 μM H2O2 or an exogenous free radical–generating system consisting of purine (3.06 mM) and xanthine oxidase (10 units/l) in the presence or absence of drugs acting on adenosine A1 or A2 receptors. H2O2 alone produced a greater than 90% loss in contractility concomitant with a threefold elevation in resting tension, although these effects occurred in the absence of ultrastructural damage. Two A1 receptor agonists N6-cyclopentyladenosine (CPA, 1 μM) and R(-)-N6-(2-phenylisopropyl)adenosine (R-PIA, 1 μM) significantly attenuated the cardiodepressant effects of H2O2 and depressed the elevation in resting tension; however, only the effect of CPA was found to be significant with regard to the latter parameter. A similar concentration of S(+)-N4-(2-phenylisopropyl)adenosine (S-PIA), a markedly less potent A1 receptor agonist, was found to be without beneficial effect. However, a significant protective effect against both the reduction in contractility and the elevation in resting tension was seen with a 10-fold elevation in the concentration of S-PIA (10 μM). The protective effects on functional parameters were associated with preservation of high-energy phosphate and adenine nucleotide contents after 30 minutes of H2O2 treatment. The salutary effects of all drugs were reversed in the presence of the A1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (0.5 μM). An A2 receptor agonist 2-[(p-(carboxyethyl)phenethylamino)-5'-N-ethylcarboxamidoadenosine, termed CGS 21680 (1 μM), failed to alter the cardiac response to H2O2 with regard to all parameters studied. Neither a 50% reduction in external CaCl2 concentration nor treatment with 10 μM DL-propranolol exerted salutary effects against H2O2-induced dysfunction. None of the A1 receptor agonists modulated the response to purine plus xanthine oxidase. Our results demonstrate a selective protective effect of adenosine A1 receptor activation against the cardiac toxicity of H2O2 and provide, at least in part, a basis for the cardioprotective actions of adenosine and its analogues. *(Circulation Research 1992;71:1101–1110)*

**KEY WORDS**  • H2O2  • oxidative stress  • heart  • myocardial contractility  • energy metabolism  • cardiac protection

Adenosine, a coronary vasodilator, has been proposed as an important regulator of coronary vascular tone, particularly during hypoxia.  

More recently, substantial evidence has been presented describing salutary effects of adenosine against cardiac injury produced by ischemia and reperfusion. These beneficial effects of adenosine are likely mediated by A1 receptor activation, in contrast to the A2-mediated coronary vasodilation, and indeed, the ventricular myocyte is thought to possess primarily the A1 receptor subtype.  

The availability of potent A1-selective agonists has resulted in substantial evidence documenting beneficial effects of this group of compounds against myocardial reperfusion injury. Very recently, it has been suggested that A1 receptor activation accounts for the phenomenon of myocardial preconditioning that bestows cardioprotection against reperfusion injury after brief periods of ischemia. Although the beneficial effects of A1 activation have been well documented, the mechanisms underlying this phenomenon are not completely understood. The generation of reactive oxygen-derived species has been established as a major mechanism of cardiac dysfunction produced by reperfusion, particularly with regard to mediation of myocardial stunning. Although the toxic effects of these compounds are minimized by endogenous scavenging systems, these protective mechanisms can be overwhelmed under conditions when the generation of free radicals and other reactive species, including hydrogen peroxide (H2O2), is elevated, as may occur after reperfusion of the ischemic myocardium. In view of the importance of these reactive species to the reperfused myocardium, we hypothesized that a potential explanation for the therapeutic effects of A1 receptor activation could be related to attenuation of oxidative stress. In this regard, we used pharmacological approaches to assess the influence of A1 receptor activation on injury produced by 1) an exogenous free radical–generating system consisting of purine plus xanthine oxidase as well as 2) the adminis-
tration of H$_2$O$_2$, both of which have been demonstrated to produce myocardial injury.$^{14,15}$ Our study suggests a selective cardioprotective influence of A$_1$ receptor activation against H$_2$O$_2$-induced cardiac injury.

**Materials and Methods**

**Animals**

Male Sprague-Dawley rats weighing between 275 and 325 g were purchased from Charles-River Canada Ltd., St. Constant, Canada. Animals were provided free access to food and water and were housed in the animal care facilities of the faculty of medicine, University of Western Ontario, in accordance with guidelines of the Canadian Council on Animal Care, Ottawa.

**Heart Perfusion**

Rats were killed by decapitation, and hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer to produce an immediate cessation of contraction. Hearts were squeezed a few times to dislodge any clotted blood in the coronary vasculature and immediately mounted by the aorta for perfusion through the coronary arteries by use of the Langendorff technique, as described in detail previously.$^{16}$ The perfusion fluid was Krebs-Henseleit buffer consisting of (mM) NaCl 120, NaHCO$_3$ 20, MgCl$_2$ 1.2, KCl 4.63, KH$_2$PO$_4$ 1.17, CaCl$_2$ 1.25, and glucose 8. Some experiments were performed in which the CaCl$_2$ concentration was reduced to 0.62 mM. The buffer was continuously gassed with a 95% O$_2$–5% CO$_2$ mixture; pH was 7.4. To ensure thermoregulation (37°C), the entire system was enclosed in a series of water-jacketed chambers and coils. Flow rate was maintained at 10 ml/min.

Contractile function was assessed as apicobasal displacement via a force-displacement transducer (model FT.O3, Grass Instrument Co., Quincy, Mass.) attached to the ventricular apex and positioned to yield an initial preload resting tension of 2 g.$^{16}$ This signal was further connected to a Grass 7P20 differantiator to obtain rates of force development (+dF/dt) and relaxation (−dF/ dt).$^{16}$ A side arm off the perfusion cannula was attached to a pressure transducer (model P23XL, Spectramed) for the recording of coronary pressure. Hearts were initially allowed to contract spontaneously; however, because of the negative chronotropic effect of numerous pharmacological interventions (see below), all hearts were subsequently stimulated at 3 times threshold at a rate of 5 Hz with a Grass S44 stimulator via platinum-needle electrodes inserted into the ventricular epicardium.

**Treatment Protocols**

All hearts were initially equilibrated for 15 minutes. To study the effect of A$_1$-selective agonists, N$^6$-cyclopentyladenosine (CPA, 1 μM) or either of the diastereoisomers $R$(-)- or $S$(-)-N$^6$-(2-phenylisopropyl)adenosine ($R$-PIA or $S$-PIA, respectively, 1 μM) was added for 15 minutes before the addition of purine (3.06 mM) plus xanthine oxidase (13.33 units/l) or H$_2$O$_2$ (100 μM). Hearts were then perfused for a further 30 minutes with the appropriate drug combination. We also examined the direct influence of the A$_2$ receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 0.5 μM) and the A$_2$-selective agonist 2-$p$-[carboxyethyl]phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680, 1 μM) by using a similar approach. To examine the potential reversibility of A$_1$-mediated effects, experiments were done in which DPCPX was administered 5 minutes before the addition of either CPA or PIA. Some experiments were done in which DL-propranolol (10 μM) was added 15 minutes before H$_2$O$_2$ administration by a protocol similar to that used for other agents. Adenosine receptor agonists and antagonists were purchased from Research Biochemicals Inc., Natick, Mass.; DL-propranolol was from Sigma Chemical Co., St. Louis, Mo. All of these drugs except $S$-PIA and DL-propranolol, which are soluble in water, were dissolved in absolute ethanol. The ethanol concentration in the perfusion buffer was 0.01%, which by itself was completely without effect on any parameter examined. Purine and xanthine oxidase were purchased from Sigma, and H$_2$O$_2$ was obtained from BDH Inc., Toronto, Canada.

As noted above, CPA and PIA produced marked (>90%) negative chronotropic effects that were maximal after 5–10 minutes. Therefore, in all studies, the hearts were stimulated electrically as described above, 5 minutes before the addition of purine plus xanthine oxidase or H$_2$O$_2$ and for the remainder of the perfusion period.

**Metabolite Assays**

At the end of the perfusion period, hearts were rapidly clamped between tongs precooled in liquid nitrogen. Energy metabolites were measured spectrophotometrically in 6% perchloric acid extracts according to Bergmeyer$^{17}$ and as described previously.$^{16}$ Energy charge was calculated from the following equation: ATP+$\frac{1}{2}$ADP/ATP+ADP+AMP.$^{18}$

**Electron Microscopy**

Two hearts (treated with H$_2$O$_2$ only and H$_2$O$_2$ plus CPA) were immediately removed at the end of the perfusion period and perfusion-fixed at a constant perfusion pressure of 80 cm H$_2$O with 150 ml fixative containing 0.08 M sodium cacodylate, 2% glutaraldehyde, and 1% paraformaldehyde, pH 7.4; after which, sections measuring approximately 1 cm×1 mm×1 mm were cut from the left ventricle. Subsequent processing and sectioning were performed at the Department of Pathology, University Hospital, London, Canada, using a Lynx automatic tissue processor. Tissues were post-fixed with 1% osmium tetroxide and dehydrated with graded ethanol and acetone rinses. The final ethanol/acetone solution was replaced by en bloc stain consisting of a 3:7 ratio of uranyl nitrate/saturated lead acetate solution for 1 hour. The tissues were infiltrated and embedded in Epon Araldite resin and polymerized overnight at 70°C. Thin sections (60–90 nm) were cut with a diamond microtome knife and stained with uranyl acetate and lead citrate. Samples were viewed on an electron microscope (model 109, Carl Zeiss, Inc., Thornwood, N.Y.).

**Statistical Analysis**

Data were analyzed using either a one-way or multifactorial analysis of variance for analysis of tissue metabolites or function, respectively, followed by a Student-Newman-Keuls test. Data are presented as
**Results**

The profiles of contractile changes in terms of developed force or resting tension produced by either H$_2$O$_2$ or purine plus xanthine oxidase are shown in Figure 1. This figure demonstrates the markedly greater degree of dysfunction in terms of contractile depression or resting tension elevation produced by H$_2$O$_2$. With either treatment, a significant depression in developed force was observed after 5 minutes of perfusion, whereas a significant effect (after 15 minutes) on resting tension was seen only with H$_2$O$_2$.

Figure 2 shows the influence of 1 μM CPA on functional responses of isolated rat hearts in terms of developed force (DF), resting tension (RT), and rates of force development (+dF/dt) and relaxation (−dF/dt) to 30 minutes of perfusion with H$_2$O$_2$ in the presence or absence of 1 μM N$^\circ$-cyclopentyladenosine (CPA). The results are mean±SEM of five experiments. Time refers to minutes after H$_2$O$_2$ addition. *When values were first significantly different (p<0.05) from those obtained before H$_2$O$_2$ or P+XO addition.

mean±SEM. Differences were regarded as significant at p<0.05.

**Function Data**

The protective actions of CPA are given in Figure 3. It is evident from this recording that H$_2$O$_2$ also decreased coronary pressure. None of the drugs studied modulated this effect, with the exception of CGS 21680 or 10 μM S-PIA, which alone reduced coronary pressure by 43±3% and 58±3%, respectively. After these pretreatments, H$_2$O$_2$ failed to produce further coronary relaxation (data not shown).

We also carried out a limited qualitative analysis to assess whether ultrastructural abnormalities occur as a consequence of H$_2$O$_2$ administration. As shown in Figure 4, no gross damage was observed in a heart perfused with H$_2$O$_2$ only (panels A and B) or, not unexpectedly, in one perfused with H$_2$O$_2$ plus CPA (panels C and D). Thus, the present model appears to be one in which contractile depression occurs in the absence of necrosis. To ensure that ischemia did not mediate any of the actions of H$_2$O$_2$, we also measured tissue lactate contents after various treatments. Lactate contents ranged from 2.8±1.12 μmol/g dry wt (H$_2$O$_2$ alone) to 5.87±2.03 μmol/g dry wt (H$_2$O$_2$ plus R-PIA), and no significant differences were found between any treatment group studied. Moreover, we found no relation between lactate content and contractile function, irrespective of treatment (data not shown).
To further probe the role of the A1 receptor, studies with CPA were repeated in the presence of the selective A1 receptor antagonist DPCPX (0.5 \( \mu \)M). DPCPX alone was without effect on contractile depression produced by H2O2; after 30 minutes of treatment, developed force, \(+dF/dt\), and \(-dF/dt\) were decreased to 14\(\pm\)8\%, 16\(\pm\)9\%, and 14\(\pm\)6\% of pre-H2O2 values, respectively, whereas resting tension increased by 246\(\pm\)27\% \((n=4, \text{data not shown})\). However, DPCPX almost totally reversed the salutary effects of CPA to values not significantly different from those observed with H2O2 only. Thus, as shown in Figure 5, in the presence of CPA plus DPCPX, contractility was reduced by approximately 90\% of the pre-H2O2 values, a result not significantly different from that observed with H2O2 alone. Furthermore, resting tension increased by 250\% after 30 minutes of treatment under these conditions (Figure 5). Moreover, for all parameters, significant H2O2-induced effects occurred sooner in the presence of CPA plus DPCPX.

Experiments were also conducted to examine the effects of R-PIA, as well as its substantially less potent diastereoisomer S-PIA, against H2O2-induced dysfunction. As shown in Figure 6, 1 \( \mu \)M R-PIA significantly attenuated H2O2-induced contractile depression and prolonged the time required to produce significant depression, whereas an identical concentration of S-PIA was without effect. With regard to resting tension, a trend toward reduction in resting tension elevation was evident with R-PIA; however, this failed to reach statistical significance. Nonetheless, the time required to produce a significant elevation in resting tension was increased to 25 minutes compared with 15 and 10 minutes observed with H2O2 alone or with H2O2 plus S-PIA, respectively. Surprisingly, in the presence of S-PIA, resting tension increased to values significantly greater than those seen with H2O2 alone after 20 minutes of treatment (Figure 6). Although the data are not shown, the protective effect of R-PIA was reversed in the presence of 0.5 \( \mu \)M DPCPX to values not significantly different from those obtained with H2O2 alone \((n=3)\). For example, after 30 minutes of H2O2 treatment in the presence of R-PIA and DPCPX, developed force, \(+dF/dt\), and \(-dF/dt\) were decreased to 11\(\pm\)3\%, 12\(\pm\)4\%, and 9\(\pm\)3\% of pre-H2O2 values, respectively, and resting tension was elevated to 306\(\pm\)41\%.

In view of the lower potency of S-PIA at the A1 receptor compared with R-PIA, experiments were performed using a 10-fold greater concentration of S-PIA (10 \( \mu \)M) to determine if this compound could bestow similar protection against H2O2-induced dysfunction. As shown in Figure 7, 10 \( \mu \)M S-PIA produced a marked attenuation of H2O2-induced dysfunction. In the presence of this drug, the decline in contractility was reduced to 40\%, and resting tension was elevated to 170\% after 30 minutes of H2O2 treatment. These protective effects were totally abolished in the presence of DPCPX, and the time required to produce significant effects was decreased for all parameters (Figure 7).

The effect of CGS 21680, an A1 receptor-selective agonist, was also examined. As shown in Table 1, H2O2 produced identical reductions in contractility and elevations in resting tension irrespective of CGS 21680 administration.

As shown in Table 2, neither hypocalcemic perfusion \((0.62 \text{ mM CaCl}_2)\) nor treatment with propranolol mimicked the cardioprotective effects of A1 receptor agonists.

A similar approach to that used for H2O2 was used to examine the effects of A1 receptor occupancy on cardiac dysfunction produced by the administration of purine plus xanthine oxidase. In contrast to its protective effects against H2O2, CPA failed to influence either the magnitude or the time required to produce a depression in contractility or the elevation in resting tension produced by the free radical-generating system (Figure 8). Moreover, none of the other pharmacological manipulations had any effect against purine plus xanthine oxidase-induced dysfunction (data not shown).

### Metabolite Data

Myocardial metabolic status after the various treatments is summarized in Table 3. Thirty minutes of perfusion with H2O2 resulted in a significant reduction in high-energy phosphate and adenine nucleotide contents as well as in the energy charge compared with pre-H2O2 values. These effects were generally reversed by CPA, R-PIA, and the higher concentration of S-PIA to values not significantly different from those found before H2O2 administration and significantly greater than those seen in hearts perfused with H2O2 only. The most consistent effect was seen with CPA, particularly with regard to the reversal of the H2O2-induced reduction in energy charge. Addition of DPCPX before the A1 receptor agonist partially reversed the metabolic status to values not significantly different from those
Karmazyn and Cook
A Receptor Activation Process Against H2O2

Figure 4. Transmission electron micrographs from a heart perfused for 30 minutes with H2O2 alone (panels A and B) or H2O2 plus 1 μM N6-cyclopentyladenosine (panels C and D). No gross abnormalities are evident. Mitochondria appear intact, and sarcomeres are relaxed. Intercalated disc (panel A) as well as nucleus and nuclear material (panel C) appear normal. Bar, 1 μm.
produced by H$_2$O$_2$ alone. CGS 21680 was without effect on any parameter.

Table 3 also shows the effects of purine plus xanthine oxidase on myocardial energy metabolism. Although a trend toward a reduction in high-energy phosphates and adenosine nucleotides was evident, this was not significant, either in the presence or absence of CPA.

**Discussion**

Adenosine exerts numerous cardiac effects, including coronary vasodilation, which has led to the hypothesis that the nucleoside represents an important regulator of coronary vascular reactivity. Moreover, adenosine produces negative inotropic, chronotropic, and dromotropic effects and attenuates the myocardial response to catecholamines. The effects of adenosine are mediated by specific membrane-bound receptors, specifically, A$_2$ receptors in vasculature and A$_1$ receptors in cardiac myocytes, some of which are linked to stimulation or inhibition of adenylate cyclase, respectively. Recent evidence has suggested that adenosine may also act as an endogenous cardioprotective agent, particularly in the ischemic and reperfused myocardium. For example, it has been shown in isolated rat hearts that adenosine and R-PIA delay the time to onset of ischemic contracture and enhance recovery on reperfusion. Moreover, Norton et al., using an in vivo rabbit model, recently demonstrated a protective effect of adenosine infusion in terms of infarct size reduction after coronary artery occlusion and reperfusion. These authors have also demonstrated a protective effect of low doses of CPA and high doses of CGS 21680. Interestingly, adenosine has also been shown to protect the reperfused myocardium when administered at the time of reflow, thus precluding the necessity for pretreatment and potentially enhancing its clinical usefulness in reduction of postreperfusion myocardial dysfunction. Aside from the salutary effects of exogenous adenosine, it has also been reported that maintenance of extracellular adenosine concentration by prevention of adenosine catabolism with adenosine deaminase inhibitors or by the use of nucleoside transport inhibitors similarly produced salutary effects in the ischemic and reperfused rat and rabbit hearts.

Although the beneficial effects of adenosine and its analogues against myocardial ischemia and reperfusion injury have been demonstrated, the mechanisms underlying these effects are uncertain. A proposed mechanism for the beneficial influence of adenosine is based on its reintroduction into the adenosine nucleotide pool, thus resulting in improved de novo synthesis and ATP
repletion on reflow. In this regard, an active myocardial adenosine transport system that mediates the rapid uptake and phosphorylation of exogenous adenosine has been demonstrated. Dependent on receptor specificity, adenosine can also produce various other effects, such as the inhibition of the release of, or the action of, norepinephrine, as well as attenuation of platelet or neutrophil activation. For obvious reasons, modulation of blood-borne factors can be excluded as a potential mechanism for the beneficial effects of adenosine or adenosine analogues under in vitro conditions, which suggests that these compounds are also able to protect the myocardium directly. In addition, attenuation of either platelet or neutrophil activation is mediated via $A_2$ receptor activation, which would not explain the beneficial effects of selective $A_2$ receptor agonists. Such effects on $A_2$ receptors offer the most likely explanation for the beneficial effect of high doses of CGS 21680 under in vivo conditions versus the lack of effect in the present report.

To overcome the potential problem of adenosine uptake and its subsequent metabolism to adenosine nucleotides, we used relatively specific adenosine receptor modulators to probe the possible mechanisms of cardiac protection afforded by adenosine and its analogues. To our knowledge, the present report demonstrates for the first time that adenosine $A_2$ receptor activation selectively inhibits the cardiotoxic effects of $H_2O_2$, a finding that could be of substantial importance in understanding the mechanisms of the cardioprotective actions of endogenous adenosine. In addition, the study suggests that $A_2$ receptor activation represents a useful approach in the development of strategies aimed at reducing postreperfusion dysfunction mediated by $H_2O_2$-induced oxidative stress, particularly since this selective approach would limit $A_2$ receptor-mediated vasodilatation. The evidence for implicating $A_2$ receptor activation in the attenuation of $H_2O_2$-induced injury is based on a number of observations. First, cardioprotection was evident with three specific $A_2$ receptor agonists, and the degree of protection was consistent with the known rank order of potencies of these agonists at the $A_2$ receptor: CPA > R-PIA > S-PIA. Second, the salutary effects of all agonists were reversed by DPCPX, an $A_2$ receptor-selective antagonist that alone had no effect against $H_2O_2$-induced dysfunction. The inability of DPCPX to modulate $H_2O_2$-induced injury likely reflects the fact that in a well-oxygenated heart there is insufficient extracellular adenosine present to protect against $H_2O_2$. However, it should be emphasized that in the present study we used a $H_2O_2$ concentration that produced contractile depression in the absence of gross cell damage. Whether $A_2$ receptor agonists possess similar protective actions vis-à-vis inhibition of cell

![Graph showing the reversal of protective effects](image-url)

**Figure 7.** Graphs showing the reversal of protective effects of 10 μM S(+)-N6(2-phenylisopropyl)adenosine (S-PIA) against $H_2O_2$-induced contractile depression by 0.5 μM 1,3-dipropyl-8-cyclopentylxanthine (DPCPX). DF, developed force; RT, resting tension; $dF/dt$, rates of force development (+) and relaxation (−). The results are mean ± SEM of five experiments. Time refers to minutes after $H_2O_2$ addition. *p<0.05 from those obtained before $H_2O_2$ addition. 

| Table 1. Effect of 1 μM $A_2$ Receptor Agonist CGS 21680 on Contractile Depression and Elevation in Resting Tension Produced by 100 μM $H_2O_2$ |
|---------------------|---------------------|---------------------|
| Time (minutes)      | $H_2O_2$ only       | $H_2O_2$+CGS 21680  |
|                     | DF                  | RT                  | $dF/dt$ | $dF/dt$ |
|                     | DF                  | RT                  | $dF/dt$ | $dF/dt$ |
| 5                   | 71±3*               | 138±11              | 76±1*   | 62±1*   |
| 10                  | 56±3                | 153±12              | 58±3    | 49±3    |
| 15                  | 38±3                | 159±14*             | 43±3    | 29±3    |
| 20                  | 24±4                | 198±12              | 30±3    | 20±2    |
| 25                  | 15±4                | 273±22              | 18±3    | 10±2    |
| 30                  | 6±3                 | 316±23              | 8±1     | 5±2     |

CGS 21680, $A_2$ receptor agonist; DF, developed force; RT, resting tension; $dF/dt$, rates of force development (+) and relaxation (−); time, minutes after $H_2O_2$ addition. Values are mean ± SEM and indicate percentage of pre-$H_2O_2$ values; n=5 for both treatment groups. 

*When values were first significantly different (p<0.05) from those obtained before $H_2O_2$ addition.
necrosis produced by higher concentrations of H₂O₂ remains to be determined.

The mechanism responsible for the salutary effects of A₁ receptor agonists against H₂O₂ is uncertain. The protective actions on function were associated with improved myocardial energy status in terms of high-energy phosphate or total adenine nucleotide preservation. However, we observed little association between contractility and energy charge. This may reflect the complex association between calculated energy charge and cell damage.18 Indeed, one study reported a loss in ATP after cardiac arrest in the absence of change in the energy charge.33 Nonetheless, our study does show a significant reduction in energy charge in hearts perfused with H₂O₂ alone or H₂O₂ plus CGS 21680, conditions that produced the greatest reduction in high-energy phosphate contents. In view of the fact that the drugs used in the present study are unlikely to be incorporated into the adenine nucleotide pool as is adenosine, the preservation of high-energy phosphate or total adenine nucleotide contents probably does not reflect an increased availability of the precursor. A possible explanation may lie in the fact that H₂O₂ has been shown to enhance intracellular calcium concentration, which may contribute to its cardiotoxic properties.34 Conversely, A₁ receptor activation is linked to depressed calcium influx either through the inhibition of cAMP-dependent phosphorylation of the calcium channel or enhanced potassium conductance,35 effects that could thus oppose the actions of H₂O₂ by attenuation of the intracellular calcium overload. Indeed, the reduction in resting tension elevation by A₁ receptor agonists can also be attained, at least in the reperfused and reoxygenated heart, by calcium channel blockers,36 an observation that indirectly supports this contention. Surprisingly, however, our initial studies failed to demonstrate a protective effect of hypocalcemic perfusion against H₂O₂-induced toxicity. This may reflect the fact that 0.62 mM CaCl₂, which was used in these experiments, was a concentration sufficiently high to mediate H₂O₂-induced dysfunction. Nonetheless, this finding agrees with a recently completed study in our laboratory that showed that modulation of external calcium concentration or the use of calcium channel blockers failed to modify the cardiac response to exogenous H₂O₂ (K.M. Zimmer and M. Karmazyn, manuscript in preparation).

Further studies are clearly required to more precisely probe the relation between calcium and H₂O₂-induced injury at the cellular level. We also attempted to determine the role of adrenergic mechanisms in H₂O₂-induced toxicity by examining whether propranolol can mimic the salutary effects of A₁ receptor agonists. The inability of propranolol to modify the cardiac response to H₂O₂ suggests, but does not necessarily prove, that the salutary effects of A₁ receptor agonists are not related to an antiaadrenergic mechanism.

A surprising observation was the specificity of A₁-mediated effects for H₂O₂-mediated dysfunction despite

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Table 2. Effect of Hypocalcemic Perfusion or 10 μM DL-Propranolol on Contractile Depression and Elevation in Resting Tension Produced by 100 μM H₂O₂

Legend: DF, developed force; RT, resting tension; dF/dt, rates of force development (+) and relaxation (−); time, minutes after H₂O₂ addition. Values are mean±SEM and indicate percentage of pre-H₂O₂ values; n=3 for both treatment groups.

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</table>

Graphs showing functional responses of isolated rat hearts in terms of developed force (DF), resting tension (RT), and rates of force development (+dF/dt) and relaxation (−dF/dt) to 30 minutes of perfusion with 3.06 mM purine (P) plus 13.3 units/mg xanthine oxidase (XO) in the presence or absence of 1 μM N6-cyclopentyladenosine (CPA). The results are mean±SEM of four experiments. Time refers to minutes after P+XO addition. *When values were first significantly different (p<0.05) from those obtained before P+XO addition.
the lower toxicity produced by the exogenous free radical–generating system. This finding suggests some differences in mechanisms of H$_2$O$_2$ versus purine plus xanthine oxidase–mediated effects. For example, it has been proposed that interference with cardiac sarcoplasmic reticular function in terms of inhibition of calcium uptake and elevation in calcium efflux through ryano-dine-sensitive calcium channels may account for the negative inotropic effects of a superoxide anion–generating system consisting of xanthine plus xanthine oxidase, but not that due to H$_2$O$_2$. Nonetheless, future studies are needed to precisely explain these apparently selective salutary effects of A$_1$ agonists against H$_2$O$_2$–induced toxicity.

Although this cannot be answered with certainty at the present time, a pertinent question to ask is whether the protection against H$_2$O$_2$–induced toxicity contributes to the salutary effects of A$_1$ agonists in the ischemic and reperfused myocardium. Whether oxidative stress mediates reperfusion injury is a controversial issue, particularly with regard to injury associated with myocardial necrosis; however, there is good evidence that free radicals and oxidative stress are important mediators of myocardial stunning. Despite the controversies, it should be noted that H$_2$O$_2$ production and accumulation in the reperfused ischemic myocardium have been reported. Indeed, Brown et al$^{38}$ have reported a close temporal relation between loss of ventricular function and H$_2$O$_2$ production in the reperfused isolated rat heart and have implicated H$_2$O$_2$ as the primary oxygen metabolite mediating contractile depression associated with reperfusion. It is interesting to point out that manipulations that result in elevated tissue catalase contents and thereby enhance the breakdown of H$_2$O$_2$ protect the heart against reperfusion injury.$^{40,41}$

In conclusion, we have demonstrated effective protection exerted by adenosine A$_1$ receptor agonists against cardiac injury produced by exogenous H$_2$O$_2$. Although the precise mechanism(s) responsible for this phenomenon still requires elucidation, our results suggest that this property may contribute to the protective effects of adenosine and adenosine analogues against myocardial reperfusion injury. The use of adenosine itself may be problematic, since it nonselectively activates both A$_1$ and A$_2$ receptors with consequent undesirable vascular actions, such as provocation of ischemia due to coronary steal.$^{42}$ Adenosine A$_1$ receptor activators should obviate such problems and may represent a rational approach in the development of pharmacological strategies aimed at protecting against ventricular dysfunction, which may be associated with elevated H$_2$O$_2$ contents in the reperfused myocardium.

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References


### Table 3. Contents of Myocardial Energy Metabolites and Energy Charge After Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolite content (µmol/g dry wt)</th>
<th>Energy charge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP</td>
<td>CrP</td>
</tr>
<tr>
<td>30-Minute control</td>
<td>17±0.9</td>
<td>18±1.0</td>
</tr>
<tr>
<td>H$_2$O$_2$ alone</td>
<td>8.7±0.4*</td>
<td>11.8±0.8*</td>
</tr>
<tr>
<td>H$_2$O$_2$+CPA</td>
<td>13±0.3†</td>
<td>20.9±0.6†</td>
</tr>
<tr>
<td>H$_2$O$_2$+CPA+DPCPX</td>
<td>11.8±1.0</td>
<td>17.0±2.1</td>
</tr>
<tr>
<td>H$_2$O$_2$+R-PIA</td>
<td>11.7±1.3</td>
<td>16±1.9</td>
</tr>
<tr>
<td>H$_2$O$_2$+S-PIA (1 µM)</td>
<td>8.9±1.6*</td>
<td>12.4±2.0</td>
</tr>
<tr>
<td>H$_2$O$_2$+S-PIA (10 µM)</td>
<td>14.9±0.8†</td>
<td>20.0±1.5†</td>
</tr>
<tr>
<td>H$_2$O$_2$+S-PIA (10 µM)+DPCPX</td>
<td>12.5±1.5</td>
<td>17.9±2.1</td>
</tr>
<tr>
<td>H$_2$O$_2$+CGS 21680</td>
<td>9.6±1.6*</td>
<td>13.5±2.5</td>
</tr>
<tr>
<td>P+XO</td>
<td>13.5±1.0†</td>
<td>14.3±1.1</td>
</tr>
<tr>
<td>P+XO+CPA</td>
<td>12.5±2.1†</td>
<td>19.8±3.9</td>
</tr>
</tbody>
</table>

CrP, creatine phosphate; HEP, total high-energy phosphates; TAN, total adenine nucleotides; CPA, N$^6$-cyclopentyladenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; CGS 21680, A$_2$ receptor antagonist; R-PIA, R(-)-N$^6$-(2-phenylisopropyl)adenosine; S-PIA, S(+)-N$^6$-(2-phenylisopropyl)adenosine; P+XO, purine plus xanthine oxidase. Values are mean±SEM; n=4–7.

*P<0.05 vs. 30-minute control values.
†P<0.05 from H$_2$O$_2$ alone values.


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Adenosine A1 receptor activation attenuates cardiac injury produced by hydrogen peroxide.

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