Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors

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Peart, Jason N., and Garrett J. Gross. Adenosine and opioid receptor-mediated cardioprotection in the rat: evidence for cross-talk between receptors. Am J Physiol Heart Circ Physiol 285: H81–H89, 2003. First published March 13, 2003; 10.1152/ajpheart.00985.2002.—The roles of free-radical production, mitochondrial ATP-sensitive K⁺ (mitoKATP) channels and possible receptor cross-talk in both opioid and adenosine A1 receptor (A1AR) mediated protection were assessed in a rat model of myocardial infarction. Sprague-Dawley rats were subjected to 30 min of occlusion and 90 min of reperfusion. The untreated rats exhibited an infarct of 58.8 ± 2.9% [infarct size (IS)/area at risk (AAR), %] at the end of reperfusion. Pretreatment with either the nonselective opioid receptor agonist morphine or the selective A1AR agonist 2-chloro-cyclopentyladenosine (CCPA) dramatically reduced IS/AAR to 41.1 ± 2.2% and 37.9 ± 5.5%, respectively (P < 0.05). Protection afforded by either morphine or CCPA was abolished by the reactive oxygen species scavenger N-(2-mercaptopropionyl)glycine or the mitoKATP channel blocker 5-hydroxydecanoate. Both morphine- and CCPA-mediated protection were attenuated by the selective A1AR antagonist 1,3-dipropyl-8-cyclopentylnxanthine and the selective δ-opioid receptor (DOR) antagonist 7-benzylidenelactone. Simultaneous administration of morphine and CCPA failed to enhance the infarct-sparing effect of either agonist alone. These data suggest that both DOR and A1AR-mediated cardioprotection are mitoKATP and reactive oxygen species dependent. Furthermore, these data suggest that there are converging pathways and/or receptor cross-talk between A1AR- and DOR-mediated cardioprotection.

Adenosine and adenosine receptor agonists have been shown to protect the myocardium from ischemia-reperfusion injury (48–50). Earlier research focused on the role of the A1 adenosine receptor (A1AR); however, recent evidence suggests that the A3 adenosine receptor (A3AR) may also afford cardioprotection (29, 48, 50, 63). Both A1AR and A3AR activation may be important triggers of both early preconditioning (EPC) and delayed preconditioning (DPC) (4, 58, 67); however, separate signaling pathways may be involved (38). Activation of opioid receptors has also been demonstrated to trigger EPC and DPC, primarily through the δ-opioid receptor (DOR) (45, 47, 54). Indeed, blockade of the DOR abrogates the infarct-sparing effect of ischemic preconditioning (55).

The signal-transduction pathways responsible for both DOR and A1/A3AR-mediated cardioprotection appear to be similar. A1ARs and A3ARs activate phospholipase C and phospholipase D, respectively (44). Activation of the DOR has also been reported to activate phospholipase C (33, 41). Activation of phospholipase C or phospholipase D results in translocation of specific isoforms of protein kinase C (PKC) (59, 61). Translocation of PKC may then lead to opening of a mitochondrial ATP-sensitive K⁺ channel (mitoKATP) after phosphorylation of a mitochondrial site (43). However, this point is controversial because it has also been reported (36, 64) that opening of the mitoKATP channel may be upstream of PKC and that activation of this channel may lead to generation of reactive oxygen species (ROS), which may subsequently activate PKC and various downstream kinases to produce cardioprotection (7).

We and others (7, 47) have previously reported that the infarct-sparing effect of DOR activation is both mitoKATP and ROS dependent; however, there are opposing reports regarding the role of the mitoKATP channel and ROS in adenosine-mediated cardioprotection. Cohen et al. (7) observed that protection afforded by adenosine receptor activation was independent of both mitoKATP channel opening and ROS in isolated rabbit hearts, whereas other investigators reported (19, 63) that mitoKATP may be integral to the cardioprotective effects of adenosine.

Both A1AR and DOR activation appear to mediate protection via similar pathways. Indeed, there is considerable evidence in the central nervous system (CNS) that adenosine and opioid receptors are tightly coupled. Adenosine receptor activation via adenosine kinase inhibition has been shown to attenuate opiate withdrawal (26). Furthermore, inhibition of neuronal Ca²⁺ channels by opioid- and adenosine-receptor agonists was mutually exclusive, supporting the possibility of a convergence for the two stimuli (6). Adenosine and morphine inhibit Ca²⁺-dependent neurotransmitter release, and this inhibition could be blocked by the nonselective adenosine receptor antagonist theophylline (20, 53). The adenosine uptake inhibitor dipryrid...
amole potentiates adenosine- and morphine-mediated inhibition of neurotransmitter release (18, 42). Moreover, a recent study (17) determined that μ- and δ-opioid receptor activation increases adenosine concentrations in rat striatal cell extracts by inhibiting adenosine uptake. These examples suggest that important links exist between opioids and adenosine, interactions that, to date, have not been addressed in the heart.

Therefore, the goals of this study were twofold: first, to examine the controversial role of ROS and mitoKATP channels in A1AR- and DOR-mediated cardioprotection and, finally, to determine whether there is a functional coupling of adenosine and opioid receptors in the heart, leading to cardioprotection.

**METHODS**

This study was performed in accordance with the guidelines of the Animal Care Committee of the Medical College of Wisconsin, which is accredited by the American Association of Laboratory Animal Care.

**General surgical preparation.** Male Sprague-Dawley rats weighing 250–350 g were used throughout this study. The rats were anesthetized via intraperitoneal administration of thiotubaratav論 sodium (Inactin; 100 mg/kg), a long-acting barbiturate. A tracheotomy was performed, and the trachea was intubated with a cannula connected to a rodent ventilator (model CIV-101, Columbus Instruments; Columbus, OH, or model 683, Harvard Apparatus; Natick, MA). The rats were ventilated with room air supplemented with O2. Atelecstasy was prevented by the maintenance of a positive end-expiratory pressure of 5–10 mmHg. Arterial pH, Pco2, and Pao2 were monitored throughout the protocol with the use of a polygraph (model 7, Grass). The right jugular vein was cannulated with polyethylene-23 (Gould) pressure transducer connected to a maintained at 38°C with the use of a heating pad.

**Baseline function.** All rats equilibrated quickly after instrumentation. Baseline levels for all rats before experimental protocols, the coronary artery was reoccluded, and blood pressure were allowed to stabilize before the experimental protocols were initiated.

**Determination of myocardial infarction size.** On completion of the experimental protocols, the coronary artery was reoccluded, and the area at risk (AAR) was determined by negative staining. Patent blue dye was administered via the jugular vein to effectively stain the nonoccluded area of the LV. The heart was excised, and the LV was removed from the remaining tissue and subsequently cut into five thin cross-sectional pieces. This allowed for the delineation of the normal area, which was stained blue, versus the AAR, which subsequently remained pink. The AAR was excised from the nonischemic area, and the tissues were placed in separate vials and incubated for 15 min in a 1% triphenyltetrazolium chloride stain in 100 mM phosphate buffer (pH 7.4) at 37°C. Tissues were stored in vials of 10% formaldehyde overnight and the infarcted myocardium was dissected from the AAR under the illumination of a dissecting microscope (Cambridge Instruments). Infarct size (IS) and AAR were determined by gravimetric analysis. IS was expressed as a percentage of the AAR (IS/AAR).

**Experimental protocols.** After equilibration of hemodynamic parameters, the rats were divided into various experimental groups (Fig. 1). Rats were either untreated, treated with agonist alone, antagonist alone, or treated with both agonists and antagonists. Agonists were given as a bolus dose 10 min before the onset of ischemia, whereas antagonists were given 20 min before ischemia, except in the case of 5-hydroxydecanoic acid (5-HD), which was administered 15 min before agonists due to its short half-life. After treatment, hearts were occluded for 30 min, followed by 90 min of reperfusion before triphenyltetrazolium chloride staining.

**Exclusion criteria.** Rats were excluded from data analysis if they exhibited severe hypotension (<30 mmHg systolic pressure) or if we were unable to maintain adequate blood gas values within a normal physiological range due to metabolic acidosis. Exclusion of animals from the present study was evenly distributed among protocol groups.

**Chemicals.** 2-Chloro-6-N(3-iodobenzyl)-adenosine-5′-N-methyluronomide (CI-IB-MECA), 2-chloro-N6-cyclopentyladenosine (CCPA), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 5-HD, N(2-mercaptopropionyl)glycine (2-MPG), 3-propyl-6-ethyl-5[(ethylthio)carbonyl]-2-phenyl-4-propyl-3-pyridine carboxylate (MRS-1523), and morphine sulfate were obtained from Sigma (St. Louis, MO). 7-Benzylidenenaltrexone maleate (BNTX) was purchased from Tocris (Ellisville, MO).

**Statistical analysis of data.** All values are expressed as means ± SE. One-way analysis of variance with Newman-Keuls post hoc test was used to determine whether any significant differences existed in any parameter between groups. Significant differences were determined at *P* < 0.05.

**RESULTS**

**Baseline function.** All rats equilibrated quickly after instrumentation. Baseline levels for all rats before treatment were the following: heart rate, 360 ± 3 beats/min; systolic pressure, 120 ± 1 mmHg; diastolic pressure, 96 ± 1 mmHg; mean arterial blood pressure, 104 ± 1 mmHg; rate pressure product, 38 ± 1 mmHg/s/1,000. After 30 min of occlusion and 90 min of reperfusion, untreated hearts (*n* = 9) exhibited a reduction in rate pressure product (26 ± 2) due to a decrease in mean arterial blood pressure. The IS on termination of reperfusion was determined to be 58.8 ± 2.9% (IS/AAR, Fig. 2).

**Effect of A1AR and DOR activation and blockade.** Because of the hemodynamic effects of CCPA treatment (i.e., bradycardia), we attempted to find a dose where cardioprotection was still present, yet the hemodynamic effects were minimal. Three doses (10, 30, and 100 μg/kg) were examined. The effect of CCPA on heart
rate was dramatically reduced at 10 μg/kg, whereas the infarct-limiting effect was only slightly reduced from that seen following the two higher doses (Fig. 3). Thus the 10 μg/kg dose of CCPA was chosen for all future studies.

After 30-min occlusion and 90-min reperfusion, pre-treatment with 10 μg/kg CCPA \( (n = 6) \) significantly reduced IS (%IS/AAR) compared with untreated rats \( (P < 0.01 \text{ vs. untreated, Fig. 2}) \). To further eliminate the bradycardic effect of CCPA as a possible mechanism leading to the infarct-limiting effect, a subset of rats was electrically paced at 6 Hz. Pacing was achieved by placing two electrodes on the surface of the ventricle. The pacing voltage was kept <5 V, and pacing was maintained throughout the experimental protocol. No differences were noted between unpaced and paced hearts pretreated with 10 μg/kg CCPA (39.9 ± 4.4%, 35.0 ± 1.3% IS/AAR for unpaced and paced hearts, respectively). Morphine (0.3 mg/kg, \( n = 7 \)), when administered as a bolus dose 10 min before the onset of ischemia, also produced a marked reduction in IS/AAR \( (P < 0.05 \text{ vs. untreated, Fig. 2}) \). Interestingly, when morphine (0.3 mg/kg) and CCPA (10 μg/kg) were combined \( (n = 8) \) and given 10 min before occlusion, no additional protective effect was observed (Fig. 4).

Blockade of the A1ARs with the selective A1AR antagonist, DPCPX (100 μg/kg), failed to modify IS/AAR compared with untreated hearts \( (n = 5) \). When administered 20 min before occlusion, DPCPX completely abolished the cardioprotective effects of both CCPA \( (n = 6) \) and morphine \( (n = 7) \) \( (P < 0.05 \text{ vs. agonist alone, Fig. 2}) \). Furthermore, pretreatment with DPCPX also eliminated the bradycardic effects of A1AR activation (data not shown).

Interestingly, DPCPX pretreatment produced no change in the protection afforded by the combination of both CCPA and morphine \( (n = 6, \text{Fig. 4}) \). Even increasing the dose of DPCPX (1 mg/kg, \( n = 10 \)) failed to attenuate CCPA + morphine-induced cardioprotection \( (P > 0.05, \text{data not shown}) \).

To examine the effects of DOR inhibition, the selective DOR antagonist BNTX (1 mg/kg) was utilized. Pretreatment with BNTX alone 20 min before ischemia...
failed to alter IS; however, when given before morphine administration \((n = 6)\), BNTX abolished the protective effect of morphine \((P < 0.01\) vs. agonist alone, Fig. 2). Surprisingly, BNTX also abolished the protective effect of A1AR activation \((n = 7, P < 0.001\) vs. agonist alone, Fig. 2). In contrast to DPCPX, pretreatment with BNTX completely blocked protection afforded by the combination of CCPA and morphine \((n = 7, P < 0.001\) vs. combination alone, Fig. 4).

A3AR inhibition with MRS-1523 \((2\ \text{mg/kg, } n = 7)\) alone had no effect when given 20 min before occlusion. The dose of MRS-1523 used was determined as the minimum dose required to prevent histamine release from 100 \(\mu\text{g/kg Cl-IB-MECA} in the intact mouse (Dr. J. Auchampach, personal correspondence). Interestingly,
Fig. 4. Effect of A1AR inhibition (100 μg/kg DPCPX), DOR inhibition (300 μg/kg BNTX), A2AR blockade (2 mg/kg MRS-1532), ROS scavenging (20 mg/kg 2-MPG), and mitoK<sub>ATP</sub> channel inhibition (10 mg/kg 5-HD) on infarct-sparing effects of the combination of adenosine A1 (10 μg/kg CCPA) and opioid receptor activation [300 μg/kg morphine + CCPA (MC)]. IS/AAR% determined after 30-min occlusion and 90-min reperfusion. Values are means ± SE. *P < 0.05 vs. untreated; †P < 0.05 vs. combination alone.

Fig. 5. Involvement of ROS generation (A) and mitoK<sub>ATP</sub> channel activation (B) in cardioprotection mediated by A1AR (10 μg/kg CCPA) and opioid receptor (300 μg/kg morphine) activation. A: 2-MPG (20 mg/kg) given 10 min before administration of agonists. B: 5-HD (10 mg/kg) was given 15 min before the onset of occlusion, agonists given 10 min before occlusion. IS/AAR% was determined after 90 min of reperfusion. Values are means ± SE. *P < 0.05 vs. untreated; †P < 0.05 vs. agonist alone.

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Similar to that seen with A1AR blockade with DPCPX, A2AR blockade also inhibited the cardioprotective effects of CCPA (n = 6) and morphine (n = 7), both alone (P < 0.01 and P < 0.05 vs. agonist alone, respectively; Fig. 2) and in combination (n = 6, P < 0.001 vs. combination alone; Fig. 4).

MitoK<sub>ATP</sub> blockade. We examined the role of the mitoK<sub>ATP</sub> channel in mediating the cardioprotective effects of both CCPA and morphine. 5-HD (10 mg/kg) was administered 5 min before drug treatment and 15 min before occlusion. After 90 min of reperfusion, IS/AAR was unaltered by 5-HD treatment alone (n = 6, Fig. 5). However, administration of 5-HD abolished the infarct-limiting effect of CCPA (n = 7) and morphine (n = 6), alone (P < 0.001 and P < 0.01 vs. agonist alone, respectively; Fig. 5) or in combination (n = 9, n = 6, P < 0.001 vs. combination alone; Fig. 4).

ROS inhibition. The possibility of ROS production as being a trigger of the anti-infarct effects observed with A1AR and DOR activation was investigated with the use of 2-MPG, a free radical scavenger. 2-MPG (20 mg/kg) administered 15 min preocclusion did not alter IS from that of untreated rats (n = 7). When given before treatment with CCPA (n = 7) or morphine (n = 6, n = 8 in combination with CCPA), MPG negated their cardioprotective effects (P < 0.001, P < 0.01, and P < 0.001 vs. agonist/s alone, respectively; Figs. 4 and 5).

The vehicle for MRS-1532 and DPCPX, DMSO, was also administered alone or before morphine treatment, to determine whether the effects of these antagonists were due to the reported free-radical scavenging ability of DMSO. DMSO pretreatment (300 μl/kg, n = 4) did not modify IS in either the untreated rats or rats treated with morphine (n = 4).

**DISCUSSION**

The results of the present study clearly suggest that there is “cross-talk” between adenosine and opioid sig-
ously suggested for opioids and adrenergic receptors (14) and adenosine and adrenergic receptors (39).

The observed coupling of adenosine and opioid receptors in the CNS (6, 17, 18, 20, 27, 42), coupled with the known cardioprotective effects of adenosine, suggest that opioid receptors and adenosine may act in concert to provide a cardioprotective effect. Indeed, a recent study (28) of the rat heart demonstrated that the cardioprotective effects of fentanyl (a μ-opioid agonist) could be attenuated with an A1AR antagonist and a KATP channel blocker. The results of the present study support the notion that adenosine and DORs act via a converging pathway because no further protection was afforded when agonists of both receptors were applied simultaneously.

**Involvement of the mitoKATP channel.** The opening of a mitoKATP channel or a mitochondrial site of action appears to be an important trigger for the mediation of cardioprotection. Both ischemic and pharmacological preconditioning may be dependent on the activation of mitoKATP channels; however, the precise role of mitoKATP and sarcolemmal (sarc)KATP channels is controversial. Either KATP channel may trigger cardioprotection (7, 45) or be the end effector (43, 62) or, alternatively, may serve as both (65). The data presented in this study implicate an integral role for the mitoKATP channel in the signal transduction pathway after both acute opioid and adenosine receptor activation. Previous studies (13, 45, 46) from our laboratory support the observation, which suggests that DOR stimulation elicits a cardioprotective effect that is mediated via KATP channels in the intact rat heart. Similarly, Kato et al. (28) demonstrated that the protective effect of fentanyl, an opioid agonist, was blocked by 5-HD. Previous studies in our laboratory (13, 46) have demonstrated that a mitoKATP-dependent mechanism is involved as a distal effector leading to both early and delayed preconditioning conferred by DOR activation in rat hearts.

Of particular interest is our observation that A1AR-mediated protection was also abolished by 5-HD. The role of the KATP channel in mediating protection afforded by adenosine receptor activation is controversial. Whereas several studies support a role for the mitoKATP channel, Grover et al. (16) and Cohen et al. (7) failed to identify an interaction between adenosine receptor activation and KATP channel opening. The report by Cohen et al. (7) may be explained by the differential actions of adenosine itself as opposed to those of selective adenosine receptor agonists (48). In the isolated murine heart, the protective effect of transgenic overexpression of A1ARs was reduced by blockade of the mitoKATP channel (19). Furthermore, we recently reported that cardioprotection mediated via A1AR activation with cyclohexyladenosine is abolished with 5-HD treatment, whereas the protection observed after adenosine administration was not abolished by 5-HD, suggesting that adenosine-mediated cardioprotection is partly independent of mitoKATP opening (48). Indeed, evidence suggests that adenosine may even afford protection independent from that mediated by adenosine receptors (12, 48, 49). Similarly, Patel et al. (47) recently found that some of the cardioprotective effects of a selective DOR agonist are independent of opioid receptor occupation. Thus it is becoming apparent that certain “selective” receptor agonists may have effects independent of their effects on specific receptors and that the A1AR and DOR may be good examples.

**Involvement of ROS.** Much research has focused on the role of ROS as a trigger in EPC and DPC. It has been proposed that opening of the mitoKATP channel leads to a small “burst” of ROS (31). These oxygen radicals may then activate/translocate PKC and other intracellular kinases leading to an unknown effector (7), possibly the KATP channel. Our observation that opioid-induced cardioprotection is mediated via ROS is supported by the present results and the results obtained from previous studies in our laboratory (47), where the infarct-limiting effects of a selective δ- opioids agonist BW-373U86 were completely abrogated by pretreatment with 2-MPG. Of interest, however, is our finding with CCPA. This observation is in direct contrast with that of Cohen and colleagues (7), who demonstrated that protection produced by adenosine could not be inhibited by 2-MPG. Interestingly, Cohen et al. (7) also reported that adenosine-mediated protection is independent of mitoKATP channel opening. However, the opening of the mitoKATP channel appears to lead to the generation of free radicals (31) and previous studies (19, 63) have demonstrated that adenosine receptor activation mediates protection via downstream mitoKATP channel opening. Indeed, the results of the current study indicate that KATP activation is essential for A1AR-mediated protection. As mentioned earlier, this controversy may be due to differences between the effects of adenosine itself and selective adenosine receptor agonists, whereas the cardioprotective effects of adenosine receptor agonists are dependent on mitoKATP opening, whereas some effects of adenosine are only partially dependent on mitoKATP channel opening (48). Thus activation of the mitoKATP channel by CCPA may initiate the production or release of ROS, which then activate downstream kinases leading to myocardial protection. Collectively, these data demonstrate a pivotal role for the mitoKATP channel and ROS generation in protection afforded by both the A1AR and DOR.

**Receptor cross-talk.** To identify the effects of A1AR and DOR activation in the in vivo rat heart, we studied responses to a selective exogenous A1AR agonist, CCPA and the nonselective opioid agonist, morphine. The data reveal that both A1AR and opioid receptor activation result in a significant reduction in IS. Whereas morphine is a nonselective opioid agonist, it was determined that the protective effects afforded by morphine were attributed to DOR activation because the infarct-limiting effects were abolished with the use of the selective DOR antagonist BNTX. Of particular interest is the observation that the cardioprotection afforded by A1AR or DOR activation could be abolished by BNTX or DPCPX, respectively. Moreover, BNTX failed to antagonize the bradycardic effects of CCPA.
suggesting that direct A₁AR receptor activation was most likely not being inhibited by BNTX. Whereas A₁AR antagonism has previously been shown to abol-

ish opioid-mediated protection in the rat heart (28), these data are the first to suggest that DOR blockade inhibits A₁AR-mediated protection. Furthermore, an interaction between opioids and adenosine has been previously documented. Binding of [3H]DPCPX is re-
duced in the brain of μ-opioid receptor knockout mice (3). DORs are also downregulated in μ-knockout mice (30). Concentrations of cortical A₁AR sites are in-

creased after 72 h of morphine treatment in mice (27), and morphine produced a concentration-dependent re-

lease of adenosine (51). Moreover, bidirectional cross-

withdrawal syndromes were evident when adenosine agonist pretreated rats were administered the opioid antagonist naloxone and when rats pretreated with morphine were given DPCPX or DMPX (8), two adeno-

sine receptor antagonists. Whereas these studies demonstrate a tight coupling between opioids and adenosine in the CNS, the present data suggest a tangible link in cardiac tissue as well.

Simultaneous activation of both the A₁AR and DOR failed to enhance the protective effect of either agonist given alone, suggesting a converging pathway or that maximal cardioprotection was produced by either drug at the doses used. This protection was abolished by BNTX, MRS-1523, 2-MPG, and 5-HD, suggesting that this pathway is DOR, A₂AR, ROS, and mitoKATP de-

pendent; however, DPCPX failed to blunt the effect of combined A₁AR and DOR activation. This observation is of interest because MRS-1523, a selective A₁AR antagonist, also blocked the anti-infarct effects of CCPA. This occurred in the absence of any reduction in A₁AR-mediated bradycardia, suggesting that there is little or no occupation of the A₁AR by MRS-1523. Pre-

vious studies in isolated rabbit and rat hearts have demonstrated that A₂AR-mediated protection can be attenuated by A₁AR antagonists (29), where the affinity of the A₂AR for DPCPX was low (35). However, the report by Kilpatrick et al. (29) did not examine the role of the A₂AR in A₁AR-mediated protection. Shainberg et al. (56) also noted that DPCPX blocked the protection achieved by Cl-IB-MECA and that cardioprotection mediated by CCPA was unaffected by MRS-1523.

Whereas the results of Shainberg et al. (56) are in con-

trast to our findings, this may be explained by differences in the models used. Shainberg et al. (56) used cultured neonatal rat cardiomyocytes, devoid of blood-borne elements, whereas we utilized the in vivo adult rat model. The results of the present study sug-

gest a cross-talk between the A₁AR and the A₂AR as the A₂ARs are resistant to xanthine compounds (35) and the concentration of DPCPX used by Kilpatrick and colleagues (29) was well below that required to occupy A₂ARs (35), thus suggesting a relationship be-

between the A₁AR and A₂AR. Furthermore, overexpression of the A₂AR (9) resulted in a reduced basal heart rate, indicating a possible increase in A₁AR activation, due to either an increase in receptor number or endog-

enous adenosine production. Previous studies (2, 32, 36) have reported that adenosine is not required for IPC in the rat heart. These observations were based on pharmacological evidence with A₁AR antagonists and 8-SPT, which may not be inhibiting the A₂AR. In addition, CCPA may also activate the A₂AR, as it has been reported that CCPA competes with [125I]ABA binding with an inhibitor constant ~50 nM to rabbit-cloned A₂ARs (22). CCPA may activate both the A₁AR, producing both bradycar-

dia and protection, and the A₃AR, producing cardio-

protection. Indeed, MRS-1523 abolished the protection afforded by CCPA, suggesting that either CCPA occup-

es the A₃AR or that activation of the A₁AR leads to activation of the A₂AR, further supporting our hypoth-

esis of receptor cross-talk. Furthermore, Armstrong and Ganote (1) demonstrated that IPC was blocked by the nonselective receptor antagonists that occupy A₃AR but not those selective for the A₁AR.

Downey et al. (15) proposed that several triggers are required to reach threshold for activation of IPC. Ac-

cording to Downey et al. (15), two or more stimuli may be required to activate PKC to a degree that initiates the signal transduction pathway mediating IPC. Activ-

ation of A₁/A₃ARs and DOR may mediate transloca-

tion of PKC (33, 41, 44, 59, 61). Thus when CCPA and morphine are added in combination, the activation of A₃ARs via CCPA and enhanced adenosine release medi-

ated by both DOR (18, 51) and A₁AR activation (57) may be sufficient to activate PKC to the precondition-

ing “threshold.”

Finally, morphine has been shown to elevate inter-

stitial adenosine levels via an inhibition of adenosine kinase (17). Cyclohexyladenosine increases interstitial adenosine in metabolically stressed cells by a PKC-

dependent inhibition of adenosine kinase (17). Cyclo-

hexyladenosine increases interstitial adenosine levels by inhibiting endogenous ecto-5'-nucleotidase, whereas 5-HD has no such effect (52), providing evi-

dence that opening of the sarcKATP channel may me-

diate an increase in interstitial adenosine. DOR acti-

vation also opens sarcKATP channels and triggers de-

layed preconditioning (45). Thus morphine and CCPA may be enhancing endogenous adenosine concentrations to occupy the A₁AR and provide protection via A₁AR or A₂AR activation and non-receptor-mediated effects (5, 48, 50). Furthermore, adenosine deami-

nase predominates as the pathway for adenosine metab-

olism at times when adenosine levels are high (21, 25). Increased activation of adenosine deami-

nase during adenosine kinase inhibition will result in increased levels of inosine. Inosine has been shown to bind to A₂ARs at concentrations between 10 and 50 μM (24, 60).
In conclusion, these data reveal that A1AR and DOR agonists mediate cardioprotection via a mitoKATP and ROS-dependent mechanism. Our observations suggest that the A2AR is intimately involved in both A1AR- and DOR-mediated protection. Furthermore, there appears to be convergence between the pathways linking A1AR- and DOR-mediated protection. Indeed, it appears that A1AR and DOR activation may act in concert to afford cardioprotection.

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