Adenosine-mediated early preconditioning in mouse: protective signaling and concentration dependent effects

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Abstract

Objectives: Signaling in adenosine-mediated preconditioning is controversial. We examined roles of mitochondrial (mito) K\textsubscript{ATP} channels, protein kinase C (PKC) and nitric oxide (NO).

Methods: Langendorff perfused C57/Bl6 mouse hearts were subjected to 20 min ischemia and 45 min reperfusion. Effects of adenosine-mediated preconditioning were assessed in the absence and presence of signaling inhibitors.

Results: Control hearts recovered 70 ± 2 mmHg ventricular pressure, and released 18.1 ± 6.2 IU/g lactate dehydrogenase (LDH). Preconditioning with 10 μM adenosine limited necrosis (10.6 ± 1.4 IU/g) without modifying contractility (72 ± 2 mmHg) whereas 50 μM adenosine reduced necrosis (10.3 ± 1.6 IU/g) and contractile dysfunction (91 ± 2 mmHg). All protective effects of 10 and 50 μM adenosine were abrogated by mito K\textsubscript{ATP} channel blockade with 100 μM 5-hydroxydecanoate (5-HD) during the 'trigger' ATP phase, but unaltered by PKC or NO synthase inhibition with 3 μM chelerythrine or 100 μM N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), respectively. Protection against necrosis was eliminated by 5-HD but unaltered by chelerythrine or L-NAME during the 'mediation' phase (ischemia–reperfusion). Reduced contractile dysfunction with 50 μM adenosine was partially sensitive to 5-HD and chelerythrine, and only eliminated by co-infusion of the inhibitors.

Conclusions: Adenosine-mediated preconditioning is dose-dependent with high level stimulation reducing contractile dysfunction in addition to necrosis. Preconditioning is triggered by a mito K\textsubscript{ATP} channel dependent process independently of PKC and NO. Subsequent protection against necrosis is also mediated by a mito K\textsubscript{ATP} channel dependent process independent of PKC and NO. In contrast, functional protection may be mediated by parallel mito K\textsubscript{ATP} and PKC dependent paths.

Keywords: Experimental; Heart; Organ; Pathophysiology

1. Introduction

Mechanisms of adenosine-mediated cardioprotection remain controversial. Investigations implicate mito K\textsubscript{ATP} channels as downstream mediators of cardioprotective stimuli including preconditioning [1,2] and adenosine [3,4]. However, adenosine-mediated preconditioning was recently found to be mito K\textsubscript{ATP} independent, in contrast to other G-coupled stimuli [5]. Furthermore, there is evidence mito K\textsubscript{ATP} channels are uninvolved in ischemic preconditioning [6], contrasting data supporting obligatory roles for mito K\textsubscript{ATP} channels prior to and during ischemia [1,2,7,8].

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Downey and colleagues [5,6] present a scheme whereby preconditioning stimuli activate multiple paths converging on PKC and/or radical generation. However, the function of PKC is also controversial, with evidence for [9,10] and against [11,12] an essential role in cardioprotection, opposing data regarding location downstream [13] vs. upstream [14] of mito K\textsubscript{ATP} channels, and evidence adenosine may inhibit rather than activate PKC in ischemic myocardium [15]. The importance of endogenous NO is also unclear, with evidence for [16,17] and against [18,19] a role in early preconditioning, and for [20] and against [21] a role in adenosine-mediated protection. There is even evidence NO worsens ischemic injury via an adenosine-dependent mechanism [18].

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These uncertainties may reflect multiple signaling paths in preconditioning [22,23], providing ‘redundancy’. It is also possible different pathways may mediate differing protective effects. There is evidence for disparate signaling in anti-necrotic vs. anti-stunning [24,25] or anti-arrhythmic [26] effects of preconditioning. The aim of the present study was to test the hypothesis that adenosine-mediated preconditioning involves multiple signaling pathways, potentially involving mito K\(_{\text{ATP}}\) channels, PKC, and NO.

2. Methods

2.1. Perfused heart preparation

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996), and work approved by the Institutional Animal Care and Use committee. The Langendorff mouse heart model described previously was employed [27,28]. Hearts were isolated from 16 to 20-week-old male C57/B6 mice (body weight = 23.6±0.4 g, n = 333) anesthetized with 50 mg/kg sodium pentobarbital administered intraperitoneally. After a thoracotomy, hearts were excised into ice-cold perfusion buffer, the aorta cannulated (20 gauge stainless steel) and perfused at 80 mmHg with Krebs buffer containing (mM): NaCl, 118; NaHCO\(_3\), 25; KCl, 4.7; KH\(_2\)PO\(_4\), 1.2; CaCl\(_2\), 2.5; Mg\(_2\)SO\(_4\), 1.2; glucose, 11; and EDTA, 0.6. Buffer was equilibrated with 95% O\(_2\), 5% CO\(_2\) at 37°C, giving a pH of 7.4 and pO\(_2\) ≥ 550 mmHg at the cannula. The left ventricle was vented with a polyethylene drain and a fluid-filled balloon inserted into the ventricle via the mitral. The balloon was connected to a pressure transducer for measurement of ventricular pressure. Hearts were immersed in perfusate at 37°C and balloons inflated to a diastolic pressure of ~5 mmHg. Coronary flow was monitored via a Doppler flow-probe (Transonic Systems, Ithaca, NY, USA), and function recorded on a MacLab (ADInstruments, Castle Hill, Australia).

2.2. Experimental protocol

Hearts stabilized for 10 min at intrinsic heart rate were switched to pacing at 420 beats/min (ventricular pacing, 2 ms duration, 20% above threshold) for 20 min. Baseline function was assessed and 20 min global ischemia initiated following by 45 min reperfusion. Pacing was stopped during ischemia and re-instated at 2 min reperfusion [4,27,28]. The protocol is outlined in Fig. 1. Hearts were untreated or subjected to preconditioning stimuli in the absence or presence of varied inhibitors. Preconditioning response to 10 or 50 μM adenosine were assessed by infusion for 5 min followed by 10 min washout prior to ischemia. To examine signal transduction during the trigger phase (preconditioning stimulus), experiments were repeated in hearts receiving 100 μM 5-HD, 3 μM chelerythrine, or 100 μM L-NAME initiated 2.5 min prior to adenosine infusion and terminated 2.5 min after infusion. To examine signaling during the mediation phase (ischemia–reperfusion), preconditioned hearts were treated with 100 μM 5-HD (n = 8 and 9 for 10 and 50 μM adenosine, respectively), 3 μM chelerythrine, 100 μM 5-HD plus 3 μM chelerythrine, 100 μM L-NAME, or 100 μM 5-HD plus 100 μM L-NAME initiated 5 min after adenosine infusion and maintained for the duration of the experiment. Inhibitors were also infused for the same periods in non-preconditioned hearts.

To confirm inhibitory effects of 5-HD, and ability to precondition via mito K\(_{\text{ATP}}\) channel activation, we examined effects of 5 min stimulation with 50 μM diazoxide followed by 10 min washout prior to 20 min ischemia. This was repeated with 100 μM 5-HD bracketing diazoxide infusion or during ischemia–reperfusion. Finally, effects of post-ischemic supplementation with 40 μM inosine+30 μM hypoxanthine+20 μM xanthine was examined in control hearts, and hearts preconditioned with 50 μM adenosine. Levels of substrate are based on interstitial estimates from microdialysis studies of ischemic myocardium [28]. The substrate mix was infused throughout reperfusion.

Inhibitory effects of L-NAME were confirmed in preliminary experiments in which dilatory responses to an A\(_{\text{2a}}\) adenosine receptor agonist (with 1 nM CGS21680) and to ADP (100 nM) were assessed in the absence and presence of 100 μM L-NAME. Experiments revealed L-NAME abrogated dilatory responses to these maximally effective stimuli (data not shown).

2.3. Determination of viability via LDH efflux, and comparison to infarct size

To assess necrosis, effluent was collected throughout reperfusion for LDH quantitation. Samples were stored at −80°C until enzymatic analysis (Sigma, St. Louis, MO, USA). Post-ischemic LDH efflux over 45 min reperfusion (IU/g) was determined by multiplying concentration (IU/ml) by effluent volume (ml/g).

In separate experiments, the relation between infarct size and LDH efflux was assessed for hearts reperfused for 45 min after 25 min ischemia, 20 min ischemia, or 20 min ischemia with preconditioning with 10 or 50 μM adenosine, 50 μM adenosine in the presence of either 5-HD or chelerythrine during ischemia–reperfusion. Infarct size was assessed as described previously [29]. On completion of experiments 8 ml of 1% (w/v) TTC in PBS was infused at a pressure of 100 mmHg. Hearts were removed from the cannula, weighed and fixed overnight in 10% formalin. Fixed hearts were stored at −20°C until sectioned transversely. The 0.5-mm sections were placed between microscope slides and visualized using a computer imaging system.
system. Boundaries of infarct area (TTC negative) were traced in each slice, and areas determined digitally by computer-assisted planimetry. Infarct size per section was expressed as % of area at risk (sum of total ventricular area minus cavities). Infarct size was averaged from five sections.

2.4. Drugs

Drugs were purchased from Sigma/RBI (Castle Hill, Australia). Adenosine, chelerythrine, 5-HD, and L-NAME were all prepared daily in distilled water. For diazoxide, a stock solution was prepared by initially dissolving the drug in a small volume of 0.1 N NaOH and subsequently diluting in water. All drug solutions were infused at 1% of coronary flow to achieve the final concentrations indicated.

2.5. Statistical analysis

Data are presented as mean±S.E.M. Baseline data and post-ischemic functional recoveries and enzyme effluxes in different experimental groups were analyzed by one-way ANOVA with Tukey’s post-hoc test. A value of P<0.05 was considered significant.
Table 1
Pre-ischemic functional parameters in control hearts, hearts preconditioned with adenosine (10 or 50 μM) or 50 μM diazoxide, and hearts treated with 100 μM 5-HD, 3 μM chelerythrine, or 100 μM L-NAME

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diastolic pressure (mmHg)</th>
<th>Systolic pressure (mmHg)</th>
<th>+dP/dt (mmHg/s)</th>
<th>−dP/dt (mmHg/s)</th>
<th>Flow (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>4±1</td>
<td>153±6</td>
<td>6395±318</td>
<td>3535±109</td>
<td>28.5±1.5</td>
</tr>
<tr>
<td>10 μM Adenosine (n=9)</td>
<td>5±1</td>
<td>147±4</td>
<td>6716±279</td>
<td>3656±190</td>
<td>30.1±0.7</td>
</tr>
<tr>
<td>50 μM Adenosine (n=8)</td>
<td>4±1</td>
<td>146±5</td>
<td>6150±241</td>
<td>3379±147</td>
<td>30.8±1.6</td>
</tr>
<tr>
<td>Diazoxide (n=8)</td>
<td>3±2</td>
<td>142±3</td>
<td>6204±254</td>
<td>3222±148</td>
<td>26.5±0.9</td>
</tr>
<tr>
<td>5-HD (n=8)</td>
<td>4±1</td>
<td>152±5</td>
<td>6417±258</td>
<td>3802±107</td>
<td>30.0±1.1</td>
</tr>
<tr>
<td>Chelerythrine (n=8)</td>
<td>5±2</td>
<td>149±5</td>
<td>6424±189</td>
<td>3673±230</td>
<td>31.5±1.3</td>
</tr>
<tr>
<td>L-NAME (n=8)</td>
<td>4±1</td>
<td>145±6</td>
<td>6230±221</td>
<td>3205±137</td>
<td>26.2±1.0</td>
</tr>
</tbody>
</table>

Data were measured immediately prior to 20 min global normothermic ischemia. All values are means±S.E.M.

3. Results

3.1. Normoxic function

Baseline functional data are provided in Table 1. Pressure development, and inotropic and lusitropic states were high, and coronary flow sub-maximal [27]. No differences in pre-ischemic contractile function were detected between untreated hearts and hearts treated with inhibitors or pharmacologically preconditioned with adenosine or diazoxide (Table 1, Fig. 2).

3.2. Responses to ischemia–reperfusion and adenosine-mediated preconditioning

Ischemia–reperfusion induced significant necrosis, as assessed by post-ischemic efflux of LDH. Untreated hearts released ~18 IU/g LDH into the venous effluent over the 45-min reperfusion period (Fig. 2) which is ~10-fold higher than efflux of LDH in normoxic hearts (calculated to be 1.9±0.4 IU/g over a 45-min perfusion period). Preconditioning with 10 and 50 μM adenosine resulted in substantial and almost identical reductions in post-ischemic LDH efflux (Fig. 2).

In addition to necrosis, ischemia resulted in a sustained depression of contractile function during 45 min reperfusion. Diastolic pressure remained elevated at ~22 mmHg (Fig. 3A), and ventricular pressure development recovered to ~50% of pre-ischemic levels (Fig. 3B). Coronary flow recovered to slightly less than pre-ischemic levels (Fig. 3C). Preconditioning with 50 μM adenosine significantly improved recovery of diastolic and developed pressures whereas preconditioning with 10 μM adenosine exerted a modest effect on diastolic pressure and no effect on overall pressure development. Coronary reflow was not altered by 10 μM adenosine but was significantly enhanced by preconditioning with 50 μM adenosine (Fig. 3C).

3.3. Signaling involved in triggering and mediating anti-necrotic and functional effects of adenosine-mediated preconditioning

The reduction in LDH efflux in preconditioned hearts was abolished by 5-HD during either the trigger (adenosine treatment period) or mediation (subsequent ischemia–reperfusion) periods (Fig. 2A). In contrast, neither chelerythrine or L-NAME modified necrosis when supplied during the trigger or mediation phases (Fig. 2B and C).

Effects of signaling inhibitors on functional recoveries did not mirror effects on necrosis. Again, 5-HD during the trigger phase abrogated functional protection with preconditioning (Fig. 3). However, 5-HD during the mediation phase only partially limited functional protection. While chelerythrine during the trigger phase failed to alter functional recoveries, chelerythrine during the mediation phase also partially limited functional protection (Fig. 4). Inhibition of NO synthase with L-NAME during either phase did not modify functional recoveries (Fig. 5). While neither 5-HD or chelerythrine alone abolished functional protection, co-treatment with 5-HD plus chelerythrine during the mediation phase effectively abrogated protection with 50 μM adenosine (Fig. 6). Co-treatment with 5-HD plus L-NAME was no more effective than 5-HD alone (Fig. 6).

3.4. Effects of mito K<sub>ATP</sub> channel activation and adenosine catabolites

Pre-treatment with the putative mito K<sub>ATP</sub> opener diazoxide induced a preconditioning response (Fig. 7). Effects of diazoxide were largely restricted to reduced necrosis, with modest effects on contractile function. Protection was eliminated by 5-HD during both trigger and mediation phases (Fig. 7). Supplying hearts with elevated inosine, hypoxanthine and xanthine during reperfusion...
Fig. 2. Anti-necrotic effects of adenosine-mediated preconditioning and effects of mito K\textsubscript{ATP} channels inhibition (A), PKC inhibition (B), or NO synthase inhibition (C) during trigger or mediation phases. Post-ischemic LDH efflux was determined in non-preconditioned hearts and hearts preconditioned with 10 or 50 μM adenosine. Hearts were untreated, or treated with 100 μM 5-HD, 3 μM chelerythrine (Chel), or 100 μM L-NAME during the adenosine stimulus or subsequent ischemia–reperfusion. Values are means ±S.E.M. * \( P<0.05 \) vs. corresponding values from the non-preconditioned group; † \( P<0.05 \) vs. untreated hearts within the same group.
Fig. 3. Functional protection with adenosine-mediated preconditioning and effects of mito K$_{\text{ATP}}$ channel inhibition during trigger or mediation phases. Recoveries are shown for diastolic pressure (A), left ventricular pressure development (B), and coronary flow (C) in non-preconditioned hearts and hearts preconditioned with 10 or 50 μM adenosine. Responses were acquired in untreated hearts and hearts treated with 100 μM 5-HD during the adenosine stimulus or subsequent ischemia–reperfusion. Values are means ± S.E.M. * $P<0.05$ vs. corresponding values from the non-preconditioned group; † $P<0.05$ vs. untreated hearts within the same group.
Fig. 4. Functional protection with adenosine-mediated preconditioning and effects of PKC inhibition during trigger or mediation phases. Recoveries are shown for diastolic pressure (A), left ventricular pressure development (B), and coronary flow (C) in non-preconditioned hearts and hearts preconditioned with 10 or 50 μM adenosine. Responses were acquired in untreated hearts and hearts treated with 3 μM chelerythrine (Chel) during the adenosine stimulus or subsequent ischemia–reperfusion. Values are means±S.E.M. *P<0.05 vs. corresponding values from the non-preconditioned group; †P<0.05 vs. untreated hearts within the same group.
Fig. 5. Functional protection with adenosine-mediated preconditioning and effects of NO synthase inhibition during trigger or mediation phases. Recoveries are shown for diastolic pressure (A), left ventricular pressure development (B), and coronary flow (C) in non-preconditioned hearts and hearts preconditioned with 10 or 50 μM adenosine. Responses were acquired in untreated hearts and hearts treated with 100 μM L-NAME during the adenosine stimulus or subsequent ischemia–reperfusion. Values are means±S.E.M. * P<0.05 vs. corresponding values from the non-preconditioned group; † P<0.05 vs. untreated hearts within the same group.
failed to alter ischemic tolerance (in terms of necrosis or functional recovery) in non-preconditioned or preconditioned hearts (Fig. 8).

3.5. Relationships between infarct size, LDH efflux, and functional recovery

Data in Fig. 9 depict relationships between infarct size, post-ischemic LDH efflux and functional recovery. A linear correlation was obtained between infarct size and LDH efflux (Fig. 9A). The same patterns of changes were observed with LDH efflux and infarct size; preconditioning with 10 and 50 μM adenosine comparably reduced both measures, 5-HD during ischemia–reperfusion eliminated effects of adenosine on infarction while chelerythrine was ineffective. A longer period of ischemia (25 min) exaggerated LDH efflux and infarction proportionately. In terms of functional recovery, there was a general linear relation between infarct size and post-ischemic diastolic dysfunction (Fig. 9B). However, recovery of developed pressure was not consistently linked to infarct size (Fig. 9C). Infarction was similar in hearts preconditioned with 10 and 50 μM adenosine whereas contractile recoveries differed substantially. Treatment of 50 μM adenosine preconditioned hearts with 5-HD abolished effects on infarction (to control levels) but did not eliminate functional protection.

4. Discussion

Our primary goal was to test whether adenosine-mediated preconditioning involves multiple signaling pathways. Data reveal ischemic tolerance in murine hearts is enhanced by pre-treatment with adenosine, and this is triggered by a mito K$_{ATP}$ channel dependent process independently of PKC or NO. Subsequent mediation of anti-necrotic effects during ischemia–reperfusion also requires mito K$_{ATP}$ channel activity. However, protection against contractile dysfunction with high level adenosine appears mediated by parallel mito K$_{ATP}$ channel and PKC dependent paths.

4.1. Adenosine-mediated preconditioning

Adenosine pre-treatment in mouse hearts enhanced...
ischemic tolerance (Figs. 2 and 3), and the type of protection was dependent upon adenosine concentration employed: 10 and 50 μM adenosine equally limit necrosis whereas only 50 μM adenosine reduced contractile dysfunction (Figs. 2 and 3). This concentration dependence may stem from differing activation of receptor sub-types. Adenosine has a 10-fold higher affinity for A₁ vs. A₃ receptors [30], and these receptors couple to different signaling pathways [31] to mediate protection [32]. Given a 10-fold or higher gradient in adenosine concentration from coronary circulation to interstitium during infusion [33], receptors are predicted to be exposed to ≤1 and ≤5 μM interstitial adenosine in the 10 and 50 μM groups, respectively. Maximal activation of A₁ and sub-maximal activation of A₃ receptors is therefore predicted in the 10-μM group [30], whereas both sub-types will be maximally active in the 50-μM group. It is possible high affinity A₁ receptors preferentially limit necrosis while less sensitive A₃ receptors more effectively limit reversible injury. Interestingly, selective effects of preconditioning stimuli on necrosis vs. contractility have been documented previously [34,35].

4.2. Signaling involved in triggering preconditioning

Adenosine-mediated preconditioning appears triggered by a mito Kₐ,ATP channel dependent, PKC and NO independent process. An essential role for mito Kₐ,ATP chan-
4.3. Signaling involved in mediating protection in preconditioned hearts

In terms of protection during ischemia–reperfusion, data again support a role for mito $K_{\text{ATP}}$ channels (Figs. 2 and 3). However, 5-HD during mediation did not eliminate protection against contractile dysfunction (Fig. 3). Furthermore, in contrast to lack of effect during the trigger phase, chelerythrine during ischemia–reperfusion reduced functional protection (Figs. 2 and 3). Since co-infusion of 5-HD and chelerythrine reduced protection (Fig. 6), parallel mito $K_{\text{ATP}}$ channel and PKC dependent paths appear to contribute to this response. Collectively, data reveal different signaling in the trigger and mediation phases of preconditioning, and in protection against necrosis vs. contractile dysfunction.

The current findings agree with prior studies questioning the role of PKC in preconditioning [11,12], and supporting multiple signaling paths [22,23]. Our data also agree with findings of Toyoda et al. [24], who showed adenosine-enhanced ischemic preconditioning reduces necrosis via a mito $K_{\text{ATP}}$ channel dependent process whereas anti-stunning effects involve other paths. Other studies support multiple signaling mechanisms for protective stimuli. For example, opioid-mediated preconditioning against necrosis is PKC-dependent whereas protection against arrhythmogenesis is not [26]. Clearly, the notion of a common path of preconditioning converging on PKC [5,6] is not applicable to different stimuli or differing endpoints.

4.4. NO and adenosine-mediated preconditioning

Endogenous NO is implicated in acute ischemic preconditioning [16,17] and adenosine-mediated protection [20]. Nonetheless, there is evidence NO plays no role in these situations [19,21]. Our data support the latter observations, demonstrating that a level of 1-NAME sufficient to eliminate dilatory responses to $A_2$ agonism and ADP fails to alter protection with adenosine (Figs. 2 and 5). The inhibitor also failed to augment effects of 5-HD (Fig. 6). We conclude NO plays no obligatory role in acute adenosine-mediated preconditioning in mouse, in contrast to evidence for a role in acetylcholine and diazoxide-mediated preconditioning in chick myocytes [37] and intact rabbits [38], respectively.

Findings regarding NO and PKC raise the possibility the murine model does not have the capacity to be acutely preconditioned via NO or PKC dependent paths. However, a number of murine studies reveal this is not the case. Specific stimuli such as resveratrol elicit acute NO-dependent protection [39], and transgenic modulation of PKC signaling enhances ischemic tolerance [40] in mouse. Thus, acute NO and PKC mediated protection occur in this species. Our data regarding lack of effect of NO and PKC inhibition are consistent with findings in eNOS knockout mice demonstrating no obligatory role for NO in ischemic preconditioning [17], and partially consistent with the observation PKC knockout abrogates anti-necrotic but not functional effects of ischemic preconditioning [41].

4.5. Dissociation of contractile recovery from necrosis

As indicated, functional recovery is dissociated from necrosis in selected groups. While 10 and 50 $\mu$M adenosine equally limit LDH efflux and infarct size (Figs. 2 and 3B), only 50 $\mu$M adenosine improved contractility (Fig. 3B). Furthermore, 5-HD during the mediation phase eliminated protection against necrosis but not contractile dysfunction, and chelerythrine reduced functional recovery without altering necrosis. A dichotomy between functional recovery and necrosis has been noted recently for preconditioned hearts [35,41], and in earlier studies [42], and is verified here by measurement of infarct size (Fig. 9). These data indicate functional effects of 50 $\mu$M adenosine are distinct from necrosis, and are mediated by combined mito $K_{\text{ATP}}$ channel and PKC dependent processes. Data in Fig. 9 show a weak correlation between contractile recovery and infarction in non-preconditioned vs. preconditioned hearts. Interestingly, diastolic pressure is more consistently correlated with necrosis than pressure development, suggesting a greater dependence of diastolic stiffness on infarct size.

Selective inhibition of oxidant damage may modify contractility independently of necrosis [42]. Since adenosine-mediated preconditioning limits oxidant stress via a mito $K_{\text{ATP}}$ and PKC dependent path [43], this could selectively reduce stunning. Additionally, reduced oxidant stress could occur via reduced xanthine-oxidase derived radical formation, since preconditioning limits substrate delivery to this enzyme. The putative xanthine oxidase inhibitor allopurinol enhances post-ischemic function in control but not preconditioned hearts [44]. We examined this possibility by supplementation with purine metabolites, reasoning that if contractile recovery was sensitive to xanthine-oxidase [44], exogenous substrate should modify recovery. As shown in Fig. 8, purine substrates did not alter functional recovery in control or preconditioned
hearts, inconsistent with selective effects of xanthine-oxidase derived radicals on contractility [42,44]. As discussed previously [45], the xanthine-oxidase reaction should be substrate saturated during ischemia and early reperfusion, and even a 2-fold reduction in substrate is unlikely to limit oxidant formation. Furthermore, uric acid formed exerts beneficial anti-oxidant effects [46]. Recent observations of Gelpi et al. [44] may reflect other effects of allopurinol, which protects in xanthine-oxidase deficient species [47,48], exerts positive inotropic effects [49], and is a non-specific radical scavenger [47].

4.6. Study limitations

A limitation in our study relates to use of LDH efflux as the primary indicator of necrosis. It might be argued this is not a reliable indicator of necrosis. To test this, we examined the relation between infarcton and LDH efflux in selected groups. These experiments reveal an excellent linear correlation between LDH loss and infarct size in control and preconditioned hearts (Fig. 9), consistent with previous work [29]. Importantly, key changes in infarct size mirror those in LDH efflux: 10 and 50 μM adenosine produced comparable reductions in infarct size (verifying selective modulation of contractility independently of necrosis in the latter group), and anti-infarct effects of adenosine were eliminated by 5-HD but not chelerythrine during the mediation phase (again verifying dissociation of contractile recovery from necrosis). The data confirm post-ischemic LDH efflux is a reliable indicator of necrosis, and specifically support our major conclusions.

4.7. Conclusions

Our observations reveal adenosine-mediated preconditioning impairs necrosis and additionally limits contractile dysfunction with higher level stimulation in mouse heart. Both effects are triggered by a mito K trapped channel-dependent process independently of PKC or NO. The anti-necrotic effect is mediated by a mito K trapped channel dependent process during ischemia–reperfusion. Partial resistance of functional protection to mito K trapped channel blockade, and partial sensitivity to PKC inhibition, suggest a specific contractile effect of preconditioning distinct from anti-necrotic effects. This protection appears mediated by parallel mito K trapped channel and PKC dependent pathways, and is unrelated to xanthine-oxidase activity. Collectively, our data support signaling paths for adenosine-mediated preconditioning which differ from other stimuli, and involves distinct effects on necrosis vs. contractility.

Acknowledgements

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