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A_1 adenosine receptor overexpression decreases stunning from anoxia-reoxygenation: role of the mitochondrial K_{ATP} channel

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Abstract Myocardial A_1 adenosine receptor (A_1AR) overexpression protects hearts from ischemia-reperfusion injury; however, the effects during anoxia are unknown. We evaluated responses to anoxia-reoxygenation in wild-type (WT) and transgenic (Trans) hearts with ~200-fold overexpression of A_1ARs. Langendorff perfused hearts underwent 20 min anoxia followed by 30 min reoxygenation. In WT hearts peak diastolic contracture during anoxia was 45 ± 3 mmHg, diastolic pressure remained elevated at 18 ± 3 mmHg after reoxygenation, and developed pressure recovered to 52 ± 4 % of pre-anoxia. A_1AR overexpression reduced hypoxic contracture to 29 ± 4 mmHg, and improved recovery of diastolic pressure to 8 ± 1 mmHg and developed pressure to 76 ± 3 % of pre-anoxia. Mitochondrial K_{ATP} blockade with 100 μM 5-hydroxydecanoate (5-HD) increased hypoxic contracture to 73 ± 6 mmHg in WT hearts, reduced post-hypoxic recoveries of both diastolic (40 ± 5 mmHg) and developed pressures (33 ± 3 %). In contrast, 5-HD had no effect on hypoxic contracture (24 ± 8 mmHg), or post-hypoxic diastolic (10 ± 2 mmHg) and developed pressures (74 ± 3 %) in Trans hearts. In summary, (i) A_1AR overexpression improves myocardial tolerance to anoxia-reoxygenation, (ii) intrinsic mitochondrial K_{ATP} channel activation decreases hypoxic contracture and improves functional recovery in wild-type hearts, and (iii) mitochondrial K_{ATP} channels do not appear to play a major role in the functional protection from anoxia afforded by A_1AR overexpression.

Key words Heart – A_1 adenosine receptor – anoxia – mitochondrial K_{ATP} channel – mouse

Introduction

Myocardial injury occurs from both anoxia and ischemia; however, their mechanisms of tissue injury may differ. While anoxia is characterized by decreased O_2 delivery, ischemia has both decreased O_2 delivery as well as decreased substrate as a result of no flow. Anoxia and reoxygenation expose the myocardium to extremes in redox stress (22), which can result in the initiation of a series of cellular pathways leading to tissue injury and death (21, 22). During anoxia there is depression or cessation of mitochondrial oxidative phosphorylation (22) with resultant depression of energy metabolism (2, 16, 27) and an increase in intracellular Ca^{2+} and impaired Ca^{2+} handling (16, 23). Myocardial anoxia reduces left ventricular contractile performance (6, 16, 27), decreases action potential duration (3, 5) and reduces levels of intracellular redox buffers. Reoxygenation is associated with additional damage to the myocardium by oxidation of cellular components and activation of inflammatory cascades (33).

A variety of endogenous cardioprotective mechanisms exist within the heart, which provide protection

from ischemic and anoxic or hypoxic injury. These include antioxidant enzymes, heat shock proteins, A_1, A_2 and A_3 adenosine receptor systems and K_{ATP} channels. Activation of A_1 adenosine receptors by exogenous agonists or endogenous adenosine protects the heart from ischemia-reperfusion injury (8, 15, 16) and anoxia/hypoxia (27) while also playing an important role in the protection afforded by ischemic preconditioning (15, 37). Exogenous activation of K_{ATP} channels improves functional and metabolic tolerance to ischemia-reperfusion and anoxia (5, 7, 13) and K_{ATP} channel blockade reduces the beneficial effects of preconditioning (1, 35) and adenosine receptor activation (24, 35). We have recently shown that intrinsic activation of K_{ATP} channels plays a role in the enhanced protection from ischemia-reperfusion injury afforded by A_1 adenosine receptor (A_1AR) overexpression (18). Based on these observations, we hypothesized that hearts overexpressing A_1ARs would also have increased functional tolerance and improved tissue viability from anoxia, and that mitochondrial K_{ATP} channels would play an integral role in this protection. The purpose of this study therefore was twofold: (i) to investigate whether transgenic A_1AR overexpression provides increased functional tolerance to anoxia-reoxygenation injury, and (ii) to investigate whether intrinsic activation of mitochondrial K_{ATP} channels enhances myocardial tolerance to anoxia-reoxygenation in wild-type hearts and transgenic hearts overexpressing A_1ARs. To achieve these goals, we examined responses to anoxia-reoxygenation in isovolumically contracting Langendorff perfused wild-type and transgenic hearts in the absence and presence of the selective mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate sodium (18, 24, 25).

Materials and methods

Transgenic murine model

All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) and the work was approved by the Institutional Animal Care and Use committee. Transgenic mice with the rat A_1 adenosine receptor cDNA under the control of the cardiac-specific α-myosin heavy chain promoter were used as described previously (28).

Langendorff perfused murine heart model

Hearts were isolated from 16 – 20 week old male and female wild-type (body weight 25 ± 3 g, n = 22) and transgenic mice (body weight 24 ± 2 g, n = 22) overexpressing A_1 adenosine receptors. Mice were anesthetized with 50 mg/kg sodium pentobarbital administered intraperitoneally, a thoracotomy performed and hearts rapidly excised into heparinized ice-cold perfusion buffer. The aorta was cannulated (20 gauge stainless steel blunt needle) and the hearts retrogradely perfused at a constant pressure of 80 mmHg with modified Krebs buffer containing (in mM): NaCl, 118; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.2; $CaCl_2$, 2.5; Mg_2SO_4, 1.2; glucose, 11; and EDTA, 0.6. Buffer was equilibrated with 95 % O_2, 5 % CO_2 at 37 °C. The left ventricle was vented with a small polyethylene apical drain and a fluid-filled balloon constructed of plastic film inserted into the left ventricle via the mitral valve. The balloon was connected to a pressure transducer by a polyethylene tube permitting continuous measurement of left ventricular pressure. Hearts were immersed in perfusate in a water-jacketed bath maintained at 37 °C and the ventricular balloon inflated to yield a left ventricular diastolic pressure of 2 – 5 mmHg. Coronary flow was continuously monitored via a Doppler flow-probe (Transonic Systems Inc., Ithaca, NY, U.S.A.) located in the aortic perfusion line. Aortic and left ventricular developed pressures were recorded on a MacLab data acquisition system (ADInstruments, Castle Hill, Australia) connected to an Apple 7300/180 computer. The ventricular pressure signal was digitally processed on-line (using MacLab Chart 3.5.6, ADInstruments, Castle Hill, Australia) to yield diastolic and systolic pressures, heart rate and ±dP/dt.

Experimental protocol

Hearts were allowed to stabilize for 20 min at intrinsic heart rate. All hearts (wild-type and transgenic) were then paced at a constant rate of ~425 bpm (ventricular pacing with 5 ms square waves, ~30 % above threshold, typically 4 – 5 V) for a further 10 min of equilibration. Baseline diastolic and systolic pressures, heart rate and coronary flow were recorded and 20 min normothermic anoxia was initiated by switching to perfusate which had been equilibrated with 95 % N_2, 5 % CO_2 at 37 °C. Ventricular pacing was stopped on initiation of anoxia and the fluid-filled bath surrounding the heart was gassed with the same anoxic gas mixture described above to ensure the bath perfusate was anoxic. Following anoxia, hearts were reoxygenated for 30 min with ventricular pacing resumed after 2 min. To verify the role of A_1 adenosine receptor activation in this model of anoxia-reoxygenation, a separate group of experiments was performed in which hearts were treated with the specific A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). DPCPX (100 nM) was infused 10 min prior to global anoxia at ~1 % of coronary flow and continued for the remainder of the experiment (n = 6 for both wild-type and transgenic hearts).

To assess the role of mitochondrial K_{ATP} channels in anoxia-reoxygenation injury, studies were performed in untreated hearts (n = 8 for both wild-type and transgenic) and hearts treated with 100 μM 5-hydroxydecanoate sodium (5-HD) to block activation of the mitochondrial K_{ATP} channel (n = 8 for both wild-type and transgenic). 5-HD infusion was initiated 10 min prior to global anoxia at ~1% of coronary flow and continued for the duration of the experiment. An initial pilot study of 5-HD treated wild-type and transgenic hearts found that coronary flow during reoxygenation was lower in the treated hearts (results not shown). Since this may cause inhibition of reoxygenation-induced hyperemia and therefore effect functional recovery of 5-HD treated hearts, all 5-HD treated hearts were perfused at coronary flow rates comparable to those observed in untreated hearts (Fig. 1). This was accomplished by using a peristaltic pump to perfuse the hearts at constant flow. Coronary flow was adjusted every 2 min to reflect coronary flow in untreated hearts (28). Perfusion pressures increased slightly during this procedure, but were not different from the untreated groups (results not shown). There were no significant differences in coronary flow rates between untreated and treated hearts when the experiments were performed in this manner (Fig. 1).

Statistical analysis

All results are expressed as means ± SEM. Functional parameters during anoxia-reoxygenation were analyzed by multivariate ANOVA for repeated measures with Bonferroni's correction for multiple comparisons and Student Neuman-Keuls post hoc test. Statistical significance was accepted for $p < 0.05$.

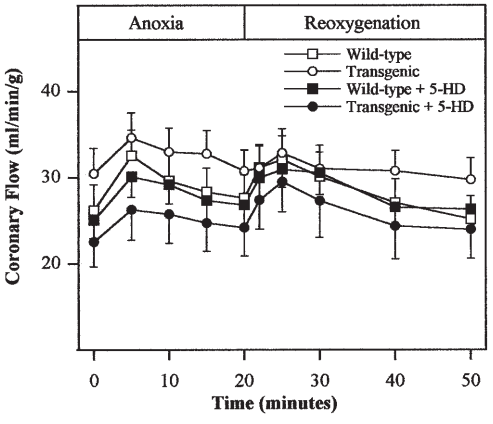


Fig. 1 Time course of coronary flow changes in wild-type (squares) and transgenic (circles) hearts during 20 min anoxia and 30 min reoxygenation. Hearts were either untreated (open symbols; n = 8 for wild-type and transgenic) or treated with 100 μM 5-HD (closed symbols; n = 8 for wild-type and transgenic). No significant difference was observed at any time point.

Table 1 Baseline functional parameters in paced Langendorff perfused hearts from wild-type and transgenic mice overexpressing myocardials A_1ARs

	Wild-type			Transgenic		
	CTL (n = 8)	5-HD (n = 8)	DPCPX (n = 6)	CTL (n = 8)	5-HD (n = 8)	DPCPX (n = 6)
HR (beats/min)	425 ± 1	427 ± 1	426 ± 3	427 ± 1	424 ± 1	426 ± 4
CF (ml/min/g)	26 ± 2	24 ± 1	26 ± 3	23 ± 3	22 ± 3	23 ± 3
EDP (mmHg)	4 ± 1	4 ± 1	3 ± 1	5 ± 1	4 ± 1	3 ± 1
LVDP (mmHg)	131 ± 5	129 ± 7	121 ± 3	121 ± 4	119 ± 9	123 ± 6

Functional parameters were measured after 30 minutes of normoxic perfusion using a standard Langendorff preparation at a perfusion pressure of 80 mmHg. *HR* heart rate; *CF* coronary flow; *EDP* end diastolic pressure; *LVDP* left ventricular developed pressure; *CTL* control untreated hearts; *5-HD* hearts treated with 100 μM 5-hydroxydecanoate; *DPCPX* hearts treated with 500 nM 8-cyclopently-1,3-dipropylxanthine. All values are means ± SEM

Results

Baseline function

Baseline functional data for wild-type (heart wt 128 ± 7 mg, n = 22) and transgenic hearts (heart wt 130 ± 9 mg, n = 22) are shown in Table 1. All hearts were paced at ~425 bpm to correct for lower intrinsic heart rates in the transgenic mice (10, 11, 28). No differences in pre-anoxic functional parameters were observed between wild-type and transgenic hearts. Coronary flow rates were also similar at baseline and remained so during anoxic dilation and reactive hyperemia following reoxygenation (Fig. 1).

Functional effects of anoxia-reoxygenation in wild-type and transgenic hearts

Anoxia abolished contractile function in the majority of hearts within 5 min and caused a rapid rise in diastolic pressure (Fig. 2). The maximum degree of left ventricular end diastolic pressure achieved during anoxia was ~45 mmHg in wild-type hearts versus only ~29 mmHg in transgenic hearts (Fig. 2). Left ventricular developed pressure (expressed as percent change from pre-anoxia) during anoxia-reoxygenation is shown in Fig. 3A. All hearts demonstrated an immediate decline in left ventricular developed pressure and arrest within ~5 min. Upon reoxygenation, all hearts resumed spontaneous contraction within 30 – 60 s and exhibited an immediate recovery of left ventricular developed pressure. There

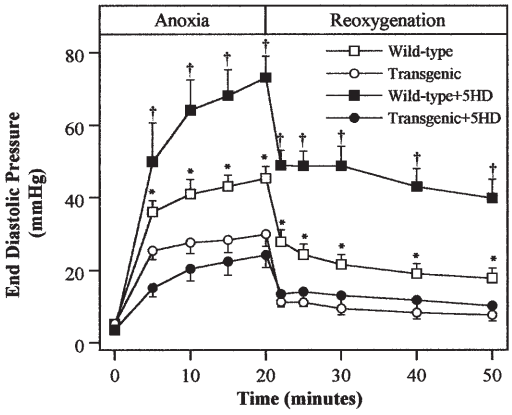
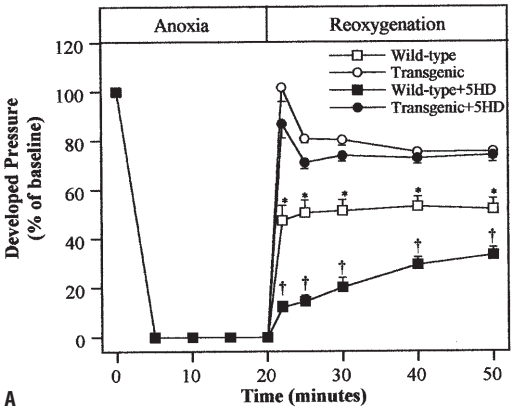


Fig. 2 Time course of left ventricular diastolic pressure in wild-type (squares) and transgenic (circles) hearts during 20 min anoxia and 30 min reoxygenation. Hearts were either untreated (open symbols; n = 8 for wild-type and transgenic) or treated with 100 μM 5-HD (square symbols; n = 8 for wild-type and transgenic). Values shown are means ± SEM. *P < 0.05, transgenic vs wild-type; †P < 0.05, 5-HD treated vs untreated hearts.



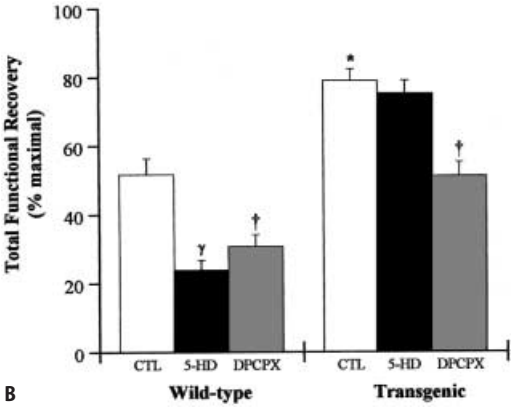


Fig. 3 **A** Effect of 20 min normothermic anoxia and 30 min reoxygenation on myocardial function (expressed as percent change from baseline developed pressure) in wild-type (squares) and transgenic (circles) mouse hearts. Hearts were either untreated (open symbols; n = 8 for wild-type and transgenic) or treated with 100 μM 5-HD (closed symbols; n = 8 for wild-type and transgenic). Values shown are means ± SEM. *P < 0.05, transgenic vs wild-type; †P < 0.05, 5-HD treated vs untreated hearts. **B** Total functional recovery of developed pressure (expressed as a percent of maximal) in wild-type and transgenic mouse hearts subjected to 20 min anoxia and 30 min reoxygenation. Hearts were either untreated (CTL: n = 8 for wild-type and transgenic), treated with 100 μM 5-HD (n = 8 for wild-type and transgenic) or treated with 100 nM DPCPX (n = 8 for wild-type and transgenic). Values shown are means ± SEM. *P < 0.05, transgenic vs wild-type; γ P < 0.05, 5-HD treated vs untreated hearts; †P < 0.05, DPCPX treated vs untreated.

was an immediate decrease in diastolic pressure and a sustained depression in left ventricular developed pressure from pre-anoxic levels for the duration of reoxygenation (Figs. 2 and 3A). Diastolic pressure remained elevated at 18 ± 3 mmHg in wild-type hearts, whereas it recovered to 8 ± 1 mmHg in the transgenic hearts, which was not significantly different from pre-anoxic values. Transgenic hearts demonstrated a significantly improved recovery of left ventricular developed pressure compared with wild-type hearts at all times during reoxygenation (Fig. 3A). This difference was particularly pronounced at 2 min of reoxygenation, when transgenic hearts recovered to almost 100% of pre-anoxia compared to only ~59% in wild-type hearts ($p < 0.05$). This difference is reflected in the calculation of total functional recovery (expressed as the percent of maximal) of left ventricular developed pressure shown in Fig. 3B. Effects of A_1AR blockade on total functional recovery of developed pressure are also shown in Fig. 3B. Treatment with DPCPX prior to anoxia-reoxygenation decreased total functional recovery of developed pressure (expressed a percent of maximal) in wild-type hearts from 52 ± 4 % to 31 ± 3% of maximal. DPCPX treatment in transgenic hearts decreased total functional recovery from 80 ± 2 to 51 ± 4% of maximal (Fig. 3B).

Functional effects of K_{ATP} blockade with 5-HD

Pre-anoxic function was not significantly altered by 5-HD pre-treatment in wild-type or transgenic hearts (Table 1). Blockade of K_{ATP} channels with 5-HD had a marked effect on contracture during anoxia and reduced recovery of contractile function during reoxygenation in wild-type hearts (Figs. 2 and 3). At the end of reoxygenation, end diastolic pressure was increased from a value of 18 ± 3 mmHg in untreated wild-type hearts to 40 ± 5 mmHg in 5-HD treated hearts. 5-HD markedly reduced recovery of left ventricular developed pressure with a final recovery of 52 ± 4% in wild-type untreated hearts compared to only 33 ± 3% with 5-HD treatment (Fig. 3A). This was also reflected in the total functional recovery, which was reduced from 52 ± 5% to 24 ± 3% of maximal, with 5-HD treatment (Fig. 3B). 5-HD had no effect on diastolic or developed pressures in transgenic hearts during anoxia or reoxygenation (Figs. 2 and 3). Left ventricular end diastolic pressure peaked at 24 ± 8 mmHg in 5-HD treated hearts compared to 29 ± 4 mmHg in untreated transgenic hearts. Recovery of post-anoxic

diastolic pressure was 10 ± 2 mmHg compared to 8 ± 1 mm Hg in untreated hearts and developed pressures were 74 ± 3% in treated and 76 ± 3% in untreated transgenic hearts, respectively (Figs. 2 and 3). Likewise, total functional recovery of developed pressure was also unaltered by 5-HD treatment with a value of 75 ± 4% compared to 79 ± 3% in untreated hearts.

Discussion

The purposes of this study were to examine (i) whether transgenic overexpression of cardiac A_1 adenosine receptors provides cardioprotection during anoxia-reoxygenation in the murine myocardium, and (ii) whether intrinsic activation of mitochondrial K_{ATP} channels enhances tolerance to anoxia-reoxygenation injury in either wild-type hearts or hearts overexpressing A_1ARs. Examining anoxia-reoxygenation in wild-type hearts and hearts with ~200-fold overexpression of A_1ARs, we demonstrate that increasing the density of cardiac A_1ARs provides increased functional tolerance to either (i) anoxia induced stunning or (ii) partial irreversible tissue injury (necrosis) (Figs. 2 and 3). Selective A_1 adenosine receptor antagonism confirms that the protection afforded by A_1AR overexpression is mediated via endogenous A_1AR activation (Fig. 3B). We also provide evidence for the beneficial effects of intrinsic activation of mitochondrial K_{ATP} channels in wild-type hearts, consistent with recent observations in ischemic hearts (18). However, activation of K_{ATP} channels does not appear to play a significant role in the improved functional tolerance to anoxia-reoxygenation observed in the transgenic hearts overexpressing A_1ARs.

Myocardial protection in transgenic hearts with increased A_1 adenosine receptors

Considerable evidence exists for the cardioprotective role of endogenous adenosine during ischemia-reperfusion (8, 15, 16), anoxia/hypoxia (27) as well as involvement of adenosine receptors in ischemic preconditioning (15, 37). We have previously suggested that varied effects of either exogenous adenosine agonists, or pharmacological enhancement of endogenous adenosine, in ischemic myocardium may be limited by normally maximal or near maximal receptor occupancy and activation during such injury (28). Therefore, we developed a transgenic murine model of cardiac A_1AR overexpression, and have subsequently documented enhanced functional and metabolic tolerance to ischemia-reperfusion injury (17, 28) and improved tissue viability (31) with A_1AR overexpression. Since anoxia, like ischemia, elicits large increases in endogenous adenosine within the interstitial compartment (27), we examined whether increasing the density of A_1ARs would also provide protection against anoxia-reoxygenation in the murine myocardium. Our data show that A_1AR overexpression provides increased tolerance to anoxia-reoxygenation injury in isovolumically contracting hearts (Figs. 2 and 3). This protection is evident as reduced hypoxic contracture and improved post-hypoxic recovery of diastolic and developed pressures. The pronounced increase in function immediately upon reoxygenation in transgenic hearts (Fig. 4A) likely reflects a marked reduction in the severity of the hypoxic insult, as has been implicated for ischemia-reperfusion (26). Using DPCPX, a selective and potent A_1AR antagonist, we demonstrate that enhanced functional tolerance in transgenic hearts to anoxia is the result of activation of endogenous A_1AR activation, consistent with similar observations in ischemia-reperfusion (28) and in ischemic preconditioning (31). Interestingly, we were not able to decrease the recovery in Trans hearts with DPCPX to the same levels as wild-type hearts with DPCPX. This may be secondary to not blocking all receptors and may indicate chronic protective mechanisms in the Trans model. Furthermore, cardioprotection occurs with no alteration in baseline contractile function (Table 1), as previously observed (10, 31).

The cardioprotective role of K_{ATP} channels in wild-type hearts

Exogenous activation of mitochondrial K_{ATP} channels may be protective in ischemia-reperfusion injury (9, 12, 30, 36) and appears to play a role in both ischemic preconditioning (12, 19, 24, 32, 34) and cardioprotection resulting from exogenously applied adenosine receptor agonists (24, 30, 35). However, the effect of endogenously activated mitochondrial K_{ATP} channels (in the absence of such applied stimuli) remains controversial, and the protective functions of these channels during anoxia-reoxygenation also remain unclear. In the murine myocardium, selective mitochondrial K_{ATP} channel blockade with 5-HD (24, 25, 34) failed to alter baseline function or coronary flow (Table 1), in agreement with our own recent observations (18) and those of others (19). Blockade with 5-HD markedly exacerbated diastolic dysfunction during anoxia and depressed recovery of contractile performance upon reoxygenation (Figs. 2 and 3). Furthermore, inhibition of mitochondrial K_{ATP} channels significantly increased myocardial cell death assessed by efflux of LDH. These data collectively indicate that intrinsic activation of mitochondrial K_{ATP} channels plays an important role in improving functional tolerance of the murine heart to anoxia and reoxygenation. These findings are consistent with recent observations in ischemia-reperfusion (18) and are similar to data regarding responses to acute anoxia in guinea pig heart (5), chronic

anoxia in rabbit heart (3, 4) and hypoxic preconditioning in the dog (29).

The role of K_{ATP} channels in the functional protection afforded by transgenic A_1AR overexpression

While we provide evidence for increased functional tolerance and improved tissue viability to anoxia-reoxygenation with A_1AR overexpression, the precise mechanism(s) remain unidentified. Since there is evidence of a link between adenosine and K_{ATP} channel activation (24, 30), and since we have established a role for mitochondrial K_{ATP} channel activity in the cardioprotection afforded by A_1AR overexpression in ischemia-reperfusion (18), it seemed reasonable to propose that K_{ATP} channels may mediate the cardioprotection afforded by A_1AR overexpression during anoxia-reoxygenation. However, the present observations suggest that mitochondrial K_{ATP} channels are not integral to the functional protection from anoxia-reoxygenation in hearts with A_1AR overexpression. 5-HD exerted no effects on contractile function during either anoxia or reoxygenation in transgenic hearts, in contrast to the effects of mitochondrial K_{ATP} channel blockade in wild-type hearts (Figs. 2 and 3).

Our observations that mitochondrial K_{ATP} channels are important in the protection afforded by A_1AR overexpression during ischemia-reperfusion (18), and in protection from anoxia in wild-type but not transgenic hearts may seem paradoxical. However, these observations are in keeping with the different metabolic effects of anoxia versus ischemia, and with the effects of adenosine receptor activation on myocardial energy state during such injury. K_{ATP} channels are activated by localized reductions in ATP and we and others have shown that the ATP loss during anoxia is much less than that during similar periods of global ischemia (2, 16). In rat hearts for example, ATP levels approximate 3 mM after ~10 – 15 min of global ischemia (16), while levels in anoxia are more than 2-fold higher than this (2, 16) after the same duration. Therefore, the overall level of energy depletion is much lower during anoxia versus ischemia (16). Thus, the trigger for K_{ATP} channel activation may be less effective and the role of these channels quite different in anoxia-reoxygenation versus ischemia-reperfusion injury. Perhaps more importantly, we have recently shown that overexpression of A_1ARs produces a remarkable degree of preservation of ATP and bioenergetic state during ischemia-reperfusion injury (17). This is consistent with our observations that endogenous adenosine receptor activation protects myocardial energy state in ischemic, adrenergically stimulated or anoxic/hypoxic hearts from various species (14, 15, 27). Thus, the lower overall level of de-energization in anoxia versus ischemia, coupled with likely preservation of ATP in transgenic hearts, may lead to minimal mitochondrial K_{ATP} channel activation in transgenic hearts subjected to anoxia. This may explain the lack of effect of 5-HD in A_1AR overexpressing hearts in contrast to depression of recovery with 5-HD in wild-type hearts. Since adenosine is thought to modulate the mitochondrial K_{ATP} channel by enhancing its ability to either open and/or close, some alternative explanations might be as follows: (i) it is more than likely that there is less tissue adenosine in anoxic hearts and therefore less adenosine to modulate the channel, and (ii) this modulatory effect is different in anoxic insults compared to ischemic insults. Furthermore, in moderate insults like anoxia, the adenosine A_1 receptor protection system may be more sensitive to $G\alpha$ signaling via cAMP → leading to protection by improved sarcolemmal Ca^{2+} handling than to the $G\beta\gamma$ signaling via PLC → leading to protection via the mitochondrial K_{ATP} channel. That is, the mitochondrial K_{ATP} channel may not be the primary end effector in these Trans hearts subjected to moderate insults such as anoxia and therefore blockade of these channels may have little or no significant effect on the observed functional protection.

Conclusions

The present study examines the effects of cardiac A_1AR overexpression on myocardial tolerance to anoxia-reoxygenation and the role of intrinsically activated mitochondrial K_{ATP} channels in anoxia. Our data demonstrate that ~200-fold overexpression of A_1ARs reduces diastolic and systolic dysfunction and improves post-ischemic contractile function in hearts subjected to anoxia-reoxygenation. Moreover, they also show that while intrinsically activated mitochondrial K_{ATP} channels are protective in wild-type hearts under these conditions, they appear to play no major role in the pronounced cardioprotection afforded by A_1AR overexpression. Our findings therefore demonstrate that targeting the A_1AR may be a useful strategy for increasing tolerance to anoxic injury in the myocardium, however, the mechanism of the protection remains to be elucidated.

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