Interactions between A$_{2A}$ adenosine receptors, hydrogen peroxide, and K$_{ATP}$ channels in coronary reactive hyperemia

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THE HEART responds to acute ischemia by transiently increasing blood flow in a phenomenon called reactive hyperemia (8). This temporary reduction in coronary vascular resistance is mediated by chemical signals released into blood (38), including adenosine (35), which activates A$_1$, A$_2A$, A$_2B$, and A$_3$ receptors (29). Both A$_2A$ and A$_2B$ adenosine receptors (ARs) are expressed on endothelial and smooth muscle cells (2, 29, 33, 43). Pharmacological studies indicate that A$_2A$ and A$_2B$ receptors are the subtypes most likely involved in coronary reactive hyperemia (6, 10, 51); however, undesirable overlap in the pharmacological profiles of adenosine receptor antagonists obfuscates their relative contribution. This issue could be resolved with the gene-targeted approaches we have used previously to demonstrate pivotal roles for A$_{2A}$ receptors in the regulation of coronary vascular function (28, 39, 41). Thus, to specifically determine the roles of A$_{2A}$ and A$_{2B}$ ARs in coronary reactive hyperemia, in addition to distinguishing their role from A$_1$ and A$_3$ ARs, we used Langendorff-perfused hearts from wild-type (WT), A$_{2A}$ knockout (KO), A$_{2B}$ KO, and A$_{2A}$/A$_{2B}$ double knockout (DKO) mice, respectively.

Numerous mediators and end effectors of coronary reactive hyperemia have been proposed to function downstream of adenosine receptors, including H$_2$O$_2$ and ATP-dependent K$^+$ (K$_{ATP}$) channels. The role of K$_{ATP}$ channels in coronary reactive hyperemia is firmly established (3, 12), while the contribution of H$_2$O$_2$ and interactions with K$_{ATP}$ channels are less clear. Some support for H$_2$O$_2$ in reactive hyperemia has been provided by studies of the reactive dilation of isolated coronary arterioles (18) and mesenteric reactive hyperemia (49). Additionally, it is reported that K$_{ATP}$ channels mediate, to some degree, H$_2$O$_2$-induced dilation of arterioles from skeletal muscle and brain (24, 44). Thus we determined whether H$_2$O$_2$ couples adenosine receptor stimulation to K$_{ATP}$ channel activity in coronary reactive hyperemia.

MATERIALS AND METHODS

Animals. An Institutional Animal Care and Use Committee at West Virginia University School of Medicine approved all experimental protocols. We followed guidelines set forth by the American Physiological Society and National Institutes of Health regarding the care and use of laboratory animals. A$_{2A}$ and A$_{2B}$ KO mice, both backcrossed 12 generations to the WT C57BL/6 background (Jackson Laboratory; Bar Harbor, ME), were bred to generate A$_{2A}$/A$_{2B}$ double heterozygotes. Double heterozygotes were intercrossed, 1/16 of the offspring were A$_{2A}$/A$_{2B}$ DKO, and A$_{2A}$/A$_{2B}$ knockout breeding pairs were established. Mice were caged in a 12:12-h light-dark cycle with free access to standard chow and water.

Langendorff-perfusion. Mice (10–14 wk) were anesthetized with pentobarbital sodium (50 mg/kg ip) and hearts were excised into heparinized (5 U/ml) ice-cold Krebs-Henseleit buffer containing (in mM) 119 NaCl, 11 glucose, 22 NaHCO$_3$, 4.7 KCl, 1.2 KH$_2$PO$_4$, 1.2 MgSO$_4$, 2.5 CaCl$_2$, 2 pyruvate, and 0.5 EDTA. Hearts beat spontaneously when retrogradely perfused (80 mmHg) with 37°C buffer bubbled with 95% O$_2$-5% CO$_2$. The left atrium was removed and the left ventricle drained. A fluid-filled balloon was inserted into the left ventricle and connected to a transducer for pressure measurements. Left ventricular diastolic pressure was adjusted to 2–5 mmHg. Coronary flow was measured with a probe (Transonic Systems; Ithaca, NY) in the aortic perfusion line. Hearts were paced to 420 beats/min and function allowed to stabilize. Hearts with persistent

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The heart responds to acute ischemia by transiently increasing blood flow in a phenomenon called reactive hyperemia. This temporary reduction in coronary vascular resistance is mediated by chemical signals released into blood, including adenosine, which activates A$_1$, A$_2A$, A$_2B$, and A$_3$ receptors. Both A$_2A$ and A$_2B$ adenosine receptors (ARs) are expressed on endothelial and smooth muscle cells. Pharmacological studies indicate that A$_2A$ and A$_2B$ receptors are the subtypes most likely involved in coronary reactive hyperemia; however, undesirable overlap in the pharmacological profiles of adenosine receptor antagonists obfuscates their relative contribution. This issue could be resolved with the gene-targeted approaches we have used previously to demonstrate pivotal roles for A$_{2A}$ receptors in the regulation of coronary vascular function. Thus, to specifically determine the roles of A$_{2A}$ and A$_{2B}$ ARs in coronary reactive hyperemia, in addition to distinguishing their role from A$_1$ and A$_3$ ARs, we used Langendorff-perfused hearts from wild-type (WT), A$_{2A}$ knockout (KO), A$_{2B}$ KO, and A$_{2A}$/A$_{2B}$ double knockout (DKO) mice, respectively.

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arhythmias or developed pressure less than 80 mmHg were excluded from the study. Coronary flow and cardiac function were measured using a Power Lab data acquisition system (AD Instruments; Colorado Springs, CO). Hearts were subjected to 15 s of total flow occlusion to elicit hyperemia. Catalase, glibenclamide, and \( \text{N}^\text{O} \)-nitro-L-arginine methyl ester (L-NAME) (Sigma Chemical; St. Louis, MO) were delivered into the aortic perfusion line using a microinjection pump (Harvard Apparatus; Holliston, MA) as 1% of coronary flow to achieve a final concentration of 1,250 U/ml (46, 49).

Patch clamp. Single smooth muscle cells were enzymatically isolated from mouse aortas. For cell isolation, a HEPES-buffered saline was used and contained (in mM) 135 NaCl, 5 KCl, 1 MgCl\(_2\), 0.36 CaCl\(_2\), 10 glucose, 10 HEPES, and 5 Tris; pH 7.4. The aorta was placed in this solution plus (in mg/ml) 2 collagenase, 1 elastase, 2 bovine serum albumin, and 1 soybean trypsin inhibitor for 15 min at 37°C. The tissue was passed through the tip of a fire-polished Pasteur pipette to liberate single cells. Smooth muscle cells were resuspended in enzyme-free buffer, stored on ice, and used within 8 h. Cells were placed in a recording chamber atop an inverted microscope and were delivered into the aortic perfusion line using a microinjection pipette to isolate single cells. Smooth muscle cells were resuspended in enzyme-free buffer, stored on ice, and used within 8 h. Cells were placed in a recording chamber atop an inverted microscope and were delivered into the aortic perfusion line using a microinjection pipette to liberate single cells. Smooth muscle cells were resuspended in enzyme-free buffer, stored on ice, and used within 8 h.

Flow debt (equal to baseline flow rate \( \times \) occlusion time) and repayment area [equal to integral of hyperemic area above baseline flow during the first minute of reactive hyperemia] were calculated as previously reported (6, 10, 51). Since absolute coronary flow rates change proportionally with heart mass and metabolic rate, the repayment area and debt area data are represented as milliliters per gram of wet heart weight (ml·min\(^{-1}\)·g\(^{-1}\)). Each reactive hyperemia graph represents the average of all studies.

Statistical analysis. Statistical analyses were made with \( t \)-test and one-way analysis of variance (ANOVA) as indicated. Results were considered significant when \( P < 0.05 \) vs. WT. Values are means ± SE from \( n \) number of animals.

RESULTS

\( \text{A2A} \) receptors are important in coronary reactive hyperemia. We used hearts from WT, \( \text{A2A} \) and \( \text{A2B} \) KO mice to test the involvement of adenosine receptor subtypes in coronary reactive hyperemia. Hearts from \( \text{A2A/2B} \) DKO mice were used to test the combined role of \( \text{A2A} \) and \( \text{A2B} \) ARs known to induce coronary vasodilation. Baseline flow in WT hearts (\( n = 6 \)) was \( 19.8 \pm 0.9 \) ml·min\(^{-1}\)·g\(^{-1}\), and 4.9 ± 0.2 ml of flow debt was incurred during a 15-s occlusion. Peak hyperemic flow was 42.7 ± 2.0 ml·min\(^{-1}\)·g\(^{-1}\), and the repayment volume was 6.3 ± 0.4 ml/g. Thus flow debt was repaid 128 ± 8% in hearts from WT mice (Fig. 1). Repayment volume was reduced in \( \text{A2A KO} \) mice (\( n = 8 \); Fig. 1). Specifically, while baseline flow (19.4 ± 1.6 ml·min\(^{-1}\)·g\(^{-1}\)), debt volume (4.9 ± 0.4 ml/g), and peak flow (42.9 ± 17 ml·min\(^{-1}\)·g\(^{-1}\)) were similar to WT, the repayment volume was only 4.5 ± 0.2 ml/g (\( P < 0.05 \) vs. WT). That is, flow debt was repaid only 98 ± 9% in hearts from \( \text{A2A KO} \) mice (just 77 ± 7% of the response seen in WT).

In contrast, hyperemic responses in hearts from \( \text{A2B KO} \) mice (\( n = 5 \)) were indistinguishable from those in WT (Fig. 1). There were no differences in baseline flow (20.3 ± 1.1 ml·min\(^{-1}\)·g\(^{-1}\)), debt volume (5.1 ± 0.3 ml/g), peak flow (44.2 ± 0.5 ml·min\(^{-1}\)·g\(^{-1}\)), or repayment volume (6.1 ± 0.1 ml/g). Flow debt in hearts from \( \text{A2B KO} \) mice was repaid (123 ± 13%), representing 95 ± 9% of the response in hearts from WT mice. Responses in hearts from \( \text{A2A/2B} \) DKO mice (\( n = 8 \)) were similar to \( \text{A2A KO} \) mice (Fig. 1). That is, repayment volume (4.9 ± 0.2 ml/g) and the repayment of flow debt (105 ± 6%) were reduced (\( P < 0.05 \) vs. WT). Resting flow (18.6 ± 0.6 ml·min\(^{-1}\)·g\(^{-1}\)), debt volume (4.7 ± 0.1 ml/g), and peak flow (6.1 ± 2.1 ml·min\(^{-1}\)·g\(^{-1}\)) were not different from WT, but the repayment of flow debt in hearts from \( \text{A2A/2B} \) DKO mice was only 82 ± 5% of the response seen in WT (Table 1).

Adenosine activates \( \text{K}_{\text{ATP}} \) channels through \( \text{A2A} \) receptors. We tested the effect of \( \text{K}_{\text{ATP}} \) channel blockade on coronary reactive hyperemia response of WT isolated hearts. Glibenclamide (10 \( \mu \)M) significantly reduced WT (\( n = 5 \)) baseline flow from 17.3 ± 0.5 to 12.8 ± 0.5 ml·min\(^{-1}\)·g\(^{-1}\) and flow debt from 4.3 ± 0.1 to 3.2 ± 0.1 ml/g. Peak hyperemic flow and the repayment volume were reduced by glibenclamide from 35.9 ± 0.8 to 29.3 ± 1.1 ml·min\(^{-1}\)·g\(^{-1}\) and 6.9 ± 0.4 to 2.7 ± 0.2 ml·min\(^{-1}\)·g\(^{-1}\).
0.4 ml/g, respectively. Thus flow debt was repaid 161 ± 12% in hearts from WT mice while this was reduced to 87.5 ± 11.2% in the presence of glibenclamide (Fig. 2).

We isolated smooth muscle cells from the aortas of WT, A2A and A2B KO, and A2A/2B DKO mice and used whole cell patch-clamp to determine whether A2A receptors couple to KATP channels. Recordings were made in symmetrical 140 mM KCl so that inward linear KATP current was more easily resolved. Effects of adenosine (10 μM) were compared with pinacidil (10 μM) to confirm that KATP channels mediated them. Adenosine activated current in smooth muscle cells from WT mice (Fig. 3A), but not A2A KO mice (Fig. 3B). Responses in smooth muscle cells from A2B KO mice (Fig. 3C) were similar to WT, while responses in cells from A2A/2B DKO mice (Fig. 3D) were like those from A2A KO mice. Group data (cells from n = 4–5 mice in each group; Fig. 3E) show that adenosine-induced KATP current was diminished in smooth muscle cells from mice lacking the adenosine A2A receptor (P < 0.05 vs. WT by one-way ANOVA). Importantly, however, functional KATP channel expression was not different as pinacidil-activated KATP current was similar in smooth muscle cells from all four strains of mice.

H2O2 activates KATP current in smooth muscle. We isolated smooth muscle cells from the aortas of WT mice and used whole cell patch clamp to determine whether H2O2 activates KATP current. Current was recorded in the symmetrical 140 mM KCl solutions described above. Figure 4A shows current sensitive to glibenclamide (10 μM) under control conditions and when activated by H2O2 (1 mM) and pinacidil (10 μM). Group data (cells from n = 5 mice) are shown in Fig. 4B: KATP conductance (nS/pF) was 0.018 ± 0.006 under control conditions and significantly increased to 0.070 ± 0.026 and 0.142 ± 0.033 with 1 mM H2O2 and 10 μM pinacidil, respectively (both P < 0.05 vs. control by one-way ANOVA). To determine the role of KATP channels in H2O2-mediated coronary vasodilation, we examined the effect of glibenclamide (10 μM) on changes in flow induced by H2O2 (10 μM). In our isolated heart system, glibenclamide significantly decreased the baseline coronary flow (79 ± 6%) and consistent with the patch-clamp data, our data showed that glibenclamide significantly reduced the H2O2-induced increase in coronary flow from 164 ± 16 to 79 ± 6% (n = 4, Fig. 4C).

Adenosine-induced coronary dilation involves H2O2. We used catalase to determine the role of endogenous H2O2 production in adenosine-induced coronary vasodilation. Adenosine-induced coronary vasodilation is catalase-sensitive (Fig. 5A), indicating a role for endogenous H2O2 production. Specifically, adenosine (1 μM) increased coronary flow (284 ± 53%) and catalase (1,250 U/ml) reduced the baseline flow to 86 ± 2%; however, catalase reduced the adenosine response to 89 ± 13% (P < 0.05 vs. control by paired t-test). Catalase reduced left ventricular developed pressure (Fig. 5C), but had no effect on heart rate (Fig. 5B). These data suggest that 1) H2O2 is involved in adenosine-mediated effects on cardiac contractility or 2) cardiac function decreased secondary to a reduction in coronary flow. Since we have previously shown that adenosine does not increase the coronary flow in A2A/2B KO mice (39), this experiment was not performed on double knockout mice.

H2O2 contributes to coronary reactive hyperemia. We used hearts from WT and A2A KO mice to determine whether H2O2 links A2A receptor activation to coronary flow during reactive hyperemia. To do so, reactive hyperemia in hearts from both strains was compared with and without catalase (1,250 U/ml)
to degrade H$_2$O$_2$ (Fig. 6). In hearts from WT mice, catalase significantly decreased the repayment of flow debt (from 128 ± 8 to 84 ± 9%; P < 0.05; n = 6). In hearts from A$_{2A}$ KO mice, catalase had no effect on the already diminished repayment of flow debt (98 ± 9 vs. 98 ± 7%; n = 6, Table 1).

H$_2$O$_2$ contributes to reactive coronary hyperemia independent of nitric oxide (NO). Adenosine A$_{2A}$ and A$_{2B}$ receptors can also signal through NO (32). NO is a well-known vasodilator involved in regulating baseline coronary flow and reactive hyperemia. Thus, to separate the role of H$_2$O$_2$ from NO, we performed reactive hyperemia experiments on hearts from WT mice with L-NAME (50 μM, a NO synthase inhibitor) and L-NAME plus catalase (Fig. 7). L-NAME significantly decreased the repayment of flow debt (73 ± 8% of control; n = 5, P < 0.05). Importantly, however, addition of catalase to L-NAME further reduced the repayment of flow debt (57 ± 9%; P < 0.05). These data indicate that the effect of catalase is on H$_2$O$_2$ signaling, not NO signaling.

**DISCUSSION**

Our study provides several new substantial lines of evidence regarding mechanisms of coronary reactive hyperemia. First, using gene-targeted mice, we offer a definitive comparison of the roles of A$_{2A}$ and A$_{2B}$ receptors in coronary reactive hyperemia. Our finding that A$_{2A}$ receptor plays a dominant role in adenosine-mediated coronary reactive hyperemia substantially advances knowledge gained from studies using adenosine receptor antagonists (6, 10, 51). Second, with our catalase experiments, we provide the first evidence of a role for H$_2$O$_2$ in reactive hyperemia in the intact heart. These data add significantly to previous observations of roles for H$_2$O$_2$ in mesenteric reactive hyperemia (49) and reactive dilation in isolated coronary arterioles (18). Third, through a combination of experiments, we offer the original observation that H$_2$O$_2$ couples A$_{2A}$ receptor stimulation to K$_{ATP}$ channel activation in coronary reactive hyperemia.

Previously, two general strategies have been employed to determine the role of adenosine and its receptors in coronary reactive hyperemia: 1) enzymatic degradation of adenosine (e.g., infusing adenosine deaminase) or 2) pharmacological antagonism of adenosine receptors (e.g., administering amrinophylline). With these tools, it had been demonstrated previously that adenosine plays a small, but significant role, in coronary reactive hyperemia in dogs, lambs, and pigs (4, 6, 10, 23). Because two of the four known adenosine receptor subtypes, A$_{2A}$ and A$_{2B}$, are linked to coronary vasodilation, a logical question centers on which receptor subtype(s) is/are involved. Gene deleted knockout mice have been an important tool to dissect physiological signaling pathways, e.g., elucidating the role of receptor through its absence. Using PCR, we confirmed the absence of A$_{2A}$ and A$_{2B}$ receptors in knockout mouse tail, aorta and coronary arteries (data not shown). Therefore, the present results obtained using adenosine receptor knockout mice support the view that adenosine and the A$_{2A}$ receptor mediate a portion of coronary reactive hyperemia.

A$_{2A}$ and A$_{2B}$ receptors both function in signaling in coronary smooth muscle and endothelium (31, 37, 40), but the relative role of these two receptor subtypes in coronary reactive hyperemia has remained, until now, obscure. Using an antagonist of A$_{2A}$ receptors (SCH58261), it was suggested that 30–50% of flow repayment could be attributed to A$_{2A}$ receptor activation (6, 51). Our results with hearts from A$_{2A}$ KO and A$_{2A}$/A$_{2B}$ DKO mice indicate that the A$_{2A}$ receptor is responsible for 22–29% of flow repayment, which is toward the lower end of the previous pharmacological estimates. Our results from A$_{2B}$ KO and A$_{2A}$/A$_{2B}$ DKO mice did not suggest a significant role for A$_{2B}$ receptors in reactive hyperemia, similar to previous pharmacological studies (6, 51). Due to its low affinity for adenosine, A$_{2B}$ receptors are thought to be activated under more severely ischemic conditions, such as during ischemia/reperfusion and preconditioning (13, 15).

We tested the involvement of H$_2$O$_2$ in reactive hyperemia and adenosine-induced coronary vasodilation. We demonstrate that catalase, which breaks down H$_2$O$_2$ into water and oxygen, significantly attenuates adenosine-induced coronary vasodilation. This suggests that H$_2$O$_2$ plays a critical role in adenosine-
mediated signaling pathways. We also demonstrate that the repayment of flow debt was reduced by catalase, showing the contribution of endogenous H$_2$O$_2$ production to coronary reactive hyperemia. Furthermore, we suggest that H$_2$O$_2$ released during coronary reactive hyperemia is, at least partly, mediated through activation of A$_2A$ adenosine receptors since catalase did not further decrease the flow repayment in A$_2A$ KO hearts. This is not likely due to changes in H$_2$O$_2$ degradation in A$_2A$ KO mice, as the concentration-response curve to exogenous H$_2$O$_2$ (100 nM to 80 $\mu$M) is unchanged compared with WT control or A$_2B$ KO (data not shown). In the vasculature, NAD(P)H oxidases (Nox) are responsible for the majority of ROS production (21). Nox4 is shown to be the dominant isoform producing H$_2$O$_2$ and also expressed in vascular smooth muscle cells (45). Therefore, expression level of factors involved in vascular H$_2$O$_2$ production such as NOX4 in A$_2A$ KO remain to be examined in future studies. Nevertheless, the idea that H$_2$O$_2$ is a mediator of coronary metabolic (36, 48) and endothelium-dependent (22, 27) vasodilation is gaining traction; however, no previous study had indicated a role for H$_2$O$_2$ in intact coronary reactive hyperemia. Two related observations had, however, been made in other vascular beds. Video microscopy of the cremasteric microcirculation indicated striking similarities in the pharmacological sensitivity of reactive hyperemia and exogenous H$_2$O$_2$ (46). A study using microspheres in the mouse mesenteric circulation indicated that ~40% of hyperemic flow was catalase-sensitive, i.e., attributable to endogenous H$_2$O$_2$ production (49). Our data are in agreement and indicate that ~35% of hyperemic flow in the mouse heart is catalase-sensitive.

We demonstrated previously that A$_2A$-mediated coronary vasodilation depends upon K$_{ATP}$ channels, as it is blocked by

![Figure 4](http://ajpheart.physiology.org/)

**Fig. 4.** H$_2$O$_2$ activates K$_{ATP}$ channels in smooth muscle cells. **A:** representative trace showing effect of H$_2$O$_2$ (1 mM) to increase K$_{ATP}$ current. **B:** group data (cells from $n = 5$ mice) illustrate effect of H$_2$O$_2$ on K$_{ATP}$ current relative to pinacidil (10 $\mu$M). **C:** group data ($n = 4$) demonstrate the effect of glibenclamide (10 $\mu$M) on H$_2$O$_2$-mediated increase in coronary flow of WT hearts. * and # indicate $P < 0.05$ vs. WT and H$_2$O$_2$ effect, respectively.

![Figure 5](http://ajpheart.physiology.org/)

**Fig. 5.** H$_2$O$_2$ dilates the coronary circulation and adenosine-induced vasodilation is catalase-sensitive. **A–C** show group data ($n = 4$) illustrating the catalase sensitivity of adenosine-induced coronary dilatation (A), contractility (C), and heart rate (B) in WT mice. LVDP, left ventricular developed pressure. *$P < 0.05$ vs. adenosine alone.
Here we show that adenosine-induced coronary vasodilation depends upon H2O2, H2O2 increases KATP channel current, and increase in coronary flow by H2O2 is mediated through KATP channels. The latter finding extends the knowledge that KATP channels mediate, at least in part, H2O2-induced dilation of arterioles from skeletal muscle and brain (20, 24, 44). Further, it has been shown that H2O2 activates KATP channels in pancreatic beta cells and cardiac myocytes (16, 30). Nevertheless, the evidence of this mechanism in smooth muscle and the role of adenosine were lacking. We show for the first time that KATP current in smooth muscle cells is activated by H2O2. This adds to previous literature indicating that H2O2 activates K+ channels in coronary vascular smooth muscle cells including Ca2+-activated K+ channels (5, 42, 52) and voltage-dependent K+ channels (34). Additionally, glibenclamide and catalase both decreased the baseline coronary flow, with glibenclamide effect being about 7% more than catalase, suggesting that KATP channels may have additional pathways of activation in addition to adenosine-induced H2O2 although H2O2 may be the major mediator in this activation.

Perhaps a better approach would be to study smooth muscle cells isolated from mouse coronary arteries or arterioles. This is because differences between large- and small-caliber vessels exist (e.g., effects on intravascular pressure and relative responses to metabolic, neurohumoral, myogenic, and flow-mediated mechanisms) (7, 11). Moreover, it has been demonstrated that differences in K+ channel expression may explain some segmental differences in vascular reactivity (1, 25).

Importantly, however, there is no a priori reason to assume that, in this context, KATP channels in smooth muscle cells from the aorta and coronary arterioles would respond differently to adenosine and H2O2 signaling. Specifically, both conduit and resistance arteriolar smooth muscle cells express KATP channels (17, 19), adenosine A2 receptors (9, 47), and relax or dilate to H2O2 (26, 27). In fact, we demonstrate here that KATP electrophysiology from aortic smooth muscle cells (Figs. 3 and 4) matches very well with functional responses from coronary resistance vessels (Fig. 4C). Thus, until demonstrated otherwise, it is justifiable to conclude that aortic smooth muscle cells serve as an excellent electrophysiological model for coronary arteriolar dilation, at least with regard to glibenclamide (39). Here we show that 1) adenosine-induced coronary vasodilation depends upon H2O2, 2) H2O2 increases KATP channel current, and 3) increase in coronary flow by H2O2 is mediated through KATP channels. The latter finding extends the knowledge that KATP channels mediate, at least in part, H2O2-induced dilation of arterioles from skeletal muscle and brain (20, 24, 44). Further, it has been shown that H2O2 activates KATP channels in pancreatic beta cells and cardiac myocytes (16, 30). Nevertheless, the evidence of this mechanism in smooth muscle and the role of adenosine were lacking. We show for the first time that KATP current in smooth muscle cells is activated by H2O2. This adds to previous literature indicating that H2O2 activates K+ channels in coronary vascular smooth muscle cells including Ca2+-activated K+ channels.

Fig. 6. Catalase (Cat)-sensitive portion of reactive hyperemia is absent in hearts lacking the adenosine A2A receptor. A: group data show baseline flow and hyperemic responses in hearts from WT and A2A KO mice in the absence or presence of catalase (1,250 U/ml). Hyperemic responses were reduced by catalase in hearts from WT (n = 7) but not A2A KO (n = 6) mice. Catalase reduced the repayment of flow debt 30% in hearts from WT (B) mice (*P < 0.05 by unpaired t-test), but had no effect in hearts from A2A KO mice (C).

Fig. 7. Catalase-sensitive portion of reactive hyperemia is independent of nitric oxide. A (n = 4–9) shows group data of baseline flow and hyperemic responses in hearts from WT mice in the presence and absence of Nω-nitro-l-arginine methyl ester (l-NAME) (5 × 10^-5 M, nitric oxide synthase inhibitor) or l-NAME + catalase (1,250 U/ml). B shows the further reduced hyperemic response in the presence of both l-NAME and catalase (68.5%) relative to l-NAME alone (53.1%) compared with WT. *P < 0.05 and #P < 0.05 vs. control and l-NAME effect, respectively.
adenosine signaling, reactive oxygen species, and \( K_{ATP} \) channel regulation.

With regard to the limitations of our study, since we have used isolated heart system, the contribution of flow/shear stress- or pressure-dependent coronary mechanisms in the development of reactive hyperemia could not be clearly elucidated or isolated from the effect of local metabolic factors. Additionally, Krebs buffer used in crystalloid-perfused mouse heart, unlike blood, does not have \( O_2 \)-carrying capacity and hence some may suggest that the heart would be ischemic (by definition) or hypoxic compared with circulating blood. Regardless, constantly oxygenated ex vivo isolated heart model is a well-established method that has been used to answer mechanistic questions. Data gathered from isolated heart reflect the overall end effect of shear/pressure stress- and metabolites-induced effects via eliminating the complexity of the role of neuronal and hormonal effects during coronary reactive hyperemia, thus allowing a better understanding of the coronary flow regulation.

Our study provides novel data to further understand mechanisms of coronary reactive hyperemia and adenosine-induced vasodilation. Using adenosine receptor KO mice, we show that \( \alpha_{2A} \) receptors, but not \( \alpha_{2B} \) receptors, are critical to coronary reactive hyperemia. This finding supports and extends previous studies using pharmacological tools. Additionally, we demonstrate a role for \( H_2O_2 \) in reactive hyperemia in the intact heart. This finding supports observations made in the microcirculation of skeletal muscle and brain and represents the first such report for the coronary circulation. Finally, our data suggest that \( H_2O_2 \) is a signaling molecule coupling the stimulation of \( \alpha_{2A} \) receptors to the opening of \( K_{ATP} \) channels. This finding is entirely novel and will require additional research to unravel molecular mechanisms linking \( \alpha_{2A} \) receptor activation to \( H_2O_2 \) production and finally to \( K_{ATP} \) channel opening. At present, a list of likely mechanisms might include \( \alpha_{2A} \)-induced activation of NADPH oxidases (14), bioconversion of superoxide to \( H_2O_2 \), and redox regulation of the \( K_{ATP} \) channel or proteins that regulate it (50).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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