Original Research

Contribution of IK_{Ca} channels to the control of coronary blood flow

Michelle M Kurian, Zachary C Berwick and Johnathan D Tune

Department of Cellular and Integrative Physiology, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46202, USA
Corresponding author: Johnathan D Tune. Email: jtune@iupui.edu

Abstract

The purpose of this investigation was to elucidate the contribution of intermediate conductance calcium-activated potassium channels (IK_{Ca}) to the regulation of coronary blood flow *in vivo*. We hypothesized that IK_{Ca} channels modulate coronary arteriolar resistance at rest and contribute to vasomotor responses to changes in coronary perfusion pressure and/or in response to cardiac ischemia. Experiments were conducted in open-chest anesthetized dogs in the absence and presence of IK_{Ca} channel inhibitor, TRAM-34 (1 μg/min, intracoronary), and the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine-methyl ester (L-NAME) (150 μg/min, intracoronary). We found that administration of the potent SK_{Ca} and IK_{Ca} channel agonist NS309 dose-dependently increased coronary blood flow and that inhibition of IK_{Ca} channels with TRAM-34 attenuated this response by ~90%. The increase in coronary blood flow to NS309 was also decreased ~100% by the inhibition of NO production with L-NAME. Multiple linear regression analysis demonstrated that TRAM-34 diminished the autoregulatory capability of the coronary circulation at coronary pressures ranging from 60 to 120 mmHg. However, inhibition of IK_{Ca} channels did not affect coronary vasodilation in response to a transient 15 s coronary artery occlusion (i.e. reactive hyperemia). Our data reveal that IK_{Ca} channels are functionally expressed in the coronary circulation and that activation of these channels produces marked coronary vasodilation *in vivo*, primarily via increases in endothelial NO production. In addition, IK_{Ca} channels modestly contribute to changes in coronary vascular resistance in response to alterations in coronary perfusion pressure but do not contribute to the reactive hyperemic response following a brief coronary artery occlusion.

Keywords: coronary vasodilation, pressure-flow autoregulation, reactive hyperemia, ischemia

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Introduction

Coronary blood flow is tightly regulated by the resistance vessels of the coronary circulation, which are largely under the control of neuro-humoral, metabolic, and endothelial-derived factors.[1] These factors modulate microvascular tone and thus coronary blood flow through the activation of downstream ion channels, in particular K^+ channels, which are primarily responsible for establishing the vascular smooth muscle membrane potential.[2] Four types of K^+ channels have been identified in the coronary circulation, including voltage-gated K^+ channels (K_v), inward-rectifying K^+ channels (K_{ir}), ATP-dependent K^+ channels (K_{ATP} – a subfamily of K_{ir}) and Ca^{2+}-activated K^+ channels (K_{Ca}). Earlier investigations have implicated smooth muscle K_{ATP} and K_v channels as critical players in the control of coronary blood flow at rest,[3–6] during increases in myocardial metabolism,[4,5,7,8] as well as during episodes of cardiac ischemia.[3] Alternatively, large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) have been shown to contribute to coronary endothelial-dependent dilation[9–11] but play little if any role in the regulation of coronary blood flow at rest,[6,10,12,13] during exercise[13] or in response to ischemia.[6]

Two additional types of K_{Ca} channels expressed in the coronary circulation include small-conductance (SK_{Ca}) and intermediate-conductance (IK_{Ca}) channels.[9,14–16] Under normal conditions, these channels are predominantly expressed in endothelial cells and when activated mediate endothelial-dependent vasodilation via stimulation of nitric oxide (NO) and hyperpolarizing factor(s) (EDHF) production.[17] More specifically, IK_{Ca} channels ($K_{Ca}3.1$) have been found to contribute to vasodilation of isolated coronary arterioles in response to bradykinin,[9,18,19] substance P[15] and increases in shear stress, i.e. flow-induced dilation.[16,20] Additional studies have documented that IK_{Ca} channel expression is significantly upregulated in

vascular smooth muscle in response to injury and disease.[21–25] Inhibition of IK_{Ca} channels with TRAM-34[26] markedly attenuates phenotypic modulation of coronary smooth muscle as well as proliferation, migration and neointimal formation in models of re-stenosis.[23,24,27] A recent study by the Sellke laboratory also showed that coronary microvascular dysfunction following cardioplegic arrest is related, at least in part, to the impairment of IK_{Ca} channel function.[28] Although these earlier investigations have established the contribution of IK_{Ca} channels to coronary endothelial-dependent and atherogenic mechanisms, the role of these channels in the regulation of coronary blood flow *in vivo* has yet to be elucidated.

Accordingly, the present study was designed to test the hypothesis that IK_{Ca} channels contribute to the control of coronary blood flow at rest, in response to activation of endothelial cells, during changes in coronary perfusion pressure and/or following a brief coronary artery occlusion. The rationale for these studies is based on previous findings implicating endothelial-dependent responses in the modulation of coronary pressure-flow autoregulation[29,30] and reactive hyperemia.[3,31] To examine this hypothesis, experiments were conducted in open-chest anesthetized dogs in the absence and presence of TRAM 34 (1 μg/min, intracoronary) and the NO synthase inhibitor, L-NAME (150 μg/min, intracoronary). Our findings provide critical *in vivo* evidence for the functional expression of IK_{Ca} channels in the coronary circulation.

Methods

This investigation was approved by the Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85–23, Revised 1996). All dogs studied were lean mongrel dogs weighing between 20 and 30 kg. Following completion of experimental protocols, hearts were fibrillated and excised as recommended by the American Veterinary Medical Association Guide on Euthanasia (June 2007).

Surgical preparation

Dogs ($n = 10$) were initially sedated with morphine (3 mg/kg, subcutaneously) and anesthetized with α-chloralose (100 mg/kg, intravenously). The animals were then intubated and mechanically ventilated (Harvard respirator) with room air supplemented with oxygen. A catheter was placed into the thoracic aorta via the right femoral artery to measure aortic blood pressure and heart rate. The left femoral artery was catheterized to supply blood to an extracorporeal perfusion system used to perfuse the left anterior descending (LAD) artery at a controlled pressure. A catheter was also inserted into the right femoral vein for injection of supplemental anesthetic, heparin and sodium bicarbonate. Arterial blood gases were analyzed periodically throughout the experimental protocol and adjustments were made as needed to maintain blood gas parameters within normal physiological limits. A left lateral thoracotomy was performed to expose the heart, and the LAD was isolated distal to its first major diagonal branch. Following heparin administration (500 U/kg, intravenously), the LAD was cannulated with a stainless steel cannula connected to an extracorporeal perfusion system. Coronary perfusion pressure was regulated by a servo-controlled roller pump. Coronary blood flow was continuously measured by an inline Transonic Systems flow transducer (Ithaca, NY, USA). Data were continuously recorded on IOX data acquisition software from Emka Technologies (Falls Church, VA, USA). Hemodynamic parameters were allowed to stabilize for ~30 min before initiation of the experimental protocol.

Experimental protocol

Following the recovery period, a continuous intracoronary infusion of the potent SK_{Ca} and IK_{Ca} channel agonist, NS309,[32,33] was initiated (20–600 μg/min) before and during the subsequent administration of the IK_{Ca} antagonist, TRAM-34 (1 μg/min, intracoronary; $n = 5$). Dose response studies to acetylcholine (0.3–30 μg/min, intracoronary; $n = 4$) were also conducted in the absence and presence of TRAM-34 (1 μg/min, intracoronary; $n = 4$). In select animals, coronary reactive hyperemia was assessed by a 15-s occlusion of the LAD ($n = 4$), and/or pressure-flow autoregulation was assessed by 10 mmHg increment changes in coronary perfusion pressure (range: 40–140 mmHg) before and during administration of TRAM-34 ($n = 5$). Most animals received either NS309 or acetylcholine infusions followed by subsequent reactive hyperemia and/or autoregulatory studies. Additional NS309 concentration response studies were also conducted in separate animals following administration of the NO synthase inhibitor L-NAME (150 μg/min, intracoronary; $n = 3$). Time control experiments revealed no tachyphylaxis to repeated administration of the highest dose (600 μg/min) of NS309 (coronary flow $= 16 \pm 1$ to 33 ± 4 versus 15 ± 2 to 37 ± 6 mL/min; $n = 3$). Previous time-control studies revealed no tachyphylaxis to acetylcholine.[34]

Drugs

NS309 and TRAM-34 were dissolved in equal parts of ethanol, propylene glycol and 1 N NaOH. NS309 was then diluted 1:10 in saline. Control studies revealed no effect of vehicle on coronary blood flow responses *in vivo*. Acetylcholine and L-NAME were both dissolved in saline. All drugs were infused into the coronary perfusion circuit at a continuous rate (0.01–0.60 mL/min) and data recorded when coronary blood flow was stabilized at each dose (~5 min infusion/dose).

Statistical analyses

Data are presented as mean $\pm$ SE for n dogs. Statistical comparisons were made by a one- or two-way repeated measures analysis of variance as appropriate (Sigma Plot 11.0 Software). If statistical differences ($P < 0.05$) in these analyses were noted, a Student–Newman–Keuls multiple comparison test was performed. Multiple linear regression analysis was conducted to compare changes in coronary

blood flow with changes in perfusion pressure (GB Stat Software). Reactive hyperemic volumes were calculated as area under the curve using Prism software (GraphPad Software).

Results

IK_{Ca} channel-mediated coronary vasodilation *in vivo*: contribution of endothelium

The effects of IK_{Ca} channel inhibition on baseline hemodynamic variables for each of the experimental protocols performed are given in Table 1. Data are provided for baseline, baseline just prior to Tram-34 infusion (i.e. following dilator agonist protocol and stabilization period) and baseline during Tram-34 administration. We found that inhibition of IK_{Ca} channels with TRAM-34 tended to reduce mean arterial pressure and reflexively increase heart rate; however no statistically significant differences were detected. Although baseline coronary blood flow tended to be higher following TRAM-34 administration, no significant differences were noted relative to baseline values obtained immediately prior to the initiation of the Tram-34 infusion. TRAM-34 vehicle had no effect on baseline blood pressure, heart rate, coronary perfusion pressure or coronary blood flow (data not shown).

Table 1 Effects of TRAM-34 on baseline hemodynamic variables in anesthetized, open-chest dogs

	Mean blood pressure (mmHg)	Heart rate (beats/min)	Coronary pressure (mmHg)	Coronary flow (mL/min)
NS309 protocol				
Baseline ($n = 5$)	98 ± 7	91 ± 10	101 ± 1	17 ± 2
Pre-Tram-34 ($n = 5$)	93 ± 7	117 ± 7	101 ± 1	21 ± 3
Post-Tram-34 ($n = 5$)	88 ± 8	113 ± 7	101 ± 1	23 ± 4
Autoregulation protocol				
Baseline ($n = 5$)	104 ± 7	125 ± 16	100 ± 1	24 ± 1
Pre-Tram-34 ($n = 5$)	97 ± 7	120 ± 8	100 ± 1	26 ± 4
Post-Tram-34 ($n = 5$)	85 ± 1	125 ± 6	101 ± 1	28 ± 3
Acetylcholine protocol				
Baseline ($n = 4$)	101 ± 7	106 ± 14	99 ± 1	20 ± 1
Pre-Tram-34 ($n = 4$)	91 ± 3	127 ± 6	100 ± 1	22 ± 3
Post-Tram-34 ($n = 4$)	91 ± 4	126 ± 8	98 ± 1	28 ± 1*
Reactive hyperemia protocol				
Baseline ($n = 4$)	91 ± 9	100 ± 11	100 ± 1	19 ± 3
Pre-Tram-34 ($n = 4$)	92 ± 10	115 ± 8	101 ± 1	21 ± 4
Post-Tram-34 ($n = 4$)	81 ± 5	125 ± 5	102 ± 1	24 ± 5

Values are mean ± SE from *n* dogs
*$P < 0.05$ versus baseline

Administration of the potent SK_{Ca} and IK_{Ca} channel agonist NS309 dose-dependently increased coronary blood flow under control conditions (Figure 1; $P < 0.001$) and inhibition of IK_{Ca} channels with TRAM-34 markedly attenuated this increase in coronary blood flow ~90% at the highest dose of NS309 ($P = 0.002$). In order to assess whether IK_{Ca} channel-mediated coronary vasodilation is endothelial-dependent, NS309 dose-response studies were also conducted in separate animals in the presence of the NO synthase inhibitor L-NAME (150 μg/min, intracoronary). L-NAME had no effect on baseline blood pressure (95 ± 7 mmHg; $P = 0.60$) or on heart rate (92 ± 10 bpm; $P = 0.32$). We found that the increase in coronary blood flow in response to activation of SK_{Ca} and IK_{Ca} channels was decreased by the inhibition of NO production with L-NAME (Figure 1; $P < 0.001$). Importantly, the ~100% reduction in the coronary blood flow response to the highest dose of NS309 produced by L-NAME was similar to the degree of inhibition produced by TRAM-34. Additional experiments also revealed that endothelial-dependent increases in coronary flow to acetylcholine were significantly diminished by the administration of TRAM-34 (Figure 2; $P = 0.003$).

Contribution of IK_{Ca} channels to coronary pressure-flow autoregulation

Pressure-flow autoregulation was assessed in our preparation via servo-control of coronary perfusion pressure over a range of 140–40 mmHg. Under untreated control conditions, coronary blood flow averaged 27 ± 2 mL/min at a coronary perfusion pressure of 120 mmHg versus 16 ± 2 mL/min at a coronary perfusion pressure of 60 mmHg (Figure 3); i.e. coronary flow varied ~10 mL/min over the classic autoregulatory range.[35] Inhibition of IK_{Ca} channels with TRAM-34 significantly diminished the

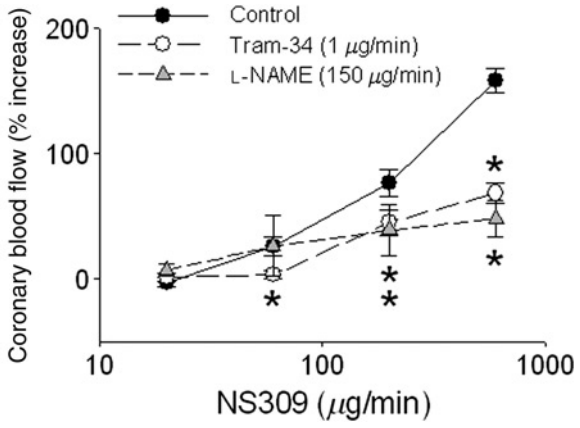

Figure 1 Administration of the potent SK_{Ca} and IK_{Ca} channel agonist NS309 dose-dependently increased coronary blood flow under control conditions ($P < 0.001$). This increase in coronary blood flow was significantly attenuated by the inhibition of IK_{Ca} channels with TRAM-34 ($P = 0.002$; $n = 5$). A similar degree of attenuation in coronary blood flow was also seen during inhibition of endothelial-derived nitric oxide synthase with L-NAME ($P < 0.001$; $n = 3$). *Indicates $P < 0.05$ versus control. SK_{Ca}, small conductance calcium-activated potassium channels; IK_{Ca}, intermediate conductance calcium-activated potassium channels

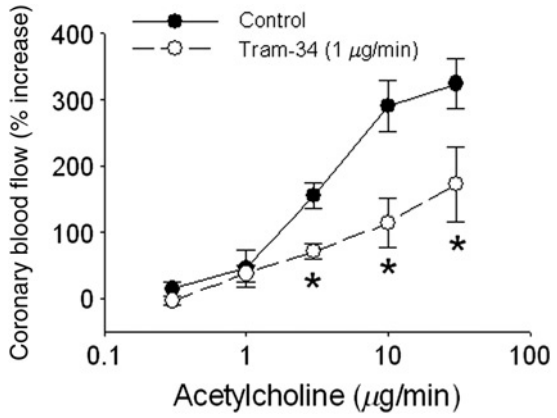

Figure 2 Administration of the endothelial-dependent agonist acetylcholine dose-dependently increased coronary blood flow under control conditions ($P < 0.001$). TRAM-34 significantly attenuated this endothelial-dependent increase in coronary blood flow ($P = 0.003$, $n = 4$) *indicates $P < 0.01$ versus control

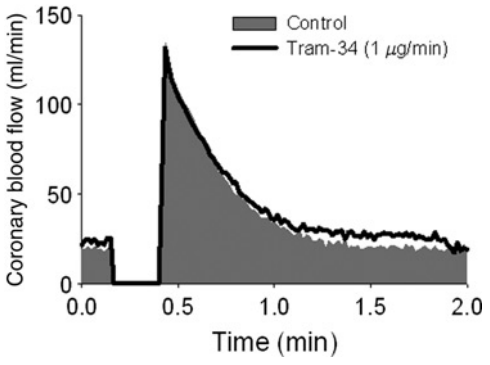

Figure 4 Inhibition of IK_{Ca} channels with TRAM-34 has no significant effect on the reactive hyperemic response of the coronary circulation ($P = 0.78$; $n = 4$)

autoregulatory capability as coronary flow changed from 35 ± 3 to 14 ± 4 mL/min; i.e. the change in coronary flow doubled to ~20 mL/min over the same range of coronary perfusion pressures when IK_{Ca} channels were inhibited. These data correspond with a significant increase in the slope ($P < 0.0001$) of the pressure–flow relationship following the administration of TRAM-34 (Figure 3b). This increase in slope was evidenced over coronary pressure ranges from 40 to 140 mmHg as well as from 60 to 120 mmHg. Coronary zero flow pressure (Pzf) was also increased from 28 ± 2 to 32 ± 2 mmHg following IK_{Ca} channel inhibition with TRAM-34 ($P = 0.009$).

Contribution of IK_{Ca} channels to coronary reactive hyperemia

The role of IK_{Ca} channels in coronary reactive hyperemia was assessed by the coronary flow response following a 15-s occlusion before and during infusion of TRAM-34 (Figure 4). We found that inhibition of IK_{Ca} channels did not significantly impact coronary vasodilation in response to a brief episode of cardiac ischemia as TRAM-34 did not affect the peak hyperemic response ($P = 0.78$), the duration of the hyperemic response ($P = 0.85$) or the volume of repayment ($P = 0.77$) following the 15-s coronary artery occlusion (Table 2). The repayment of coronary flow debt was also not significantly affected by the inhibition of IK_{Ca} channels with Tram-34 (Table 2; $P = 0.31$).

Discussion

The purpose of this investigation was to elucidate the contribution of IK_{Ca} channels to the control of coronary blood flow *in vivo*. Since previous studies have implicated IK_{Ca} channels in coronary endothelial-dependent vasodilation *in vitro*,[9,15,16,18–20] and endothelial-derived factors have been shown to contribute to pressure-flow autoregulation[29,30] as well as ischemic vasodilation,[3,10] we hypothesized that IK_{Ca} channels modulate coronary microvascular resistance at rest and contribute to vasomotor responses to changes in coronary perfusion pressure and/or in response to cardiac ischemia. The major findings of this study were: (1) IK_{Ca} channels are functionally expressed in the coronary microcirculation and activation of these channels produces significant increases in coronary blood flow *in vivo*; (2) IK_{Ca} channel-induced coronary vasodilation is primarily mediated via endothelial-dependent production of NO; (3) IK_{Ca} channels do not significantly contribute to the regulation of baseline coronary vasomotor tone;

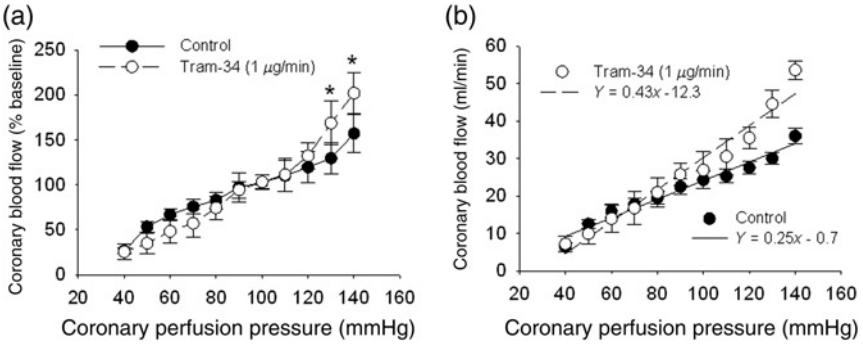

Figure 3 (a) TRAM-34 increased the coronary flow response at coronary perfusion pressures >120 mmHg ($P < 0.001$) and tended to exaggerate reductions in coronary blood flow as perfusion pressures were lowered from 70 to 50 mmHg ($P = 0.09$). (b) Multiple linear regression analysis demonstrated that TRAM-34 diminished the autoregulatory capability of the coronary circulation as evidenced by the significant increase in the slope of the coronary flow versus pressure relationship ($P < 0.0001$; $n = 5$)

Table 2 Effects of TRAM-34 on coronary reactive hyperemic response in anesthetized, open-chest dogs

	Peak flow (mL/min)	Duration (s)	Volume repayment (mL)	Repayment/debt (%)
Control	137 ± 12	52 ± 4	33.5 ± 3.4	700 ± 68
Tram-34	135 ± 10	53 ± 8	32.9 ± 4.6	592 ± 70

Values are mean ± SE from $n = 4$ dogs

(4) inhibition of IK_{Ca} channels with TRAM-34 moderately impairs coronary pressure-flow autoregulation; and (5) IK_{Ca} channels play little/no role in the coronary reactive hyperemic response.

IK_{Ca} channels in control of coronary blood flow at rest

Our data indicate that IK_{Ca} channels do not contribute to the active regulation of baseline coronary tone, as TRAM-34 did not significantly reduce coronary flow under resting conditions (Table 1). These data are consistent with previous studies in isolated coronary arterioles which documented no changes in baseline tone in response to K_{Ca} channel inhibition.[9,16,36] Based on numerous earlier studies which have demonstrated that inhibition of NO synthesis has little/no effect on baseline coronary blood flow,[37–40] it is not surprising that inhibition of IK_{Ca} channels failed to substantially alter baseline coronary blood flow. The lack of a distinct role for IK_{Ca} channels in the regulation of basal vasomotor tone has also been reported in other vascular beds.[17,41,42]

Mechanisms of IK_{Ca} channel-mediated coronary vasodilation

Earlier studies have demonstrated that IK_{Ca} channels mediate vasodilation of isolated vessels from various vascular beds via an endothelial-dependent mechanism.[17] In particular, inhibition of IK_{Ca} channels has been shown to diminish dilation of isolated coronary arterioles in response to bradykinin[9,18,19] and substance P[15] as well as flow-mediated increases in shear stress.[16,20] However, the extent to which IK_{Ca} channels modulate coronary blood flow *in vivo* has not been examined. Data from the present study indicate that IK_{Ca} channels are functionally expressed in the coronary microcirculation and, when stimulated, are capable of mediating significant increases (>100%) in coronary blood flow. In particular, we found that the potent SK_{Ca} and IK_{Ca} channel agonist, NS309, dose-dependently increased coronary blood flow and that this response was markedly attenuated by inhibition of IK_{Ca} channels with TRAM-34 (Figure 1). Furthermore, our data support that IK_{Ca} channels mediate coronary vasodilation predominantly via endothelial-dependent increases in NO production as inhibition of NO synthesis with L-NAME attenuated NS309 dilation to a similar extent as that observed during TRAM-34 administration (Figure 1). This hypothesis is further supported by the significant reduction in acetylcholine-induced coronary vasodilation in the presence of TRAM-34 (Figure 2). Given that these endothelial-dependent responses were not completely abolished by L-NAME or TRAM-34, we submit that IK_{Ca} channels likely also mediate coronary vasodilation *in vivo* through the production of EDHFs such as CO, H_2S and/or arachadonic acid metabolites.[32,43] The extent to which these specific EDHFs contribute to IK_{Ca} channel-mediated coronary vasodilation merits further investigation.

Functional contribution of IK_{Ca} channels to the regulation of coronary blood flow

There is evidence for a direct role of NO in modulating coronary blood flow in response to changes in coronary perfusion pressure[29] and following a brief coronary artery occlusion.[3,31] However, the extent to which IK_{Ca} channels contribute to coronary pressure-flow autoregulation and/or reactive hyperemia has not yet been elucidated. Our data are the first to document that inhibition of IK_{Ca} channels impairs the autoregulatory capability of the coronary circulation as the change in coronary flow within the 'classic' autoregulatory range (60–120 mmHg) was doubled in the presence of TRAM-34 (Figure 3b). In order to account for differences in baseline coronary blood flow between control-baseline and TRAM-34 treatment, changes in coronary blood flow to alterations in perfusion pressure were analyzed both as absolute coronary blood flow and normalized as percent of baseline. This analysis revealed that inhibition of IK_{Ca} channels exaggerated the flow response, primarily to elevations in coronary perfusion pressure (Figure 3a). The mechanism underlying this paradoxical effect is unclear but is likely unrelated to increases in myocardial metabolism as the double-product of arterial pressure and heart rate (index of myocardial oxygen consumption) was markedly lower during TRAM-34 administration. Importantly, decreases in coronary blood flow also tended to be greater as coronary pressures were lowered from 70 to 50 mmHg in the presence of TRAM-34 (Figure 3a), but these differences did not reach statistical significance ($P = 0.09$). In contrast, Pzf was increased ~15% by the inhibition of IK_{Ca} channels ($P < 0.05$). Taken together, these data suggest that IK_{Ca} channels play a moderate role in the regulation of coronary blood flow with alterations in perfusion pressure. The extent to which NO modulates this effect merits further investigation.

Additional studies were also conducted to examine the relative contribution of IK_{Ca} channels to increases in coronary blood flow following a brief coronary artery occlusion, i.e. reactive hyperemia. We found that inhibition of IK_{Ca} channels with TRAM-34 had no effect on key variables of the coronary reactive hyperemic response (Table 2; Figure 4). Importantly, alteration in the repayment of coronary flow debt (i.e. debt to repayment ratio) following Tram-34 administration ($P = 0.31$) is primarily related to an increase in coronary flow debt (increase in baseline flow) and not to a significant reduction of the hyperemic response (duration and volume of repayment; Table 2). Based on our present findings we propose that the contribution of NO to ischemic dilation in the coronary circulation[3,31] is not dependent on the activation of IK_{Ca} channels. These data are consistent with a recent study from our laboratory which demonstrated that BK_{Ca}

channels are not involved in coronary reactive hyperemia.[6] Therefore, there is little support for the contribution of K_{Ca} channels to the coronary reactive hyperemic response. However, additional studies are needed to address whether the limited effect of K_{Ca} channel inhibition could be related to the activation of compensatory parallel (redundant) pathways, e.g. K_v, K_{ir} and/or K_{ATP} channels, that mask the overall effect of K_{Ca} channel blockade on coronary blood flow responses.

In summary, this study importantly demonstrates that IK_{Ca} channels are functionally expressed in the coronary microcirculation and that activation of these channels produces marked increases in coronary blood flow, primarily via increases in endothelial NO production. Our data indicate that IK_{Ca} channels modestly contribute to changes in coronary vascular resistance in response to alterations in coronary perfusion pressure but do not modulate coronary vasodilation in response to a brief coronary artery occlusion. Since earlier studies have established that NO is not required for coronary vasodilation in response to increases in myocardial oxygen demand,[37,38,40] we postulate that IK_{Ca} channels are unlikely to significantly contribute to local metabolic control of coronary blood flow. We propose that IK_{Ca} channels represent an important mechanism in the regulation of endothelial-dependent responses *in vivo* and that alterations in channel expression and/or activity could significantly contribute to the development of endothelial dysfunction observed in disease states such as hypertension, obesity-insulin resistance and heart failure.[17,43]

Author contributions: All authors participated in the design and conduction of experiments and were directly involved with interpretation of the studies, analysis of the data and review of the manuscript.

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