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CONTRIBUTION OF ADENOSINE A_{2A} AND A_{2B} RECEPTORS TO ISCHEMIC CORONARY DILATION: ROLE OF K_V AND K_{ATP} CHANNELS

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Abstract

This study was designed to elucidate the contribution of adenosine A_{2A} and A_{2B} receptors to coronary reactive hyperemia and downstream K⁺ channels involved. Coronary blood flow was measured in open-chest anesthetized dogs. Adenosine dose-dependently increased coronary flow from 0.72 ± 0.1 to 2.6 ± 0.5 ml/min/g under control conditions. Inhibition of A_{2A} receptors with SCH58261 (1 μM) attenuated adenosine-induced dilation by ~50%, while combined administration with the A_{2B} receptor antagonist alloxazine (3 μM) produced no additional effect. SCH58261 significantly reduced reactive hyperemia in response to a transient 15 sec occlusion; debt/repayment ratio decreased from 343 ± 63 to 232 ± 44%. Alloxazine alone attenuated adenosine-induced increases in coronary blood flow ~30% but failed to alter reactive hyperemia. A_{2A} receptor agonist CGS21680 (10 μg bolus) increased coronary blood flow by 3.08 ± 0.31 ml/min/g. This dilator response was attenuated to 0.76 ± 0.14 ml/min/g by inhibition of K_V channels with 4-aminopyridine (0.3 mM) and to 0.11 ± 0.31 ml/min/g by inhibition of K_{ATP} channels with glibenclamide (3 mg/kg). Combined administration abolished vasodilation to CGS21680. These data indicate that A_{2A} receptors contribute to coronary vasodilation in response to cardiac ischemia via activation of K_V and K_{ATP} channels.

Keywords

Reactive hyperemia; coronary vasodilation; adenosine receptor; potassium channel; canine

INTRODUCTION

Since adenosine was first proposed as a constituent of coronary vasomotor regulation by Berne in 1963 (4), the role of this local purinergic metabolite has evolved considerably. Although studies have demonstrated that adenosine is not required for the regulation of coronary blood flow at rest or during increases in myocardial metabolism (2;12;44), adenosine has been shown to contribute to coronary vasodilation when myocardial oxygen requirements are not sufficiently met during episodes of ischemia (11;14;29). In particular, coronary vasodilation in response to exercise is diminished by combined enzymatic degradation and non-selective inhibition of adenosine receptors in dogs with a coronary

stenosis (coronary perfusion pressure = 40 mmHg) (29). This finding is in agreement with data from Stepp and Feigl who demonstrated that cardiac adenosine production is markedly elevated by reductions in coronary perfusion pressure below 60 mmHg (40). Other studies indicate that adenosine contributes to coronary vasodilation in response to brief episodes of cardiac ischemia, i.e. coronary reactive hyperemia (2;39), however, this is not a consistent finding (5;10).

The cardiovascular effects of adenosine are mediated via four extracellular receptor subtypes; A₁, A_{2A}, A_{2B} and A₃ (31). Although coronary microvascular vasodilation in response to adenosine occurs primarily through the activation of A_{2A} and A_{2B} receptor subtypes (3;22;26;27;30;41;43), the relative contribution of these subtypes to ischemic coronary vasodilation has not been clearly defined. Recently, Zatta and Headrick (51) found that selective inhibition of adenosine A_{2A} receptors reduced coronary reactive hyperemia in isolated, buffer-perfused mouse hearts by ~20-30%. The contribution of A_{2A} receptors to microvascular regulation was also demonstrated by Frobert et al. who found that the selective A_{2A} antagonist ZM241385 attenuated hypoxic dilation of isolated porcine coronary arteries by ~30% (18). A_{2B} receptors have also been shown to contribute to coronary vasodilation in response to adenosine (26;30;32), although, not in response to hypoxia (18). At present, it is unclear whether A_{2A} and/or A_{2B} receptors regulate coronary blood flow during cardiac ischemia *in vivo*.

Earlier studies demonstrate that both A_{2A} and A_{2B} receptor subtypes converge on downstream ATP-dependent K⁺ channels (K_{ATP}) to induce coronary vasodilation (7-9;20-22;24;27;45). More recent data from our laboratory also indicate that voltage-dependent K⁺ channels (K_v) play a significant role in the coronary vascular response to adenosine (10). Importantly, both K_{ATP} and K_v channels have been shown to modulate coronary vasodilation in response to cardiac ischemia (7;10;25;51). However, the extent to which A_{2A} and/or A_{2B} receptor activation contributes to this effect of K⁺ channels has not been investigated. Accordingly, the purpose of this investigation was to examine the hypothesis that adenosine A_{2A} and/or A_{2B} receptors mediate coronary vasodilation in response to cardiac ischemia via activation of downstream K_v and/or K_{ATP} channels. Our findings provide important new data on the functional contribution of adenosine receptor subtypes to increases in coronary blood flow in response to endogenously produced ischemic metabolites *in vivo*.

METHODS

All protocols were 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). Male mongrel dogs (n = 16) weighing between 20-30 kilograms were administered morphine (3 mg/kg, s.c.) as a sedative, pre-anesthetic before inducing anesthesia with α -chloralose (100 mg/kg, iv). 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

Following induction of anesthesia, dogs were intubated and ventilated with room air supplemented with O₂. 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 coronary artery (LAD). 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 made as needed to maintain blood gas parameters within normal physiological limits. Following a left lateral thoracotomy, a proximal portion of the LAD was isolated distal to its first major diagonal branch. Following heparin administration (500 U/kg), the LAD was cannulated with a stainless steel cannula connected to an extracorporeal perfusion system. Coronary perfusion pressure was maintained at 100 mmHg throughout the experimental protocol by a servo-controlled roller pump. Hemodynamic parameters were allowed to stabilize for ~30 min before initiation of the experimental protocol.

Experimental Protocol

Adenosine (3 – 30 $\mu\text{g}/\text{min}$) was infused at a constant rate into the LAD perfusion circuit before and during administration of the selective A_{2A} receptor antagonist SCH58261 (1 μM , i.c.), followed by the subsequent inhibition of A_{2B} receptors with alloxazine (3 μM , i.c.). Coronary reactive hyperemia was assessed by a 15 sec occlusion of the LAD before and during administration of SCH58261 (1 μM , i.c.) followed by alloxazine (3 μM , i.c.). Adenosine dose-response and reactive hyperemia studies were also performed in the absence and presence of the A_{2B} antagonist alloxazine alone (i.e. without SCH58261 administration). Additional studies were also conducted with the selective A_{2A} agonist CGS 21680 (10 μg bolus, i.c.), before and after administration of the K_V channel antagonist 4-aminopyridine (4-AP, 0.3 mM, i.c.) and/or inhibition of K_{ATP} channels with glibenclamide (3 mg/kg, i.v.). CGS 21680 infusions were separated by at least 45 min as time control studies revealed similar coronary vasodilation in response to CGS 21680 following a 45 min washout period. Coronary reactive hyperemia studies were also conducted in the presence of 4-AP, glibenclamide, and 4-AP + glibenclamide. All drugs, with the exception of glibenclamide (dissolved in equal parts of ethanol, propylene glycol, 1N NaOH) and 4-AP (in saline) were dissolved in DMSO, diluted 1:10 with saline and infused i.c. at a constant rate (~300 $\mu\text{l}/\text{min}$) for ~5 min prior to measurements in order to achieve the desired coronary plasma concentration. LAD perfusion territory was estimated as previously described by Feigl *et al.* (15).

Statistical analyses

Data are presented as mean $\pm$ SE from n dogs. Reactive hyperemic volumes were calculated as area under the curve using Prism software (GraphPad, San Diego, CA). Duration parameters were evaluated at 35 seconds post occlusion and the point at which hyperemic flow had returned to within 5% of baseline. Statistical comparisons were made by t-test, one-way or two-way repeated measures analysis of variance (ANOVA) as appropriate. If statistical differences ($P < 0.05$) in these analyses were noted, a Student-Newman-Keuls multiple comparison test was performed.

RESULTS

Contribution of A_{2A} and A_{2B} receptors to adenosine-mediated coronary vasodilation

Effects of A_{2A} and A_{2B} receptor blockade on baseline hemodynamic variables are listed in Table 1. Treatment with vehicle, SCH58261 and/or alloxazine did not significantly affect arterial blood pressure or coronary blood flow under baseline-resting conditions. Heart rate was modestly elevated during combined blockade of A_{2A} and A_{2B} receptors. Under control conditions, exogenous adenosine administration dose-dependently increased coronary blood flow from 0.81 ± 0.07 at rest to 2.53 ± 0.51 ml/min/g at the highest dose of adenosine (30 $\mu\text{g}/\text{min}$, i.c.). Administration of the A_{2A} receptor antagonist SCH58261 significantly reduced coronary vasodilation in response to adenosine ~70% (Fig 1, n = 4). Subsequent administration of the A_{2B} receptor antagonist alloxazine had no additional effect on adenosine dilation. In separate studies, alloxazine alone attenuated adenosine-induced increases in coronary blood flow ~30% (Fig 3A, n = 3).

Contribution of A_{2A} and A_{2B} receptors to coronary reactive hyperemia

Table 2 shows the effects of vehicle, A_{2A} and subsequent A_{2B} receptor blockade on key coronary reactive hyperemia variables. Importantly, drug-vehicle had no significant effect on the coronary reactive hyperemic response. Inhibition of A_{2A} receptors with SCH58261 significantly reduced coronary vasodilation in response to brief, 15 sec coronary artery occlusion (Fig 2A, n = 7). In particular, SCH58261 diminished the duration of the hyperemic response and the overall volume of repayment (Table 2). This reduced the repayment of coronary flow debt from $343 \pm 63\%$ in vehicle treated hearts to $232 \pm 44\%$ following SCH58261 administration (Fig 2C). Successive blockade of A_{2B} receptors with alloxazine had no additional effect on the coronary reactive hyperemic response (Fig 2B, Table 2). Sole administration of alloxazine also failed to significantly alter the hyperemic response (Fig 3B; n = 3).

A_{2A} receptor activation of coronary K⁺ channels

Experiments were conducted to assess the contribution of K_V and K_{ATP} channels to coronary vasodilation mediated by A_{2A} receptor activation. Coronary blood flow increased from 0.75 ± 0.09 to 3.32 ± 0.17 ml/min/g following intracoronary administration of the selective A_{2A} agonist CGS 21680 (10 μ g, n = 4). A_{2A}-mediated vasodilation was significantly attenuated by inhibition of either K_V (~70%, n = 3) or K_{ATP} channels (~96%, n = 3) alone (Fig 4). Combined administration of 4-AP and glibenclamide essentially abolished the response to CGS 21680 (Fig 4, n = 4). As recently demonstrated by our laboratory (6;10), inhibition of K_V (n = 5) and/or K_{ATP} (n = 3) channels markedly reduced the coronary reactive hyperemic response (Fig 5). In particular, the decrease of both peak flow and the duration of the hyperemic response following combined K⁺ channel blockade significantly reduced the repayment of coronary flow debt from $422 \pm 51\%$ to $162 \pm 40\%$.

DISCUSSION

This study examined the hypothesis that adenosine A_{2A} and/or A_{2B} receptors significantly contribute to coronary vasodilation in response to cardiac ischemia via activation of downstream K_V and K_{ATP} channels. To test this hypothesis, we measured changes in coronary blood flow in open-chest anesthetized canines in response to selective adenosine A_{2A} and A_{2B} receptor blockade during both exogenous adenosine infusion and following a brief coronary artery occlusion. In addition, the coupling of A_{2A} receptors to K_V and K_{ATP} channels was also assessed. The major findings of this study are: 1) A_{2A} receptors contribute to coronary vasodilation in response to exogenous adenosine administration and cardiac ischemia; 2) A_{2A} receptor-induced coronary vasodilation is mediated via signaling pathways that converge on K_V and K_{ATP} channels; 3) A_{2A} and A_{2B} receptors do not contribute to the regulation of coronary blood flow under baseline-resting conditions; and 4) A_{2B} receptors contribute to coronary vasodilation in response to exogenous adenosine, but are not required for dilation in response to cardiac ischemia. These data indicate that A_{2A} receptors contribute to coronary vasodilation in response to cardiac ischemia via activation of K_V and K_{ATP} channels.

Contribution of A_{2A} and A_{2B} receptors to the control of coronary blood flow

Findings from this investigation indicate that A_{2A} and A_{2B} receptors contribute to coronary vasodilation in response to exogenous adenosine *in vivo*, with the A_{2A} receptor pathway playing the predominant role. In particular, inhibition of A_{2A} receptors significantly reduced the increase in coronary blood flow to the highest dose of adenosine (30 μ g/min) ~40% beyond that of A_{2B} receptor blockade alone (Figs 1 and 3). Earlier *in vitro* studies using selective antagonists and/or genetic knockout of A₂ receptor subtypes in isolated coronary arteries (3;18;26) and buffer-perfused mouse hearts (30;41;51) support this finding. In

addition, recent *in vivo* studies have also documented marked coronary vasodilation in response to selective A_{2A} receptor agonists (23;43). Although A₂ receptors are expressed and capable of mediating vasodilation in the canine coronary circulation, it is important to recognize that inhibition of A_{2A} and/or A_{2B} receptors failed to significantly diminish resting coronary flow (Table 1). Therefore, our data do not support a role for either A_{2A} or A_{2B} receptors in the regulation of baseline coronary vasomotor tone *in vivo*. This conclusion is supported by numerous previous studies utilizing enzymatic and/or non-selective adenosine receptor antagonists (2;12;44;48).

A_{2A} and A_{2B} receptors in coronary reactive hyperemia

A primary goal of this investigation was to assess the functional contribution of A_{2A} and A_{2B} receptors to coronary vasodilation in response to endogenous metabolites produced during cardiac ischemia. We show, for the first time *in vivo*, that A_{2A} receptors contribute to coronary reactive hyperemia, as inhibition of A_{2A} receptors with SCH58261 reduced repayment of coronary flow debt ~32% (Fig 2). Although earlier studies indicate that the mechanisms of coronary reactive hyperemia are dependent on species, experimental preparation and the duration of coronary occlusion (17;19;51), our finding is consistent with data from Zatta and Headrick who reported that SCH58261 (100 nM) resulted in an ~20-30% reduction in the coronary reactive hyperemic response in isolated mouse hearts (51). In addition, data from Frobert *et al.* demonstrate that the selective A_{2A} blockade attenuates hypoxic dilation of isolated porcine coronary arteries by ~30% (18). The effect of A_{2A} receptor inhibition on coronary reactive hyperemia is in contrast with our recent study which found little/no effect of the non-selective adenosine receptor antagonist 8-phenyltheophylline on ischemic vasodilation in the canine coronary circulation (10). We hypothesize these discrepant findings are related to the opposing effects A₁ vs. A₂ receptors on coronary microvascular resistance (8;30;41;42).

Although A_{2B} receptor blockade reduced coronary vasodilation in response to exogenous adenosine (Fig 3A; see **Limitations of the study** below), alloxazine did not significantly affect the coronary reactive hyperemic response (Fig 3B). Similarly, hypoxic coronary vasodilation of isolated coronary artery rings was unaffected by selective A_{2B} receptor inhibition with MRS1754 (18). This lack of effect of A_{2B} receptor blockade is consistent with the lower affinity of A_{2B} vs. A_{2A} receptors for adenosine (K_a ranges 1-20 nM for A_{2A} vs. 5-20 μ M for A_{2B}) (16;34) as well as the differential expression of A_{2A} receptors in the coronary microcirculation vs. A_{2B} receptor expression in larger coronary arteries (22). Taken together, these findings argue against a prominent role for A_{2B} receptors in the modulation of coronary blood flow in response to endogenously produced adenosine.

A_{2A} activation of coronary K⁺ channels

Based on our initial findings, the next goal of this investigation was to determine the relative contribution of K⁺ channels to A_{2A} receptor mediated coronary vasodilation *in vivo*. This question is important because although numerous earlier studies have demonstrated that adenosine and A₂ receptor signaling converge on K_{ATP} channels (1;7;8;20-22;24;27;28;47), more recent data from our laboratory indicate that K_V channels also play a significant role (3;10). In addition, both K_{ATP} and K_V channels have been shown to modulate coronary vasodilation in response to cardiac ischemia (7;10;25;51). Accordingly, we conducted experiments to examine the effects of K_V and K_{ATP} channel inhibition on the coronary vasodilatory response to the selective A_{2A} agonist CGS 21680 (22;27;46). We found that coronary vasodilation in response to A_{2A} receptor activation is mediated via signaling pathways that converge on both K_{ATP} and K_V channels as inhibition of either K_V channels or K_{ATP} channels markedly attenuated vasodilation in response to CGS 21680 (Fig 4). Importantly, the decrease in the coronary reactive hyperemia following inhibition of K_V and

K_{ATP} channels (~60% reduction; Fig 5) was much greater than the relatively modest effect A_{2A} receptor blockade alone (~30% reduction; Fig 2A). These data support previous studies implicating a prominent role for K^+ channels in ischemic coronary vasodilation (6;10;13) and indicate that while A_{2A} receptor signaling is a component of K_V and K_{ATP} channel activation in response to cardiac ischemia, numerous other pathways must also converge on these channels to mediate the marked increase in coronary blood flow following a brief episode of cardiac ischemia.

Limitations of the study

We fully acknowledge that conclusions derived from this study regarding the relative contribution of A_{2A} vs. A_{2B} receptors to the regulation of coronary blood flow are dependent on the selectivity of the antagonists used. SCH58261 is an A_{2A} receptor antagonist that effectively inhibits A_{2A} mediated coronary vasodilation at 1 μM (36), the concentration used in the present study. Alloxazine is a non-xanthine A_2 antagonist with a K_B value of 2.3 μM for A_{2B} receptors (41); a coronary plasma concentration of 3 μM was used for these experiments. Our studies with these antagonists revealed somewhat paradoxical results in that SCH58261 reduced coronary vasodilation in response to adenosine (~70%), with no additional effect of alloxazine. However, alloxazine alone (when administered without SCH58261) also reduced adenosine-induced increases in coronary blood flow by ~30%. Since SCH58261 is ~1000-fold more selective for A_{2A} over A_{2B} receptors (33;34;49), while alloxazine has much more limited selectivity (~10-fold greater selectivity for A_{2B} vs. A_{2A} ; (17)), we propose the most likely explanation for our paradoxical results is that alloxazine also impaired A_{2A} signaling as opposed to an SCH58261-induced impairment of A_{2B} signaling. However, the extent to which SCH58261 and alloxazine selectively inhibit A_{2A} and A_{2B} induced coronary vasodilation *in vivo* requires further investigation.

Equally important in this study is the selectivity of the K^+ channel antagonists used. We recently demonstrated that 0.3 mM 4-AP, the same concentration used in the present study, significantly attenuates coronary vasodilation in response adenosine, but does not affect increases in coronary blood flow to the K_{ATP} channel agonist pinacidil (10). However, glibenclamide has been shown to attenuate voltage-gated K current in rabbit kidney (50). This could explain the similar effect of glibenclamide alone vs. glibenclamide + 4-AP on CGS 21860 dilation (Fig 4) and the coronary reactive hyperemic response (Fig 5). Regardless, the reduction of the coronary reactive hyperemic response following K^+ channel inhibition was much greater than the relatively modest effect of A_{2A} receptor blockade alone.

In conclusion, findings from this investigation indicate that adenosine A_{2A} receptors contribute to coronary vasodilation in response to exogenous adenosine infusion and cardiac ischemia. Importantly, these findings are consistent with earlier studies in A_{2A} and A_{2B} receptor knockout mice (35-38). We have also identified that A_{2A} -induced coronary vasodilation *in vivo* is mediated via activation of K_V and K_{ATP} channels. Although A_{2B} receptors are functionally expressed, our data do not support an active role for A_{2B} receptors in the endogenous regulation of coronary blood flow in healthy control subjects. However, it is important to note that Bender *et al.* recently found that the obese-metabolic syndrome augments the contribution of A_{2B} receptors to adenosine-induced dilation *in vitro* (3). Interestingly, this increase was accompanied by a decrease in coronary A_{2B} receptor protein expression as well as a loss of downstream K_{ATP} channel activation. Other studies in human atrial appendage microvessels (26) support that disease states may significantly alter the functional contribution of A_2 receptor subtypes to the control of coronary microvascular tone. The significance of these changes along with the role of alternative adenosine receptor subtypes (A_1 and A_3) remains to be elucidated.

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Figure 1. Effect of A_{2A} and A_{2B} receptor inhibition on adenosine-induced coronary vasodilation
Inhibition of A_{2A} receptors with SCH58261 (1 μ M, ic) significantly attenuated coronary vasodilation in response to exogenously infused adenosine. Combined administration of SCH58261 with the A_{2B} receptor antagonist alloxazine (3 μ M, ic) produced no additional effect. * $P < 0.05$ vs. control.

Figure 2. Role of adenosine A_{2A} and A_{2B} receptors in ischemic coronary vasodilation

A: Inhibition of A_{2A} receptors with SCH58261 (1 μ M, ic) significantly reduced coronary vasodilation in response to a 15 sec transient coronary artery occlusion. B: The coronary reactive hyperemic response was unaffected by additional blockade of A_{2B} receptors with alloxazine (3 μ M). C: Effects of A_{2A} and A_{2B} receptor blockade on the repayment of coronary flow debt. A_{2A} receptor blockade significantly reduced the debt-repayment ratio relative to vehicle-control. Addition of the A_{2B} receptor antagonist alloxazine produced no additional effect. Figures show grouped average traces for $n = 7$ dogs. * $P < 0.05$ vs. vehicle.

Figure 3. Contribution of A_{2B} receptors to exogenous adenosine and ischemia induced vasodilation

A: Alloxazine (3 μM, i.c.) significantly reduced coronary blood flow during cumulative adenosine infusions. B: No effect of A_{2B} receptor blockade on reactive hyperemia. * $P < 0.05$ vs. control.

Figure 4. Effect of selective K⁺ channel blockade on A_{2A} receptor-mediated dilation

Coronary vasodilation in response to A_{2A} receptor activation with CGS 21680 (10 µg bolus, ic) was markedly attenuated by inhibition of K_V channels with 4-AP (0.3 mM, i.c.) and K_{ATP} channels with glibenclamide (3.0 mg/kg, i.v.). Combined administration of 4-AP and glibenclamide essentially abolished A_{2A} receptor-induced coronary vasodilation. * $P < 0.01$ vs. control.

Figure 5. Effect of K^+ channel blockade on ischemic coronary vasodilation

Averaged group tracings showing impaired coronary ischemic vasodilation in response to A: K_V channel blockade with 4-AP ($n = 5$). B: K_{ATP} channel blockade with glibenclamide ($n = 3$). C: Combined inhibition of both K_V and K_{ATP} channels ($n = 3$).

TABLE 1
Effect of A_{2A} and A_{2B} receptor blockade on baseline hemodynamics

Condition	Systolic Pressure, mmHg	Diastolic Pressure, mmHg	Mean Arterial Pressure, mmHg	Heart Rate, bpm	Coronary Pressure, mmHg	Coronary Flow, ml/min/g
Control	125 ± 11	79 ± 7	98 ± 8	75 ± 12	100 ± 1	0.81 ± 0.07
Vehicle	123 ± 11	79 ± 6	97 ± 7	77 ± 12	100 ± 1	0.91 ± 0.07
SCH 58261	123 ± 7	87 ± 7	102 ± 7	97 ± 13	99 ± 1	0.96 ± 0.09
SCH + Alloxazine	115 ± 7	82 ± 3	96 ± 4	105 ± 13*	100 ± 1	1.00 ± 0.09

Values are means ± SE for n=7 dogs.

* $P < 0.05$ vs. respective vehicle control.

TABLE 2
Effect of A_{2A} and A_{2B} receptor blockade on coronary reactive hyperemia

Condition	Peak Flow, ml/min/g	Debt Area, ml/g	Repayment Area, ml/g	Δflow at 35 seconds, ml/min/g	Duration, seconds
Control	3.15 ± 0.2	0.20 ± 0.02	0.71 ± 0.07	0.92 ± 0.14	83.14 ± 6.24
Vehicle	3.26 ± 0.2	0.23 ± 0.02	0.74 ± 0.11	0.92 ± 0.12	83.57 ± 5.75
SCH 58261	3.05 ± 0.1	0.24 ± 0.02	0.51 ± 0.06 [*]	0.57 ± 0.10 [*]	65.00 ± 4.43 [*]
SCH + Alloxazine	3.29 ± 0.1	0.25 ± 0.02	0.57 ± 0.09 [*]	0.69 ± 0.17 [*]	73.29 ± 8.07

Values are means ± SE for n = 7 dogs.

^{*} $P < 0.05$ vs. respective vehicle control.