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Comparison of Three Different A_1 Adenosine Receptor Antagonists on Infarct Size and Multiple Cycle Ischemic Preconditioning in Anesthetized Dogs

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Abbreviations: AR, adenosine receptor; CPX, 1,3-dipropyl-8-cyclopentylxanthine; BG 9719, 1,3-dipropyl-8-[2-(5,6-epxynorbornyl)]xanthine; BG 9928, 1,3-dipropyl-8-[1-(4-propionate)-bicyclo-[2,2,2]octyl]xanthine; IPC, ischemic preconditioning; GFR, glomerular filtration rate; [^{125}I]ZM 241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)-3-[^{125}I]iodophenol; [^{125}I]AB-MECA, N^6-(4-amino-3-[^{125}I]iodobenzyl)adenosine-5'-*N*-methylcarboxamide; [^{3}H]MRS 1754, 1,3-dipropyl-8-[4-[(4-Cyano-[2,6-^{3}H]phenyl)carbamoylmethyl)oxy]phenyl]xanthine; DMEM, Dulbecco's modified Eagles Medium; NECA, adenosine-5'-*N*-ethylcarboxamide; LAD, left anterior descending; ECG, electrocardiogram; TTC, triphenyltetrazolium chloride; DPSPX, 1,3-dipropyl-8-sulfophenylxanthine; CCPA, 2-chloro-N^6-cyclopentyladenosine

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Abstract

A_1 adenosine receptor (AR) antagonists are effective diuretic agents that may be useful for treating fluid retention disorders including congestive heart failure. However, antagonism of A_1ARs is potentially a concern when using these agents in patients with ischemic heart disease. To address this concern, the present study was designed to compare the actions of the A_1AR antagonists CPX (1,3-dipropyl-8-cyclopentylxathine), BG 9719 (1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)]xanthine), and BG 9928 (1,3-dipropyl-8-[1-(4-propionate)-bicyclo-[2,2,2]octyl]xanthine) on acute myocardial ischemia/reperfusion injury and ischemic preconditioning (IPC) in an *in vivo* dog model of infarction. Barbital-anesthetized dogs were subjected to 60 min of left anterior descending coronary artery occlusion followed by three h of reperfusion after which infarct size was assessed by staining with triphenyltetrazolium chloride. IPC was elicited by four 5-min occlusion/5-min reperfusion cycles produced 10 min before the 60-min occlusion. Multiple cycle IPC produced a robust reduction (~65%) in infarct size; this effect of IPC on infarct size was not abrogated in dogs pre-treated with any of the three AR antagonists. Surprisingly, in the absence of IPC, pre-treatment with CPX or BG 9928 before occlusion or immediately before reperfusion resulted in significant reductions (~40-50%) in myocardial infarct size. However, treatment with an equivalent dose of BG 9719 had no similar effect at the dose tested. We conclude that the A_1AR antagonists BG 9719, BG 9928, and CPX do not exacerbate cardiac injury and do not interfere with IPC induced by multiple ischemia/reperfusion cycles. We discuss the possibility that the cardioprotective actions of CPX and BG 9928 may be related to antagonism of A_{2B}ARs.

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A_1 adenosine receptor (AR) antagonists are currently being developed for their use in humans to treat fluid retention disorders including congestive heart failure (Gottlieb, 2001). A_1AR antagonists are effective diuretic agents due to their ability to inhibit the actions of adenosine to constrict afferent arterioles and increase sodium reabsorption at the proximal and distal tubules (Holz and Steinhausen, 1987; Takeda et al., 1993; Balakrishnan et al., 1996; Wilcox et al., 1999). An additional mechanism for the renal actions of A_1AR antagonists is the interruption of tubuloglomerular feedback linked to sodium delivery to macula densa cells (Wilcox et al., 1999; Brown et al., 2001; Sun et al., 2001; Schnermann, 2002). Unlike thiazide and loop diuretics, A_1AR antagonists have the unique ability to promote natriuresis without reducing glomerular filtration rate (GFR) (Gellai et al., 1998; Wilcox et al., 1999).

BG 9719 (1,3-dipropyl-8-[2-(5,6-epoxy-*s*-norbornyl)]xanthine previously named CVT-124) is one of the most potent and selective A_1AR antagonists developed to date (Belardinelli et al., 1995; Pfister et al., 1997). It is a xanthine derivative containing a norbornyl ring at the C-8 position (Figure 1) that effectively increases A_1AR affinity while decreasing potency at A_{2A}ARs (Belardinelli et al., 1995; Pfister et al., 1997). The affinities of BG 9719 for rat and human A_1ARs are 0.67 and 0.45 nM (Pfister et al., 1997), respectively, with selectivity versus A_{2A}ARs of 1,800-fold (rat) and 2,400-fold (human). In human patients with congestive heart failure, acute administration of BG 9719 increased urine output without decreasing GFR (Gottlieb et al., 2000). In a follow-up study in congestive heart failure patients in which furosemide induced diuresis at the expense of decreased GFR, combining BG 9719 with furosemide increased urine volume additionally while preventing the deterioration in GFR (Gottlieb et al., 2002).

These studies demonstrate the efficacy of BG 9719 as a renal modulating agent *in vivo* and suggest that the combined use of A_1AR antagonists with conventional diuretics may be an effective approach for the treatment of congestive heart failure (Gottlieb et al., 2000; Gottlieb, 2001; Gottlieb et al., 2002).

Although the clinical usefulness of A_1AR-selective antagonists is promising, one concern with the use of these agents in patients with cardiovascular disease is their potential to counteract the beneficial actions of adenosine in non-renal tissues. This is especially a concern in the heart. Adenosine is produced in response to ischemic stress, which is believed to serve a protective role to limit tissue injury by multiple mechanisms (Ely and Berne, 1992; Vinten-Johansen et al., 1999). Adenosine interacting with A_1ARs also appears to be one of several endogenous mediators of ischemic preconditioning (IPC), the phenomenon in which brief periods of ischemia activate defense mechanisms within the myocardium that increases resistance to subsequent ischemic episodes. Blockade of these actions of adenosine in the heart may be undesirable in patients with ischemic heart disease. Interestingly, however, many studies in experimental animal models have found that AR antagonists have no effect on the extent of tissue injury induced by acute ischemia and reperfusion (Auchampach and Gross, 1993; Thornton et al., 1993; Zhao et al., 1994; Haessler et al., 1996; Todd et al., 1996; Auchampach et al., 1997b; Kitakaze et al., 1997; Domenech et al., 1998). On the contrary, it has recently been proposed that selective blockade of A_1ARs during reperfusion may actually be an effective means to reduce myocardial infarct size (Neely et al., 1996; Forman et al., 2000).

The goal of the present investigation was to examine the effect of BG 9719 on infarct size in an *in vivo* dog model of infarction. In addition, we examined the effects of BG 9719 on the development of IPC in a clinically relevant model of multiple ischemia/reperfusion cycles (four 5-min occlusion/5-min reperfusion cycles). We compared the effects of BG 9719 with that of the traditional A_1AR antagonist CPX (1,3-dipropyl-8-cyclopentylxathine) and a newer xanthine antagonist BG 9928 (1,3-dipropyl-8-[1-(4-propionate)-bicyclo-[2,2,2]octyl)]xanthine) being developed as a renal modulating agent. (Ticho et al., 2003) BG 9928, a C-8 substituted bicyclo-[2,2,2]octylxanthine, binds with high affinity to the A_1AR and possesses improved physiochemical properties (solubility and stability) compared to BG 9719 (Figure 1). (Ticho et al., 2003) Since the affinities of the different antagonists used in the present study for dog ARs have not been assessed previously and since the relative selectivity of the antagonists for A_1ARs versus A_{2B} and A_3ARs is unknown, radioligand binding studies were performed with recombinant dog ARs expressed in HEK 293 cells.

Methods

Materials.

All reagents were from Sigma-Aldrich (St. Louis, MO) except for the following: HEK 293 cells were from ATTC (Manassas, VA); [^{3}H]CPX and radioactive microspheres were from Perkin-Elmer Life Sciences (Boston, MA); adenosine deaminase was from Boerhinger-Mannheim Biochemicals (Indianapolis, IN); lipofectamine, G418, fetal bovine serum, cell culture media, and pcDNA3.1 were from Invitrogen Life Technologies (Carlsbad, CA); BG 9719 and BG 9928 were provided by Biogen, Inc. (Cambridge, MA); and Whatman GF/C glass fiber filters were from Brandel (Gaithersburg, MD). [^{125}I]ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)-3-[^{125}I]iodophenol) and [^{125}I]AB-MECA (*N*^{6}-(4-amino-3-[^{125}I]iodobenzyl)adenosine-5'-*N*-methylcarboxamide) were synthesized by the chloramine-T method and purified by reverse-phase high-performance liquid chromatography. [^{3}H]MRS 1754 (1,3-dipropyl-8-[4-[((4-Cyano-[2,6-^{3}H]phenyl)carbamoylmethyl)oxy]-phenyl]xanthine) was custom synthesized according to the procedure of Ji and colleagues (Ji et al., 2001).

Radioligand Binding Assay.

The affinities of the AR antagonists for recombinant canine ARs expressed in HEK 293 cells were determined by radioligand binding assay using the antagonist radioligands [^{3}H]CPX, [^{125}I]ZM 241385, and [^{3}H]MRS 1754 for the A_1, A_{2A}, and A_{2B}AR, respectively, and the agonist radioligand [^{125}I]AB-MECA for the A_3AR. The full coding region of the receptor cDNAs were subcloned into the mammalian expression vector pcDNA3.1, transfected into HEK 293 cells using Lipofectamine^{TM}, and then selected

with 2 mg/ml of G418. Following antibiotic selection, the cells were maintained in DMEM cell culture media containing 10% fetal bovine serum with 0.6 mg/ml of G418. Dr. Guy Vassart (Brussels, Belgium) provided the full-length canine A_1 and A_{2A}AR cDNAs (Libert et al., 1989) and we previously cloned the full-length canine A_3AR cDNA from a mast cell cDNA library (Auchampach et al., 1997a). The canine A_{2B}AR cDNA was obtained by RT-PCR from RNA isolated from large intestine (GenBank accession # AY313204) using primers based on the sequences of the A_{2B}AR cloned from human, rabbit, rat, and mouse. Crude membranes were prepared from transfected cells, as described previously (Auchampach et al., 1997a), and stored in aliquots at -20^o C.

Binding assays were performed in triplicate with 50 μg membrane protein in a total volume of 0.1 ml HE buffer with 1 unit/ml adenosine deaminase and 5 mM $MgCl_2$. Membranes were incubated with radioligands at room temperature for 3 h and the reactions were terminated by rapid filtration over Whatman GF/C glass fiber filters using a 48-well Brandel cell harvestor followed by four 3-ml washes with ice-cold 10 mM Tris-HCl (pH 7.4) containing 10 mM $MgCl_2$. Non-specific binding was determined in the presence of 100 μM adenosine-5'-*N*-ethylcarboxamide (NECA). Saturation binding assays were conducted first to calculate K_d and B_{max} values by incubating the membranes with 6-8 concentrations of radioligand (the specific activity of [^{125}I]AB-MECA was reduced 10-20 fold with cold ligand to achieve saturation of the A_3AR). For competition experiments, 5-10 nM [^{3}H]CPX, 0.5-1.0 nM [^{125}I]ZM 241385, 5-10 nM [^{3}H]MRS 1754, or 0.25- 0.50 nM [^{125}I]AB-MECA were incubated with cell membranes in the presence of inhibitors.

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In saturation assays, specific binding data with [^{3}H]CPX, [^{125}I]ZM 241385, and [^{3}H]MRS 1754 fit optimally to a single binding site model using Marquardt's nonlinear least squares interpolation (Marquardt, 1963), whereas [^{125}I]AB-MECA fit optimally to a two-site binding model. The two binding sites reflect binding of [^{125}I]AB-MECA to the high affinity, G protein-coupled form of the A_3AR and the low affinity, uncoupled form of the receptor since the addition of GTPγS converts all of the binding to the low affinity state (Auchampach et al., 1997a). The K_d (nM) and B_{max} (fmol/mg membrane protein) values of the radioligands for their respective receptors were as follows: [^{3}H]CPX/A_1AR, K_d = 18.1 ± 4.4, B_{max} = 23,870 ± 1,480; [^{125}I]ZM 241385/A_{2A}AR, K_d = 0.76 ± 0.02, B_{max} = 1,501 ± 269; [^{3}H]MRE 1754/A_{2B}AR, K_d = 12.8 ± 1.7, B_{max} = 8,573 ± 784; and [^{125}I]AB-MECA/A_3AR, K_{d1} = 0.84 ± 0.04, K_{d2} = 21.1 ± 1.2, B_{max1} = 781 ± 42, B_{max2} = 2,146 ± 469. For analysis of competition data, IC_{50} values of antagonist ligands were fit to:

$$SB_i = B_i - (B_i - NS) * \frac{[I]}{IC_{50} + [II]}$$

where *i* is the number of binding sites, SB is specific binding, and NS is non-specific binding. K_i values were calculated from IC_{50}, B_{max}, the concentration of radioligand, and the radioligand K_d value with correction for radioligand and competing compound depletion, as described previously by Linden (Linden, 1982). For A_3AR binding using [^{125}I]AB-MECA, we used nonlinear least squares fitting to obtain K_i values of antagonists in competition for two binding sites by solving the following four equations simultaneously:

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$$LB = \left(B_{max1} * \frac{\frac{L}{K_{d1}}}{1 + \frac{L}{K_{d1}} + \frac{C}{K_{d1}}}\right) + \left(B_{max2} * \frac{\frac{L}{K_{d2}}}{1 + \frac{L}{K_{d2}} + \frac{C}{K_{d2}}}\right) + f + C$$

$$CB = \left(B_{max1} * \frac{\frac{C}{K_{i1}}}{1 + \frac{L}{K_{d1}} + \frac{C}{K_{i1}}}\right) + \left(B_{max2} * \frac{\frac{C}{K_{i2}}}{1 + \frac{L}{K_{d2}} + \frac{C}{K_{i2}}}\right) + f + C$$

$$LT = L + B \qquad CT = C + CB$$

where LB is radioligand bound, CB is the competitor bound, L is free radioligand, C is free competitor, LT is the total ligand, CT is total competitor, and *f* is the fraction of L or C non-specifically bound (Auchampach et al., 1997a). $K_{d1\&2}$ and $B_{max1\&2}$ values are known from independent binding isotherms performed in the absence of competitor. Since antagonists are assumed to bind with similar affinity to G protein-coupled and -uncoupled receptors, a single K_i value for each competitor was obtained by setting K_{i1} and K_{i2} values within the equations to be equal.

Anesthetized Dog Model.

Surgical preparation. The open-chest, barbital-anesthetized dog model of infarction used in this investigation has been described previously in detail(Auchampach and Gross, 1993). Adult mongrel dogs (average weight 23.3; range = 19.0 - 28.0 kg) of either sex were anesthetized (mixture of sodium pentobarbital [15 mg/kg i.v.] and

barbital sodium [200 mg/kg i.v.]), ventilated (tidal volume, 15 ml/kg; 10 - 15 breaths/min), and instrumented to measure left ventricular pressure and aortic pressure by inserting a double pressure transducer-tipped catheter into the aorta and left ventricle via the left carotid artery. Left ventricular dP/dt was recorded by electronic differentiation of the left ventricular pulse pressure and heart rate was determined by a tachometer. A left thoracotomy was performed at the fifth intercostal space and a 1.0 – 1.5 cm section of the left anterior descending (LAD) coronary artery was dissected free from surrounding tissue just distal to the first diagonal branch. A calibrated electromagnetic flow probe was placed around the vessel to measure coronary flow continuously with a flowmeter. The heart was paced at 150 beats/min (2.5 Hz) with rectangular pulses of 4 ms duration and a voltage of twice the threshold via bipolar leads clipped to the atrium. All measurements were recorded throughout the experiment on a Grass Model 7 polygraph. All of the dogs received humane care in accordance with the guidelines established by the Medical College of Wisconsin, which conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Experimental protocols. Three experimental protocols were performed in the study (Figure 2). In all three protocols, the dogs were subjected to 60 min of LAD occlusion and 3 h of reperfusion. Coronary occlusion and reperfusion was performed using a micrometer-driven occluder placed around the LAD artery. At the end of reperfusion, infarct size was assessed *ex vivo* by incubating the hearts with triphenyltetrazolium chloride and expressed as a percentage of the area at risk or as a

percent of the entire left ventricle, as described previously in detail (Auchampach and Gross, 1993). Hemodynamic variables (heart rate, arterial blood pressure, left ventricular pressure, left ventricular dP/dt, and LAD coronary blood flow) were measured continuously throughout the experiments. Regional myocardial blood flow was measured by use of radioactive microspheres (Ce^{141} and Nb^{95})(Auchampach and Gross, 1993) at 30 min into the prolonged 60-min occlusion period and after 3 h of reperfusion.

Protocol I (Antagonist Pretreatment): This protocol was designed to determine whether pre-treatment with CPX, BG 9719, or BG 9928 influences the infarct size induced by acute regional myocardial ischemia and reperfusion. The dogs were subjected to 60 min of LAD occlusion and three h of reperfusion. Four groups of dogs (n = 8-12/group) were randomly assigned to receive vehicle, CPX, BG 9719, or BG 9928 beginning 10 min before the coronary occlusion. All of the antagonists were administered at a dose of 1 mg/kg as an i.v. bolus followed by an infusion of 10 μg/kg/min, which was continued until immediately before reperfusion (70 min total, total dose = 1.7 mg/kg).

Protocol II (Ischemic Preconditioning): The goal of this protocol was to determine whether pre-treating with the AR antagonist influences the development of IPC induced by multiple ischemia/reperfusion cycles. Four groups of dogs (n = 6-8/group) were subjected to 60 min of coronary artery occlusion followed by three h of reperfusion. Preconditioning was elicited by four 5-min occlusion/5-min reperfusion cycles produced

10 min before the 60-min occlusion. The dogs were randomly assigned to receive vehicle, CPX, BG 9719, or BG 9928 beginning 10 min before the first preconditioning occlusion. The antagonists were administered at a dose of 1 mg/kg i.v. bolus followed by an infusion of 10 μg/kg/min, which was continued until the release of the prolonged occlusion (115 min total; total dose = 2.15 mg/kg).

Protocol III (Antagonist at Reperfusion). Since we observed that CPX and BG 9928 reduced infarct size when administered prior to and throughout the ischemic period in Protocol I, we included a third protocol in which we determined whether administration of the AR antagonists reduced infarct size if administered just prior to and during the first h of reperfusion. Three groups of dogs (n = 8/group) were subjected to 60 min of coronary artery occlusion followed by three h of reperfusion. The dogs were randomly assigned to receive CPX, BG 9719, or BG 9928 beginning 10 min before the release of the occlusion. The antagonists were administered at a dose of 1 mg/kg i.v. bolus followed by an infusion of 10 μg/kg/min for 1 h (70 min total; total dose = 1.7 mg/kg).

Exclusion criteria. Strict criteria were used to ensure that the animals included in data analysis were healthy and exposed to similar degrees of ischemia. Dogs were excluded if heart worms were found after the animals were killed, subendocardial blood flow exceeded 0.15 ml/min/g, or more than three consecutive attempts were required to convert ventricular fibrillation with low energy DC pulses applied directly to the hearts.

Statistical Analysis. All values are expressed as the mean ± SE. Hemodynamic variables were analyzed by a two-way repeated measures ANOVA (time and drug treatment) to determine whether there was a main effect of time, a main effect of treatment, or a time-treatment interaction. If global tests showed a main effect or interaction, post hoc contrasts between time-points or treatments were performed with Student's *t* test for unpaired or paired data, as appropriate, with the Bonferroni correction. Infarct sizes and risk region sizes were compared using a one-way ANOVA followed by Student's *t* test with the Bonferroni correction.

Results

Radioligand binding data. Table 1 reports the affinity of the AR antagonists used in the present investigation for the four canine ARs. We have also listed affinity values of 1,3-dipropyl-8-sulfophenylxanthine (DPSPX) for canine AR receptors, since this xanthine antagonist has also been shown to reduce myocardial infarct size in an experimental dog model by Forman and colleagues (Forman et al., 2000). All four of the antagonists bound with highest affinity for the A_1AR with a rank order of potency of $A_1 > A_{2B} > A_{2A} > A_3$. The absolute affinity of the antagonists for the canine A_1AR were lower than that reported previously for rat and human A_1ARs (Pfister et al., 1997). This finding corresponds with previous work (Tucker et al., 1994) demonstrating that a single amino acid difference in the canine A_1AR at position 270 within the 7th transmembrane region (isoleucine to methionine) reduces binding of xanthine antagonists ~10-20-fold. One important finding of these studies is that all of the antagonists exhibited relatively high affinity for the canine A_{2B}AR. BG 9719 and BG 9928 showed the greatest overall selectivity for the A_1AR being 79-, 21-, 556- and 163-, 24-, 1,457-fold selective versus the A_{2A}, A_{2B}, and A_3AR, respectively. CPX was only 9, 6, and 119-fold selective for the A_1AR versus A_{2A}, A_{2B}, and A_3ARs. DPSPX was essentially equipotent at binding to all four AR subtypes.

Effect of the AR antagonists on A_1 and A_{2A}AR-mediated responses in anesthetized dogs. All of the antagonists were used in the present study at a dose of 1 mg/kg followed by an infusion of 10 μg/kg/min. This dose of BG 9719 and BG 9928 has previously been shown to produce a maximal natriuretic/diuretic effect in rats, non-

human primates, and humans (Gellai et al., 1998; Wilcox et al., 1999; Gottlieb et al., 2000; Gottlieb et al., 2002; Ticho et al., 2003). In preliminary studies in un-paced barbital-anesthetized dogs, we determined whether this dose of each of the AR antagonists effectively blocked changes in heart rate and blood pressure induced by bolus injection (100 μg/kg) of the A_1AR-selective agonist N^6-cyclopentyladenosine (CCPA). We also examined in preliminary studies the effect of the same dose of the antagonists (1 mg/kg followed by an infusion of 10 μg/kg/min) on changes in LAD coronary conductance (LAD blood flow/mean arterial blood pressure) induced by intracoronary administration of the A_{2A}AR-selective agonist CGS 21680. In these studies, peak changes in coronary conductance were measured after bolus injections of 100 μl aliquots of 3, 10, or 100 μM solutions of CGS 21680 given into a needle catheter inserted directly into the LAD coronary artery immediately distal to the flow probe.

In the absence of the antagonists, bolus administration of CCPA (100 μg/kg) decreased heart rate 24 ± 7% and decreased blood pressure 10 ± 2% (n = 3). Pre-treatment with each of the antagonists blocked the hemodynamic actions of CCPA completely. The effects of CPX, BG 9719, and BG 9928 on changes in coronary conductance are depicted in Figure 3. In the absence of inhibitors, bolus injection of CGS 21680 produced a dose-dependent increase in coronary conductance. The dose-response relationship of CGS 21680 on coronary conductance was shifted significantly to the right by both CPX (estimated EC_{50} values for CGS 21680: control = 37.3 ± 3.1 μM; in the presence of CPX = 125.8 ± 1.8 μM) and BG 9928 (control = 33.1 ± 1.9 μM; BG 9928 = 74.1 ± 2.3 μM), but not by BG 9719 (control = 32.1 ± 1.8 μM; BG 9719 = 41.0 ± 2.2 μM). These data demonstrate that at a dose of 1 mg/kg followed by an

infusion of 10 μg/kg/min, all three of the antagonists efficiently antagonized A_1ARs. At this dose, CPX and BG 9928 (but not BG 9719) also antagonized A_{2A}ARs in the coronary circulation. Differences in the pharmacodynamic/pharmacokinetic properties of BG 9719 likely explain its lower *in vivo* potency.

Pre-treatment with CPX or BG 9928 reduces myocardial infarct size (Protocol I).

Pre-treatment with CPX or BG 9928 resulted in a significant reduction in myocardial infarct size induced by 60 min of LAD coronary artery occlusion and 3 h of reperfusion in barbital-anesthetized dogs (Figure 4). Infarct size expressed as a percentage of the area at risk was reduced from 22.6 ± 1.3% in vehicle-treated dogs to 11.1 ± 2.2% in CPX-treated dogs (~51% reduction) and to 11.5 ± 4.6% in BG 9928-treated dogs (~49% reduction). Pre-treatment with an equivalent dose of BG 9719, however, did not produce a significant reduction in infarct size (18.8 ± 1.0% of the area at risk). The protection against infarction provided by CPX or BG 9928 was not the result of differences in the area at risk size (control, 32.5 ± 1.5%; CPX, 31.9 ± 1.8%; BG 9719, 30.9 ± 1.6%, BG 9928, 30.4 ± 1.7%), changes in hemodynamic parameters (Table 2), or increases in regional myocardial blood flow (Tables 3 and 4). When infarct size expressed as a percentage of the area at risk was plotted versus transmural collateral blood flow, an inverse relationship was evident in all of the treatment groups such that infarct size was progressively smaller with higher levels of collateral flow (Figure 4b). This relationship between collateral blood flow was shifted downward in dogs treated with CPX or BG 9928 compared to vehicle-treated control dogs, indicating that the

smaller infarcts observed in these two groups of dogs were independent of changes in collateral blood flow during the ischemic period.

Pre-treatment with CPX, BG 9719, or BG 9928 does not block IPC (Protocol II).

To determine whether any of the AR antagonists blocked the development of IPC, we subjected dogs to four 5-min coronary occlusion/5-min reperfusion cycles 10 min before the 60-min occlusion period. This protocol has been shown previously to induce a preconditioning effect that resulted in a robust reduction in myocardial infarct size (Gross and Auchampach, 1992). As shown in Figure 5, we also observed a marked reduction in myocardial infarct size in dogs subjected to multiple cycle IPC. Infarct size as a percent of the area at risk was reduced by IPC to 8.1 ± 2.3% (versus 22.6 ± 1.3% in control dogs from Protocol I) or 2.6 ± 0.7% of the left ventricle (control = 7.3 ± 0.5%). In the three groups of dogs pre-treated with CPX, BG 9719, or BG 9928, infarct size continued to be reduced by IPC (3.2 ± 1.6%, 7.4 ± 1.6%, and 2.7 ± 2.6% of the area at risk, respectively). Similar to our observations in Protocol I, we also observed that none of the antagonists influenced the area at risk size (control, 32.5 ± 1.5%; Precond, 32.0 ± 1.3%; Precond + CPX, 33.4 ± 1.6%; Precond + BG 9719, 33.7 ± 3.0; Precond + BG 9928, 33.7 ± 3.2%), systemic hemodynamics (Table 5), or regional myocardial blood flow (Tables 3 and 4). Plots of infarct size versus transmural collateral blood flow (Figure 5b) demonstrated that infarct size was reduced by IPC in a matter that was independent of changes in collateral blood flow and that none of the antagonists interfered with this relationship.

Treatment with CPX or BG 9928 reduce infarct size when administered just prior to reperfusion (Protocol III).

This protocol was designed to determine whether the AR antagonists reduce infarct size if administered just prior to the reperfusion period. In this protocol, CPX, BG 9719, or BG 9928 were administered at the same dose and duration as Protocol I, except that the drugs were given 10 min before the release of the 60-min occlusion period and continued for the first hour of reperfusion. Interestingly, the two groups of dogs that were treated with CPX or BG 9928 during reperfusion continued to exhibit smaller infarcts compared to the vehicle-treated control group from Protocol I (Figure 6; infarct sizes as a percentage of the area at risk were: CPX, 12.9 ± 2.6%; BG 9719, 20.8 ± 4.5%; BG 9928, 12.6 ± 3.0%). The relationship between transmural collateral blood flow and infarct size was similarly shifted downward in the two groups of dogs given CPX or BG 9928 (Figure 6b). None of the three antagonists influenced the area at risk size (control, 32.5 ± 1.5%; CPX, 31.4 ± 1.2%; BG 9719, 33.6 ± 2.4%; BG 9928, 36.9 ± 1.9%), systemic hemodynamics (Table 6) or regional myocardial blood flow (Tables 3 and 4).

Discussion

This study demonstrates that three different AR antagonists, with preferential affinity for the A_1AR, did not exacerbate the development of irreversible tissue injury induced by a prolonged coronary occlusion and reperfusion in an anesthetized dog model at a dose that produces maximal natriuresis/diuresis. Indeed, we found that two of the three AR antagonists that we studied, CPX and BG 9928, provided a cardioprotective effect and resulted in reductions in infarct size of ~40-50%. The protective effects of CPX and BG 9928 were evident if the drugs were administered prior to and during the ischemic period or if they were administered just prior to and during the reperfusion period, suggesting that they may act at least partially by reducing reperfusion-mediated injury. In a clinically relevant model of IPC produced by multiple ischemia/reperfusion cycles, the present study also demonstrates that blockade of ARs does not interfere the infarct size-sparing actions of IPC.

AR Antagonists During Ischemia/Reperfusion Injury

Adenosine accumulation in the ischemic myocardium is generally considered to serve a protective role by delaying the development of ischemic injury via multiple receptor and non-receptor mechanisms (Ely and Berne, 1992). Receptor-mediated mechanisms are manifest by coronary dilation which increases oxygen supply (A_{2A}ARs) and by multiple effects which decrease oxygen demand (A_1AR) including negative inotropism, chronotropism, and dromotropism (Ely and Berne, 1992). Via actions on A_1ARs and potentially A_3ARs expressed in cardiomyocytes, adenosine also provides direct cardioprotection likely to be mediated by ATP-sensitive potassium (K_{ATP})

channels. Finally, adenosine also reduces inflammation and reperfusion-mediated injury via actions on A_{2A}ARs expressed in neutrophils and other inflammatory cells (Vinten-Johansen et al., 1999). Non-receptor mediated actions of adenosine involve serving as a substrate for purine salvage to restore energy supply during reperfusion (Ely and Berne, 1992).

Based on these important actions of adenosine, it has been postulated that AR antagonism would have negative effects during acute ischemia/reperfusion injury. However, previous studies are conflicting, and have reported that AR antagonists increase, decrease, or have no effect on infarct size (Auchampach and Gross, 1993; Thornton et al., 1993; Zhao et al., 1994; Haessler et al., 1996; Neely et al., 1996; Todd et al., 1996; Auchampach et al., 1997b; Kitakaze et al., 1997; Domenech et al., 1998; Forman et al., 2000). How can these discrepant data be reconciled? Forman and colleagues (Forman et al., 2000) presented the theory that the differences may be related to the selectivity of the antagonist used in the various studies. These investigators (Forman et al., 2000) suggested that antagonists with preferential affinity for the A_{2A}AR may increase infarct size by reducing coronary blood flow and/or by inhibiting the numerous anti-inflammatory actions of adenosine mediated by A_{2A}ARs. On the other hand, since A_1ARs on neutrophils promote chemotaxis, it was suggested that antagonists with preferential affinity for the A_1AR may reduce neutrophil-mediated reperfusion injury resulting in a reduction in infarct size. Thus, even though adenosine may mediate several salutary actions during ischemia, it was suggested that it may also exert deleterious actions during reperfusion such that selective blockade of specific AR subtypes could result in effective reduction in infarct size.

The results of the present investigation are in general agreement with this theory proposed by Forman and colleagues (Forman et al., 2000). However, our results suggest that AR subtypes other than the A_1AR may be involved. This conclusion is based on our observation that only two of the three AR antagonists that we tested (CPX and BG 9928, but not BG 9719) effectively reduced infarct size, even though all three of the drugs were administered at a dose that efficiently blocked the A_1AR. We speculate, therefore, that CPX and BG 9928 reduced infarct size by blocking the A_{2B}AR rather than the A_1AR. This hypothesis is based on two pieces of evidence. First, we found in our radioligand binding studies that CPX, BG 9719, BG 9928 as well as DPSPX have relatively high affinity for the canine A_{2B}AR (Table 1). Second, we demonstrated that the dose of CPX and BG 9928 used in our *in vivo* studies was likely sufficient to antagonize A_{2B}ARs. This latter conclusion is based on our observation that both CPX and BG 9928 (but not BG 9719) antagonized CGS 21680-mediated coronary vasodilation, implying that the drugs were administered at a dose sufficient to block A_{2A}ARs and, by corollary, A_{2B}ARs (since the antagonists have higher affinity for A_{2B}ARs vs. A_{2A}ARs). The lack of effect of BG 9719 to block A_{2B}ARs at the dose utilized in our investigation may explain its ineffectiveness at reducing infarct size (although our theory suggests that higher doses of BG 9719 would be effective). Additional mechanistic studies with selective A_{2B}AR antagonists, once developed and made readily available, are necessary to test this hypothesis. Although we predict that CPX and BG 9928 reduced infarct size by blocking the A_{2B}AR, we must also consider other potential mechanisms. For instance, CPX and BG 9928 may have been effective by blocking A_3ARs. It is also possible that CPX and BG 9928 were effective by mechanisms

unrelated to AR blockade, such as inhibition of intracellular phosphodiesterases responsible for degrading cAMP. Finally, we also cannot discount the possibility that the dose of BG 9719 was too low and that CPX and BG 9928 reduced infarct size via efficient blockade of A_1ARs.

AR Antagonists and Ischemic Preconditioning (IPC)

The elucidation of the mechanisms of IPC has uncovered a new physiological role of adenosine. IPC is the phenomenon whereby brief periods of ischemia induce adaptive responses that increase the tolerance of the heart to subsequent ischemic episodes. There are two phases of IPC: an early phase that provides immediate protection (classical or "early" IPC) and lasts approximately 1-2 h and a second phase (second window of protection or "late" IPC) that develops 12-24 h later and lasts for days. The early phase involves acute changes in metabolism due to post-translational modification of protective proteins (Nakano et al., 2000). The second phase involves increased synthesis of cardioprotective proteins (Bolli, 2000). Both the early and late phases of IPC can be triggered by adenosine produced during the preconditioning ischemia since pre-treatment with AR antagonists blocks the induction of both early and late IPC (Vinten-Johansen et al., 1999). Thus, studies of IPC have demonstrated that adenosine not only provides acute cardioprotection, but that it also induces a sustained ischemia-tolerant phenotype.

In addition to adenosine, subsequent work has shown that other mediators generated during ischemia are also capable of inducing the early phase of IPC including bradykinin and opioid peptides (Nakano et al., 2000). Since all three of these receptor

systems couple to similar intracellular signaling pathways (i.e., protein kinases, ATP-sensitive potassium channels), it has been proposed (Nakano et al., 2000) that a threshold level of stimulation is necessary to precondition the myocardium. That is, the combined actions of adenosine, bradykinin, opioid peptides, and potentially other mediators released during ischemia stimulate kinase signaling pathways to a threshold level that results in the cardioprotective phenotype of IPC. This additive theory of IPC (Nakano et al., 2000) predicts that IPC induced by a mild stimulus (i.e., a single occlusion/reperfusion cycle) can be inhibited by blocking a single endogenous mediator of IPC, presumably by preventing threshold activation of cardioprotective signaling mechanisms, whereas IPC induced by a more robust stimulus (i.e., multiple ischemia/reperfusion cycles) cannot be blocked efficiently since other mediators are generated in sufficient amounts to attain threshold. This hypothesis has recently been tested directly in an *in vivo* rabbit model of infarction (Goto et al., 1995; Miki et al., 1998). In this model (Goto et al., 1995; Miki et al., 1998), blockade of opioid receptors or bradykinin receptors efficiently blocked IPC induced by a single 5-min occlusion period. However, pharmacological blockade of either type of receptor was not capable of blocking IPC induced by multiple ischemia/reperfusion cycles (Goto et al., 1995; Miki et al., 1998).

The present study provides additional support for this theory. We observed that pre-treating dogs with three potent A_1AR antagonists did not block the reduction in infarct size provided by IPC induced by multiple ischemia/reperfusion cycles. In fact, CPX and BG 9928 appeared to provide additional cardioprotection in preconditioned dogs (Figure 5). In contrast, in a previous study (Auchampach and Gross, 1993) we observed that

pre-treating dogs with a single dose of CPX completely blocked the reduction in infarct size provided by IPC induced by a single 5-min coronary occlusion. In this study, it is important to note that CPX was administered as a single bolus dose prior to the IPC stimulus (Auchampach and Gross, 1993) and was not administered during reperfusion. Collectively, the results of the present study as well as those from previous work in our laboratory (Auchampach and Gross, 1993) demonstrate that the early phase of IPC in the dog is not mediated solely by adenosine. Rather, it appears likely that multiple mediators are involved that act in concert with adenosine.

Conclusions

The present investigation unveiled several new aspects of adenosine biology during ischemia/reperfusion injury. Our results demonstrate that A_1AR blockade is not detrimental in this experimental model and does not block IPC induced by multiple ischemia/reperfusion cycles. Although caution is certainly necessary to extrapolate our findings in a dog model to humans, especially since it has been suggested that A_1AR-mediated responses in the dog may differ from that of humans (Belloni et al., 1989; Martin, 1992), our data provide evidence that the use of A_1AR antagonists as diuretics in patients with ischemic heart disease may not pose a problem. Dose-relationship studies in additional models will be necessary to address this issue further.

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Footnotes

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Legends for Figures

Figure 1: Chemical structures of A_1AR antagonists.

Figure 2: Schematic illustration of the three experimental protocols.

Figure 3. Change in left anterior descending coronary artery conductance in response to 100 μl bolus injections of CGS 21680 in vehicle-treated control dogs and in dogs pre-treated with 1 mg/kg followed by and infusion of 10 μg/kg/min of CPX **(Panel A)**, BG 9719 **(Panel B)**, or BG 9928 **(Panel C)**.

Figure 4. Myocardial infarct size data from Protocol I (Antagonist Pre-treatment). **Panel A.** Infarct size expressed as a percentage of the area at risk. **Panel B.** Plot of infarct size expressed as a percentage of the area at risk and transmural collateral blood flow measured 30 min after coronary occlusion. The data was fitted by linear regression analysis: Control, $y = -95.5x + 28.4$, $r^2 = 0.47$; CPX, $y = -89.1x + 19.3$, $r^2 = 0.68$; BG 9719, $y = -22.0x + 20.7$, $r^2 = 0.27$; BG 9928, $y = -111.5x + 21.5$, $r^2 = 0.44$. *P < 0.05 versus the vehicle control group.

Figure 5. Myocardial infarct size data from Protocol II (Ischemic Preconditioning). **Panel A.** Infarct size expressed as a percentage of the area at risk. **Panel B.** Plot of infarct size expressed as a percentage of the area at risk and transmural collateral blood flow measured 30 min after coronary occlusion. Control, $y = -95.5x + 28.4$, $r^2 = 0.47$; Precond, $y = -94.7x + 15.4$, $r^2 = 0.313$; Precond + CPX, $y = -103.4x + 9.9$, $r^2 =$

0.63; Precond + BG 9719, y = -86.4x + 15.2, r^2 = 0.32; Precond + BG 9928, y = -65.3x + 9.2, r^2 = 0.10. *P < 0.05 versus the vehicle control group.

Figure 6. Myocardial infarct size data from Protocol III (Antagonist at Reperfusion). **Panel A.** Infarct size expressed as a percentage of the area at risk. **Panel B.** Plot of infarct size expressed as a percentage of the area at risk and transmural collateral blood flow measured 30 min after coronary occlusion. Control, y = -95.5x + 28.4, r^2 = 0.47; CPX, y = -123.3x + 22.9, r^2 = 0.76; BG 9719, y = -96.6x + 29.7, r^2 = 0.29; y = -106.0x + 21.0, r^2 = 0.36. *P < 0.05 versus the vehicle control group.

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Table 1.

Dissociation constants of antagonist for recombinant canine adenosine receptors determined by radioligand binding analysis.

Compound	A_1	A_{2A}	A_{2B}	A_3
CPX	18.1 ± 4.4 (9, 6, 119)	166 ± 23	115 ± 29	2,160 ± 580
BG 9719	35.8 ± 4.0 (79, 21, 556)	2,830 ± 190	756 ± 90	19,900 ± 710
BG 9928	28.9 ± 4.1 (163, 24, 1,457)	4,720 ± 890	690 ± 115	42,110 ± 6,120
DPSPX	568 ± 118 (1, 1, 2)	710 ± 126	712 ± 175	1,400 ± 550

K_i values (nM ± SEM; n = 3-5) obtained from competition binding experiments with membranes from HEK 293 cells expressing recombinant canine adenosine receptors using [^{3}H]CPX (A_1), [^{125}I]ZM 241385 (A_{2A}), [^{3}H]MRS 1754 (A_{2B}), or [^{125}I]AB-MECA (A_3). Values in parentheses indicate the selectivity ratios of the compounds for the A_1AR versus the A_{2A}, A_{2B} and A_3ARs, respectively.

Table 2.

Hemodynamic variables from Protocol I (Antagonist Pre-treatment).

	baseline	occ30'	occ60'	rep1hr	rep2 hr	rep3hr
Vehicle						
HR (beats/min)	155 ± 3	153 ± 2	154 ± 3	154 ± 3	152 ± 2	152 ± 5
MBP (mmHg)	107 ± 5	105 ± 5	102 ± 5	104 ± 5	110 ± 6	109 ± 6
LVdP/dt (mmHg/sec)	1663 ± 89	1650 ± 121	1813 ± 119	1650 ± 76	1538 ± 87	1513 ± 75
CPX						
HR	150 ± 2	153 ± 4	152 ± 4	150 ± 5	153 ± 5	151 ± 5
MBP	90 ± 4	94 ± 7	98 ± 8	97 ± 5	102 ± 6	105 ± 6
LVdP/dt	1650 ± 106	1481 ± 146	1631 ± 92	1506 ± 77	1538 ± 74	1538 ± 135
BG 9719						
HR	155 ± 2	161 ± 4	159 ± 4	157 ± 5	160 ± 4	161 ± 4
MBP	104 ± 6	109 ± 5	103 ± 5	106 ± 3	114 ± 4	112 ± 5
LVdP/dt	1838 ± 141	1931 ± 125	1819 ± 205	1706 ± 102	1781 ± 125	1725 ± 113
BG 9928						
HR	152 ± 2	150 ± 2	151 ± 4	153 ± 4	153 ± 4	154 ± 4
MBP	87 ± 6	92 ± 5	95 ± 5	87 ± 3	97 ± 5	99 ± 4
LVdP/dt	1518 ± 154	1631 ± 115	1650 ± 136	1463 ± 62	1463 ± 141	1463 ± 84

HR, heart rate; MBP, mean arterial blood pressure; LVdP/dt, maximal left ventricular dP/dt.

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Table 3.

Regional myocardial blood flow data (ml/min/gm) from Protocols I, II, and III in the non-ischemic region (region perfused by the left circumflex coronary artery).

	Protocol I		**Protocol II**		**Protocol III**	
	occ30	rep3hr	occ30	rep3hr	occ30	rep3hr
Vehicle						
epi	0.65 ± 0.06	0.53 ± 0.05	0.66 ± 0.06	0.69 ± 0.10	0.65 ± 0.06	0.53 ± 0.05
mid	0.75 ± 0.09	0.60 ± 0.05	0.62 ± 0.07	0.57 ± 0.09	0.75 ± 0.09	0.60 ± 0.05
endo	0.76 ± 0.09	0.69 ± 0.09	0.61 ± 0.10	0.59 ± 0.11	0.76 ± 0.09	0.69 ± 0.09
trans	0.72 ± 0.07	0.61 ± 0.05	0.63 ± 0.07	0.62 ± 0.05	0.72 ± 0.07	0.61 ± 0.05
CPX						
epi	0.60 ± 0.08	0.66 ± 0.07	0.97 ± 0.20	0.85 ± 0.12	0.69 ± 0.05	0.96 ± 0.12
mid	0.66 ± 0.08	0.64 ± 0.07	0.78 ± 0.12	0.76 ± 0.12	0.67 ± 0.07	0.94 ± 0.12
endo	0.54 ± 0.04	0.61 ± 0.06	0.73 ± 0.22	0.81 ± 0.15	0.71 ± 0.07	1.02 ± 0.12
transmural	0.60 ± 0.06	0.64 ± 0.06	0.83 ± 0.20	0.81 ± 0.13	0.69 ± 0.06	0.97 ± 0.11
BG 9719						
epi	0.70 ± 0.08	0.64 ± 0.09	0.91 ± 0.22	0.83 ± 0.13	0.60 ± 0.08	0.46 ± 0.03
mid	0.77 ± 0.06	0.64 ± 0.07	0.92 ± 0.14	0.87 ± 0.11	0.66 ± 0.06	0.50 ± 0.02
endo	0.77 ± 0.08	0.67 ± 0.08	0.86 ± 0.16	0.88 ± 0.20	0.63 ± 0.06	0.59 ± 0.06
transmural	0.75 ± 0.07	0.65 ± 0.08	0.90 ± 0.13	0.86 ± 0.12	0.63 ± 0.05	0.52 ± 0.03
BG 9928						
epi	0.87 ± 0.08	0.73 ± 0.07	0.48 ± 0.14	0.45 ± 0.06	0.83 ± 0.07	0.84 ± 0.10
mid	0.80 ± 0.07	0.71 ± 0.07	0.49 ± 0.14	0.47 ± 0.12	0.87 ± 0.06	0.89 ± 0.08
endo	0.80 ± 0.11	0.79 ± 0.06	0.51 ± 0.12	0.56 ± 0.14	0.85 ± 0.06	0.88 ± 0.08
transmural	0.82 ± 0.06	0.74 ± 0.06	0.49 ± 0.13	0.50 ± 0.13	0.85 ± 0.05	0.87 ± 0.08

epi, epicardium; mid, midmyocardium; endo, endocardium; trans, transmural

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Table 4.

Regional myocardial blood flow data (ml/min/gm) from Protocols I, II, and III in the ischemic-reperfused region (region perfused by the left anterior descending coronary artery).

	Protocol I		**Protocol II**		**Protocol III**	
	occ30	rep3hr	occ30	rep3hr	occ30	rep3hr
Vehicle						
epi	0.08 ± 0.01	0.47 ± 0.10	0.10 ± 0.04	0.48 ± 0.12	0.08 ± 0.01	0.47 ± 0.10
mid	0.06 ± 0.01	0.50 ± 0.08	0.06 ± 0.02	0.35 ± 0.04	0.06 ± 0.01	0.50 ± 0.08
endo	0.05 ± 0.01	1.01 ± 0.16	0.07 ± 0.02	1.06 ± 0.13	0.05 ± 0.01	1.01 ± 0.16
trans	0.06 ± 0.01	0.66 ± 0.10	0.08 ± 0.02	0.63 ± 0..04	0.06 ± 0.01	0.66 ± 0.10
CPX						
epi	0.15 ± 0.04	0.48 ± 0.06	0.07 ± 0.03	0.62 ± 0.12	0.10 ± 0.01	0.50 ± 0.04
mid	0.08 ± 0.02	0.49 ± 0.04	0.05 ± 0.01	0.54 ± 0.11	0.07 ± 0.01	0.40 ± 0.04
endo	0.05 ± 0.01	0.90 ± 0.16	0.04 ± 0.01	0.68 ± 0.12	0.04 ± 0.01	0.93 ± 0.15
transmural	0.09 ± 0.02	0.62 ± 0.06	0.06 ± 0.02	0.61 ± 0.10	0.07 ± 0.01	0.61 ± 0.05
BG 9719						
epi	0.11 ± 0.03	0.44 ± 0.10	0.14 ± 0.04	0.63 ± 0.12	0.10 ± 0.03	0.31 ± 0.04
mid	0.06 ± 0.02	0.31 ± 0.04	0.08 ± 0.02	0.43 ± 0.04	0.07 ± 0.03	0.33 ± 0.05
endo	0.05 ± 0.01	0.77 ± 0.19	0.06 ± 0.01	0.64 ± 0.10	0.04 ± 0.01	0.72 ± 0.13
transmural	0.09 ± 0.02	0.51 ± 0.10	0.09 ± 0.02	0.56 ± 0.10	0.09 ± 0.03	0.45 ± 0.06
BG 9928						
epi	0.14 ± 0.05	0.48 ± 0.11	0.12 ± 0.04	0.45 ± 0.13	0.10 ± 0.02	0.66 ± 0.12
mid	0.09 ± 0.03	0.39 ± 0.05	0.06 ± 0.01	0.31 ± 0.10	0.08 ± 0.02	0.67 ± 0.15
endo	0.05 ± 0.01	0.73 ± 0.12	0.03 ± 0.01	0.72 ± 0.30	0.05 ± 0.01	1.20 ± 0.15
transmural	0.09 ± 0.03	0.54 ± 0.06	0.07 ± 0.01	0.49 ± 0.14	0.08 ± 0.02	0.84 ± 0.12

epi, epicardium; mid, midmyocardium; endo, endocardium; trans, transmural

Table 5.

Hemodynamic variables from Protocol II (Ischemic Preconditioning).

	baseline	occ30'	occ60'	rep1hr	rep2 hr	rep3hr
Vehicle						
HR (beats/min)	155 ± 4	153 ± 4	152 ± 4	144 ± 3	144 ± 3	146 ± 2
MBP (mmHg)	103 ± 6	101 ± 6	104 ± 6	107 ± 6	108 ± 4	106 ± 5
LVdP/dt (mmHg/sec)	1606 ± 196	1625 ± 142	1550 ± 124	1394 ± 94	1356 ± 75	1281 ± 60
CPX						
HR	151 ± 1	150 ± 3	148 ± 3	150 ± 5	151 ± 4	152 ± 4
MBP	87 ± 6	88 ± 4	96 ± 8	91 ± 5	100 ± 5	100 ± 6
LVdP/dt	1369 ± 140	1294 ± 130	1388 ± 113	1181 ± 82	1256 ± 89	1313 ± 105
BG 9719						
HR	156 ± 3	152 ± 4	152 ± 5	155 ± 7	156 ± 6	156 ± 6
MBP	105 ± 7	103 ± 5	103 ± 5	97 ± 6	99 ± 6	101 ± 5
LVdP/dt	1693 ± 121	1671 ± 111	1736 ± 130	1500 ± 164	1457 ± 153	1479 ± 155
BG 9928						
HR	149 ± 1	149 ± 2	150 ± 1	149 ± 1	148 ± 1	148 ± 1
MBP	86 ± 2	84 ± 3	84 ± 3	80 ± 5	87 ± 5	86 ± 3
LVdP/dt	1300 ± 50	1400 ± 74	1375 ± 72	1100 ± 50	1125 ± 64	1175 ± 72

HR, heart rate; MBP, mean arterial blood pressure; maximal LVdP/dt, left ventricular dP/dt.

Table 6.

Hemodynamic variables from Protocol III (Antagonist at Reperfusion).

	baseline	occ30'	occ60'	rep1hr	rep2hr	rep3hr
Vehicle						
HR (beats/min)	155 ± 3	153 ± 2	154 ± 3	154 ± 3	152 ± 2	152 ± 5
MBP (mmHg)	107 ± 5	105 ± 5	102 ± 5	104 ± 5	110 ± 6	109 ± 6
LVdP/dt (mmHg/sec)	1663 ± 89	1650 ± 121	1813 ± 119	1650 ± 76	1538 ± 87	1513 ± 75
CPX						
HR	150 ± 2	149 ± 1	151 ± 1	152 ± 3	151 ± 4	156 ± 4
MBP	102 ± 4	99 ± 7	105 ± 6	108 ± 5	112 ± 4	114 ± 4
LVdP/dt	1556 ± 85	1531 ± 159	1688 ± 105	1688 ± 97	1650 ± 57	1631 ± 72
BG 9719						
HR	150 ± 3	154 ± 3	153 ± 4	154 ± 5	155 ± 6	151 ± 4
MBP	102 ± 5	95 ± 7	101 ± 5	101 ± 3	103 ± 3	97 ± 5
LVdP/dt	1519 ± 125	1400 ± 149	1569 ± 165	1500 ± 102	1425 ± 85	1350 ± 90
BG 9928						
HR	151 ± 1	151 ± 3	150 ± 2	147 ± 2	148 ± 2	150 ± 3
MBP	90 ± 6	90 ± 5	96 ± 4	88 ± 5	92 ± 5	95 ± 4
LVdP/dt	1594 ± 106	1638 ± 132	1744 ± 69	1406 ± 49	1463 ± 74	1463 ± 79

HR, heart rate; MBP, mean arterial blood pressure; maximal LVdP/dt, left ventricular dP/dt.

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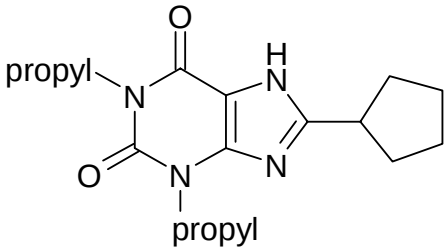
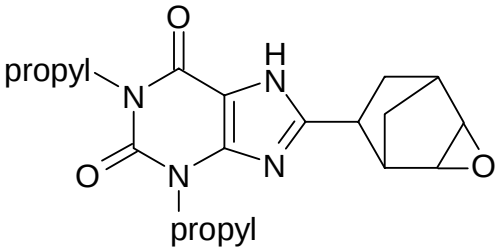


CPX



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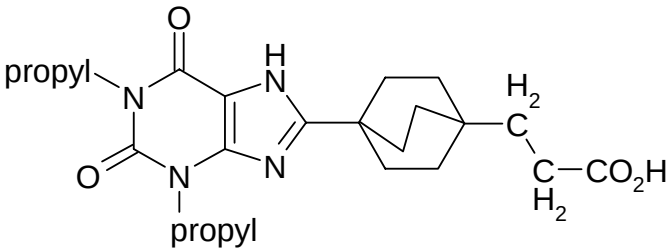


BG 9928

Figure 1

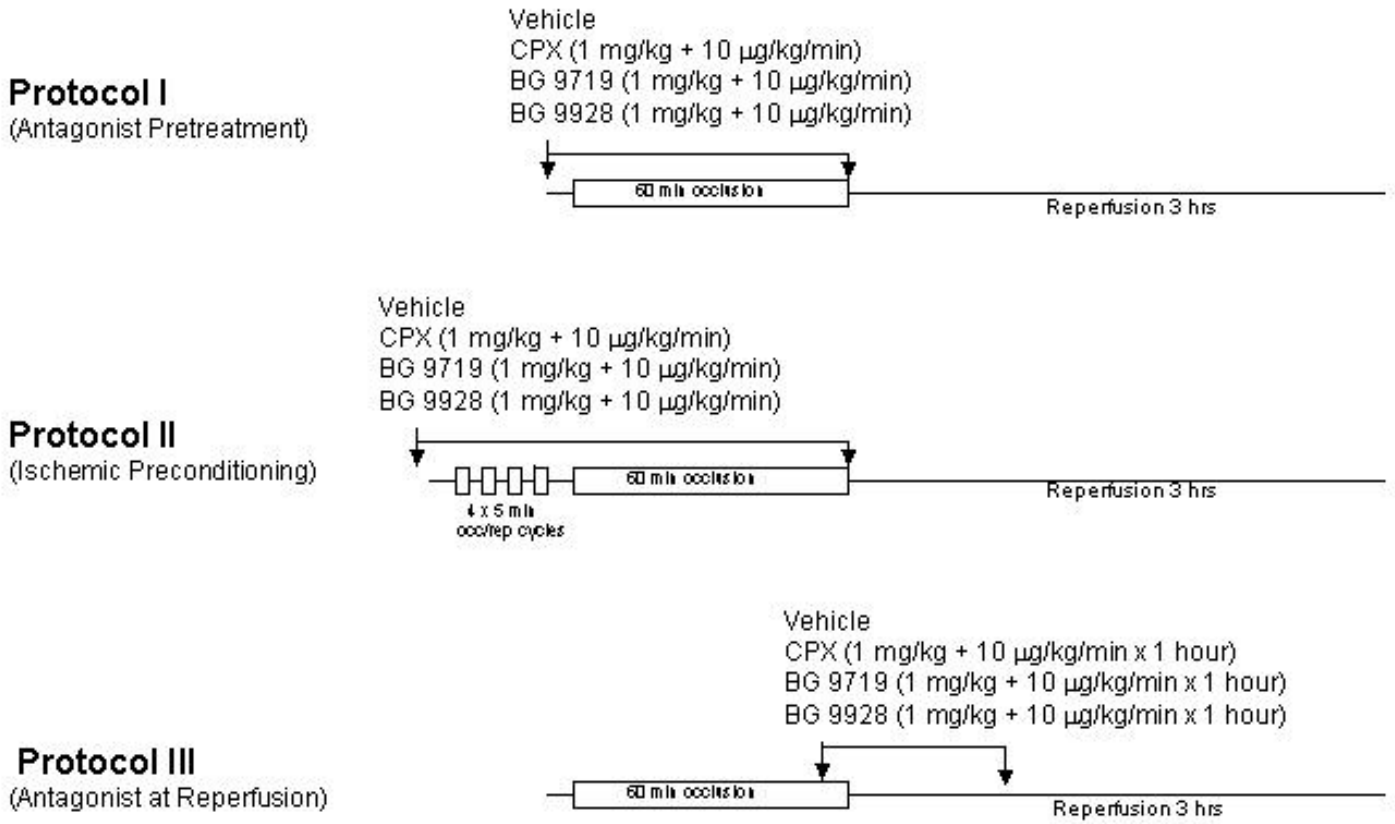
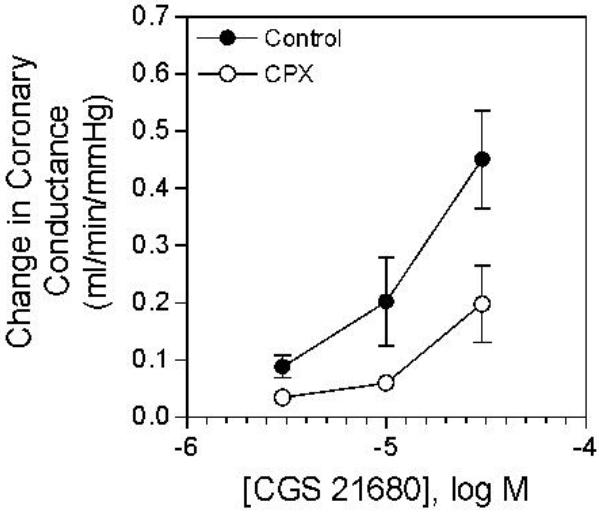
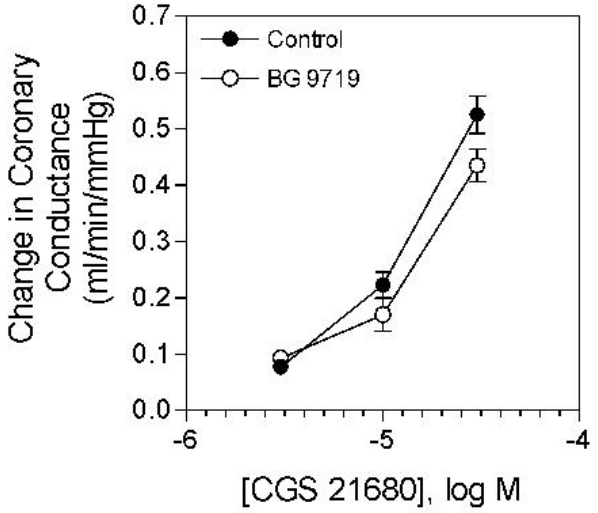


Figure 2


Control
CPX
Change in Coronary Conductance (ml/min/mmHg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-6
-5
-4
[CGS 21680], log M


Control
BG 9719
Change in Coronary Conductance (ml/min/mmHg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-6
-5
-4
[CGS 21680], log M

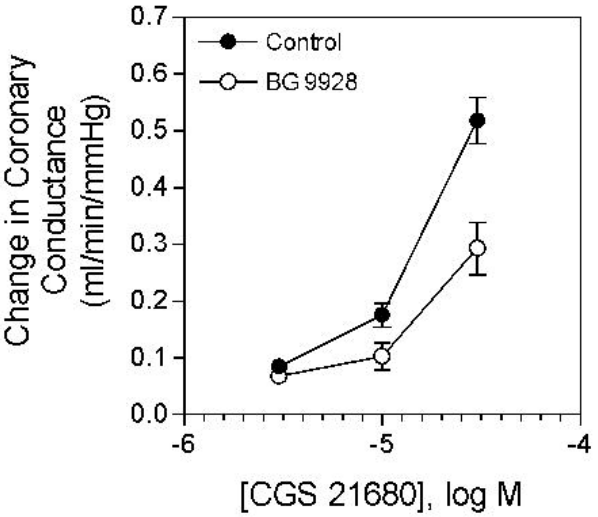

Control
BG 9928
Change in Coronary Conductance (ml/min/mmHg)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-6
-5
-4
[CGS 21680], log M

Figure 3

Protocol I
(Antagonist Pre-treatment)

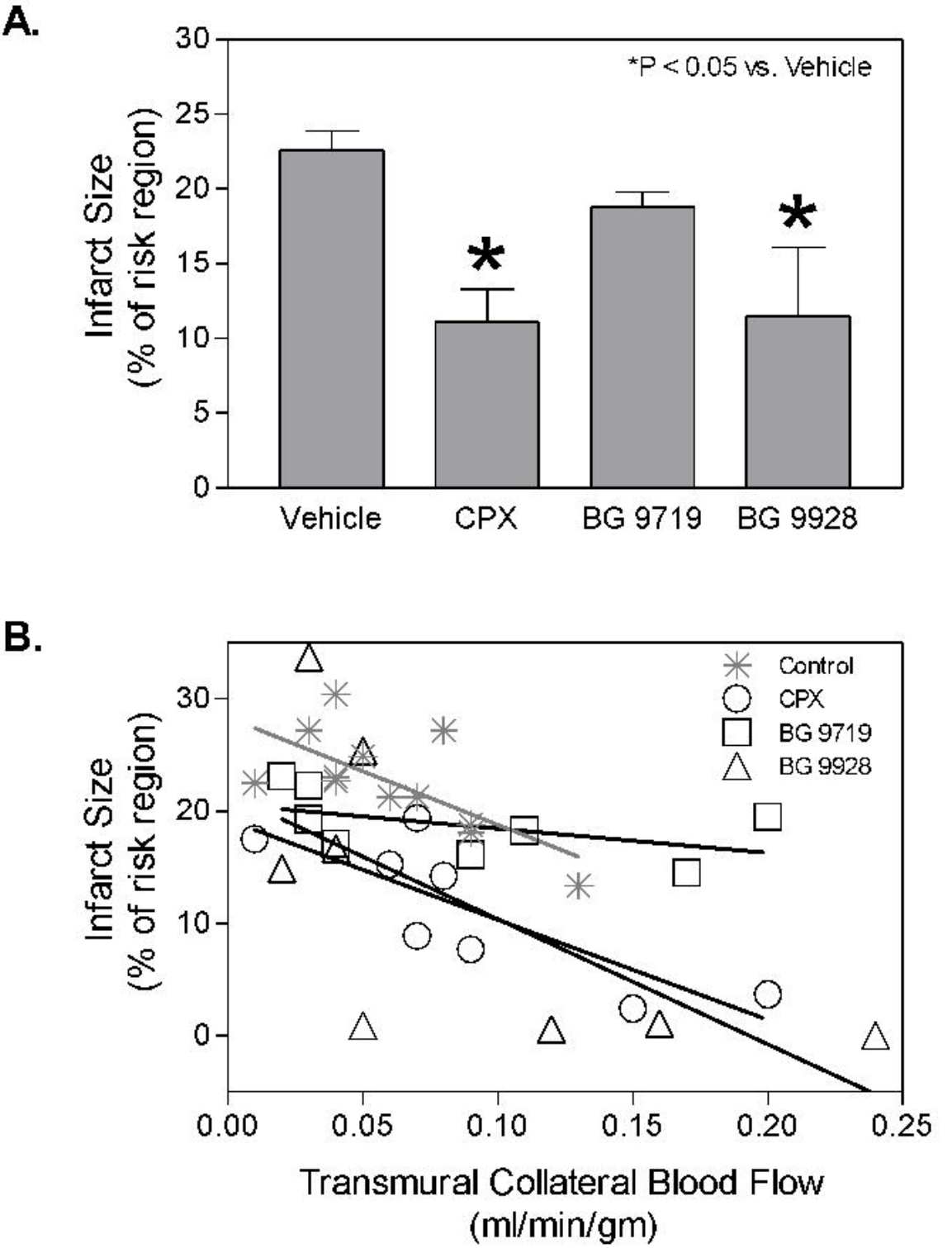


Figure 4

Protocol II (Ischemic Preconditioning)

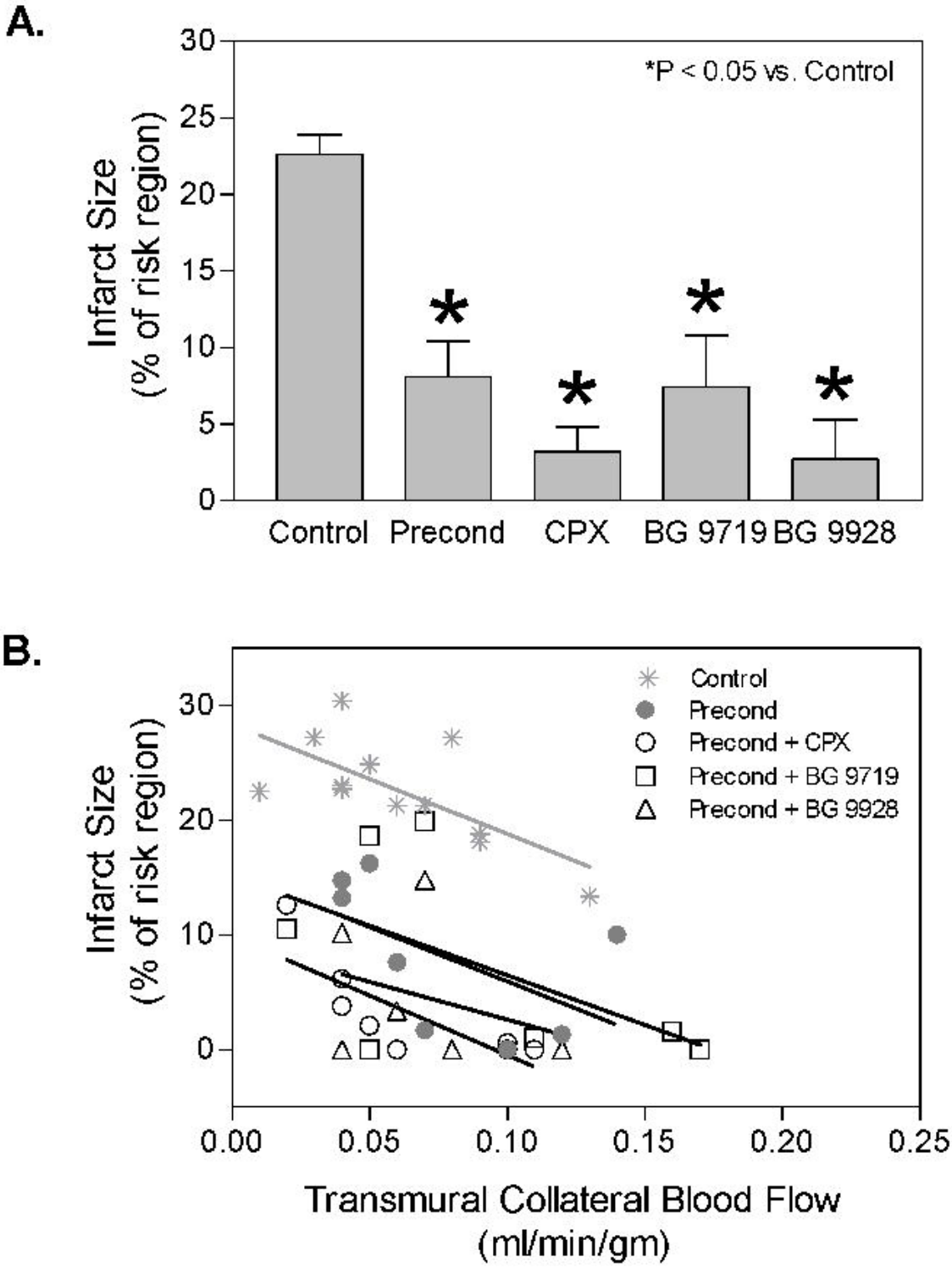


Figure 5

Protocol III
(Antagonist at Reperfusion)

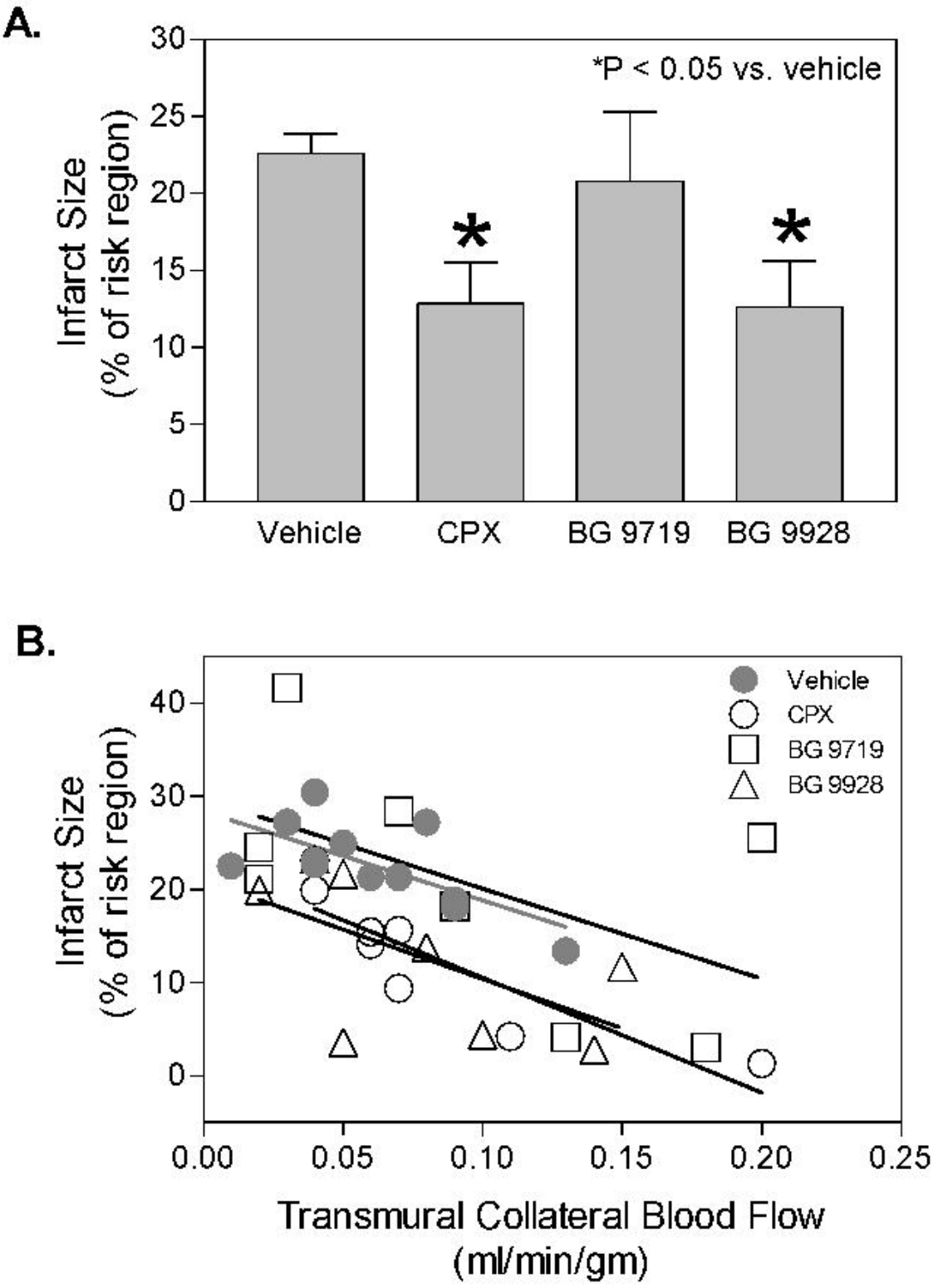


Figure 6