A3 adenosine receptor agonist IB-MECA reduces myocardial ischemia-reperfusion injury in dogs

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Auchampach, John A., Zhe-Dong Ge, Tina C. Wan, Jeannine Moore, and Garrett J. Gross. A3 adenosine receptor agonist IB-MECA reduces myocardial ischemia-reperfusion injury in dogs. Am J Physiol Heart Circ Physiol 285: H607–H613, 2003. First published April 10, 2003; 10.1152/ajpheart.01001.2002.—We examined the effect of the A3 adenosine receptor (AR) agonist IB-MECA on infarct size in an open-chest anesthetized dog model of myocardial ischemia-reperfusion injury. Dogs were subjected to 60 min of left anterior descending (LAD) coronary artery occlusion and 3 h of reperfusion. Infarct size and regional myocardial blood flow were assessed by macrohistochemical staining with triphenyltetrazolium chloride and radioactive microspheres, respectively. Four experimental groups were studied: vehicle control (50% DMSO in normal saline), IB-MECA (100 μg/kg iv bolus) given 10 min before the coronary occlusion, IB-MECA (100 μg/kg iv bolus) given 5 min before initiation of reperfusion, and IB-MECA (100 μg/kg iv bolus) given 10 min before coronary occlusion in dogs pretreated 15 min earlier with the ATP-dependent potassium channel antagonist glibenclamide (0.3 mg/kg iv bolus). Administration of IB-MECA had no effect on any hemodynamic parameter measured including heart rate, first derivative of left ventricular pressure, aortic pressure, LAD coronary blood flow, or coronary collateral blood flow. Nevertheless, pretreatment with IB-MECA before coronary occlusion produced a marked reduction in infarct size (~40% reduction) compared with the control group (13.0 ± 3.2% vs. 25.2 ± 3.7% of the area at risk, respectively). This effect of IB-MECA was blocked completely in dogs pretreated with glibenclamide. An equivalent reduction in infarct size was observed when IB-MECA was administered immediately before reperfusion (13.1 ± 3.9%). These results are the first to demonstrate efficacy of an A3AR agonist in a large animal model of myocardial infarction by mechanisms that are unrelated to changes in hemodynamic parameters and coronary blood flow. These data also demonstrate in an in vivo model that IB-MECA is effective as a cardioprotective agent when administered at the time of reperfusion.

THE PHYSIOLOGICAL ACTIONS of adenosine are mediated by four adenosine receptor (AR) subtypes, designated A1, A2A, A2B, and A3 (1, 12). In the cardiovascular system, A1ARs are classically known to be the receptor subtype expressed in cardiac myocytes responsible for the brady- and antiadrenergic actions of adenosine, whereas A2ARs are best known to be expressed in vascular smooth muscle cells, where they produce vasodilation (1). The A3AR is the most recently identified AR subtype (24). Although A3AR message is expressed in many different tissues, including the heart, its physiologic functions remain unknown (24). A3AR agonists such as N6-(4-iodobenzyl)-adenosine-5′-N′-methylcarboxamide (IB-MECA) or N6-(3-chloro-benzyl)-adenosine-5′-N′-methylcarboxamide (CB-MECA) and the 2-chloro derivative 2-CI-IB-MECA have been shown to be effective cardioprotective agents in numerous in vitro and in vivo rodent models of ischemia-reperfusion injury (5, 9, 18, 19, 21, 22, 26, 33, 39–44). However, no studies have been performed with A3AR agonists in large animal models that have variable levels of collateral blood flow similar to that found in humans with coronary artery disease. The present study examined the effect of IB-MECA in an open-chest, anesthetized dog model of infarction.

METHODS

Radioligand binding analysis. Radioligand binding studies were performed with membranes prepared from HEK-293 cells expressing canine A1ARs or A3ARs using N6-(4-amino-3-[125I]iodobenzyl)adenosine-5′-N′-methylcarboxamide ([125I]AB-MECA) as the radioligand (3, 6, 41). The full-length canine A1AR and A3AR were subcloned into the mammalian expression vector pCDNA3.1, transfected into HEK-293 cells using lipofectamine, and then selected with 2 mg/ml G418. After antibiotic selection, the cells were maintained in DMEM cell culture media containing 10% fetal bovine serum with 0.6 mg/ml G418. Cell membranes were prepared and then incubated with [125I]AB-MECA in the presence of inhibitors (3, 6, 41). The binding data were analyzed, as described previously (3, 6).

Anesthetized dog model. A standard barbital-anesthetized dog model was employed, as described previously in detail (2, 4). All dogs were subjected to 60 min of left anterior descending (LAD) coronary artery occlusion and 3 h of reperfusion. Dogs were randomly assigned to one of four experimental groups: vehicle (1 ml solution of 50% DMSO in normal saline) given 10 min before coronary occlusion, IB-MECA (100 μg/kg iv bolus) given 10 min before coronary occlusion, IB-MECA (100 μg/kg iv bolus) given 5 min before reperfusion, and IB-MECA (100 μg/kg iv bolus) given 10 min before coronary occlusion.
occlusion with pretreatment 15 min earlier with the ATP-dependent potassium (K_{ATP}) channel antagonist glibenclamide (0.3 mg/kg iv bolus). Glibenclamide was administered at a dose that we have shown previously to have no effect on infarct size in our dog model (16). In all of the groups, hemodynamic measurements and arterial blood gases were obtained before occlusion, at 30 min of the 60-min occlusion period, and every hour after reperfusion. Regional myocardial blood flow was measured at 30 min during the 60-min occlusion period and at the end of the experiment. Throughout the ischemia-reperfusion experiments, heart rate was maintained at 150 beats/min by left atrial pacing. All procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin and complied with the procedures established by National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After 3 h of reperfusion, the anatomic area at risk (AAR) and the nonischemic area were demarcated by staining with Evan’s blue dye (2, 4). The hearts were electrically fibrillated, removed, and prepared for infarct size determination (using triphenyltetrazolium chloride) and regional myocardial blood flow measurements (2, 4). Infarcted and noninfarcted tissues within the AAR were separated and determined gravimetrically. Regional myocardial blood flow was measured by the radioactive microsphere technique (2, 4). Dogs were excluded from the study if subendocardial collateral blood flow was >0.15 ml·min^{-1}·g^{-1} or if more than three consecutive attempts were required to convert ventricular fibrillation with low-energy direct current pulses.

Statistical analyses. All values are expressed as means ± SE. Hemodynamic variables were analyzed by 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. Infarct sizes and risk region sizes were compared using a one-way ANOVA, followed by Student’s t-test with the Bonferroni correction.

RESULTS

Pharmacology of IB-MECA. On the basis of the radioligand binding analysis with recombinant canine receptors, IB-MECA was found to be ~50-fold selective at binding to the high-affinity form of the A1AR versus the high-affinity form of the A3AR (Fig. 1). The dissociation constants were calculated to be 0.67 ± 0.09 and 33.8 ± 2.97 nM for the A3AR and A1AR, respectively.

In preliminary studies, we examined the actions of IB-MECA on systemic hemodynamic parameters including heart rate, mean arterial blood pressure, left ventricular (LV) pressure, first derivative of LV pressure (LV dP/dt), and LAD coronary artery blood flow in un-paced dogs. Bolus administration of IB-MECA (100 µg/kg) had no effect on any systemic hemodynamic parameter measured for at least 30 min (Fig. 2). In contrast, an equivalent dose of the A1AR agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA) caused short-lived decreases in heart rate, LV systolic pressure, LV dP/dt, and mean arterial blood pressure. CCPA also produced a marked increase in coronary blood flow.

Ischemia-reperfusion data. A total of 33 dogs was initially included in the infarction study. One dog was excluded from the control group because of intractable ventricular fibrillation, and one dog was excluded from the group given IB-MECA at reperfusion because of high collateral blood flow. Thus a total of 31 dogs was included in the data analysis.

Humodynamic and regional myocardial blood flow data are shown in Tables 1 and 2. There were no significant differences within or between groups throughout the experiment with regard to heart rate, mean arterial blood pressure, LV dP/dt, the rate-pressure product, or regional myocardial blood flow in either the nonischemic or ischemic regions, with the exception of a significant increase in mean arterial blood pressure at 3 h of reperfusion in the group of dogs treated with IB-MECA before occlusion. There were also no significant differences in blood pH, PO₂, or PCO₂ within and between groups at any of the times studied (data not shown).

Figure 3 summarizes the effect of IB-MECA on infarct size. Myocardial infarct size expressed as a percentage of the AAR was reduced significantly (~40%, P < 0.05) in the two groups of dogs treated with IB-MECA (control = 25.2 ± 3.7%, IB-MECA before occlusion = 13.0 ± 3.2%, and IB-MECA during reperfusion = 13.1 ± 3.9%). Myocardial infarct size expressed as a percentage of the entire LV was also reduced significantly by IB-MECA (8.1 ± 1.4%, 4.4 ± 1.4%, and 4.1 ± 1.2%, respectively.) Remarkably, the reduction in infarct size was equivalent in magnitude in the group of dogs treated with IB-MECA immediately before reperfusion compared with the pretreated group. Pretreatment with IB-MECA did not reduce infarct size in the group of dogs pretreated with glibenclamide (infarct size as a percentage of the AAR = 20.6 ± 5.6% and as a percentage of the LV = 7.0 ± 2.0%). Among the treatment groups, there were no significant differences with respect to the LV weight (data not shown). Importantly, there were also no differences among the groups with respect to the size of the AAR (control = 32.0 ± 3.9%, IB-MECA before...
Figure 3, B and C, illustrates the relationship between infarct size and transmural collateral blood flow measured at 30 min into the occlusion period in the four experimental groups. In control dogs, there was an inverse relationship between infarct size and collateral blood flow. This relationship was shifted downward in both of the IB-MECA-treated groups, indicating that the reduction in infarct size produced by IB-MECA occurred independently of changes in collateral blood flow. The relationship between infarct size and collateral flow was not shifted by IB-MECA in the group of dogs pretreated with glibenclamide.

DISCUSSION

Adenosinergic therapy is one of the most promising pharmacological treatments for acute ischemia-reperfusion injury (28, 46). Clinically, adenosine has been shown to be effective when used in cardioplegic solutions (30), when coadministered with thrombolytic agents (28) or when administered before coronary angioplasty (23). The clinical usefulness of adenosine, however, is limited by its hemodynamic, respiratory, and central nervous system side effects in addition to its low potency and short half-life. Many of these problems could potentially be avoided by administering synthetic adenosine receptor ligands that are more selective for the individual adenosine receptor subtypes. In experimental animal models, agonists with a high affinity for the A2AAR including CGS-21680 (20) and AMP-579 (29, 38) have been shown to be effective at reducing infarct size when administered during reperfusion by reducing neutrophil-mediated injury and inflammation (8, 32, 48). A1AR agonists are highly cardioprotective when administered before and during ischemia by producing favorable metabolic effects resulting in preservation of high-energy phosphates (46).

We have recently focused on testing the efficacy of A3AR agonists in experimental models of ischemia-reperfusion injury (5, 21, 22, 41). The A3AR is the most recently identified subtype of adenosine receptor that is coupled to Gi/o inhibitory proteins similar to the A1AR (24). With the use of a chronically instrumented conscious rabbit model, we (5, 21) observed that pretreatment with IB-MECA (100 µg/kg) produced a significant protective effect against both reversible (myocardial “stunning”) and irreversible injury (infarction).

In these studies, IB-MECA was effective when administered intravenously at a dose that had no effect on heart rate or systemic blood pressure (5, 21). The protective effects of IB-MECA were blocked by the nonspecific adenosine receptor antagonist 8-sulfo-phenytheophylline but not by the A1AR antagonist N-0861, implying the involvement of the A3AR (5, 21).
H610 EFFECT OF IB-MECA ON INFARCT SIZE IN DOGS

Table 1. Hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>MABP, mmHg</th>
<th>LV dP/dt, mmHg·s⁻¹·min⁻¹</th>
<th>RPP, (HR × SBP/1,000), mmHg·beats⁻¹·min⁻¹</th>
</tr>
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<tbody>
<tr>
<td>Preocclusion</td>
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<tr>
<td>Control</td>
<td>155 ± 5</td>
<td>111 ± 6</td>
<td>1,780 ± 125</td>
<td>19 ± 2</td>
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<tr>
<td>IB-MECA</td>
<td>152 ± 2</td>
<td>114 ± 6</td>
<td>1,590 ± 120</td>
<td>19 ± 1</td>
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<tr>
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<td>116 ± 8</td>
<td>1,760 ± 115</td>
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<tr>
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<td>18 ± 2</td>
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<tr>
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<td>116 ± 4</td>
<td>1,910 ± 105</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>IB-MECA Occ</td>
<td>153 ± 2</td>
<td>109 ± 9</td>
<td>1,690 ± 190</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>IB-MECA Occ-Glib</td>
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<td>104 ± 15</td>
<td>1,830 ± 90</td>
<td>18 ± 3</td>
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<tr>
<td>3-h Reperfusion</td>
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</tr>
<tr>
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<td>109 ± 2</td>
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<td>IB-MECA Rep</td>
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<td>104 ± 8</td>
<td>1,690 ± 120</td>
<td>18 ± 2</td>
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<tr>
<td>IB-MECA Occ</td>
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<td>104 ± 15</td>
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<td>113 ± 7</td>
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<td>IB-MECA Rep</td>
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<td>121 ± 9</td>
<td>1,656 ± 90</td>
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<td>IB-MECA Occ</td>
<td>153 ± 2</td>
<td>109 ± 9</td>
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<td>17 ± 2</td>
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<tr>
<td>IB-MECA Occ-Glib</td>
<td>160 ± 5</td>
<td>101 ± 12</td>
<td>1,670 ± 90</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = 8 control dogs, 8 dogs given IB-MECA 10 min before coronary occlusion (Oc), 8 dogs given IB-MECA 5 min before reperfusion (Rep), and 7 dogs given IB-MECA 10 min before occlusion treated 10 min earlier with glybenclamide (Oc-Glib); HR, heart rate; ABP, mean arterial blood pressure; LV dP/dt, first derivative of left ventricular pressure; RPP, rate-pressure product; SBP, systolic blood pressure. *P < 0.05 vs. control.

These results provided evidence that effective cardioprotection can be achieved after systemic administration of an A3AR agonist without causing unfavorable hemodynamic consequences.

The results of the present investigation using a dog model of ischemia-reperfusion injury extend these previous observations. Similar to our previous studies in the rabbit (5, 21), we found that pretreatment with IB-MECA effectively reduced infarct size from ~25% of the AAR in control dogs to ~13% in dogs pretreated with 100 µg/kg of IB-MECA (Fig. 3), a dose also found to be hemodynamically inactive in the dog (Fig. 2). The novel aspect of the present study using the dog model is that we were able to provide more detailed assessment of IB-MECA on hemodynamics and coronary artery blood flow. Similar analyses have not been possible in previous studies conducted in rodents. At a dose of 100 µg/kg, we found that IB-MECA did not influence blood pressure, LV pressure, LV dP/dt, or the rate-pressure product (Table 1 and Fig. 2). In addition, IB-MECA had no effect on coronary blood flow (Table 2 and Fig. 2). A lack of effect of IB-MECA on hemodynamic parameters was apparent during the ischemia-reperfusion studies as well as in preliminary studies in which the effects of IB-MECA were observed for 30 min in control barbital-anesthetized dogs. The lack of an effect of IB-MECA on coronary blood flow in these studies is an important observation because it rules against the possibility that it may cause coronary "steal," a complication that has been demonstrated to occur with dipyridamole (47). In addition, the lack of hemodynamic effects suggests that IB-MECA was administered at a dose that did not influence other AR subtypes in the heart or vasculature. By comparison, an equivalent dose of the A1AR agonist CCPA reduced heart rate, reduced systemic blood flow, and increased coronary blood flow (Fig. 2). Overall, these results clearly suggest that the protective effects of IB-MECA are not dependent on changes in the oxygen supply-demand balance. Rather, they suggest that it acts via a direct cardioprotective mechanism. Previous studies (9, 18, 19, 26, 33, 39, 40, 42–44) using isolated hearts and isolated cardiomyocytes have suggested that A3ARs are expressed in the myocardium and that they couple to cardioprotective signaling mechanisms similar to those coupled to the A1AR, presumably the K_ATP channel. We predict that pretreatment with IB-MECA provided protection in our dog model by a comparable mechanism. This theory is supported by the observation that the reduction in infarct size provided by pretreating with IB-MECA was blocked completely by glybenclamide. One interesting aspect of the present investigation is that subendocardial blood flow was increased after 3 h of reperfusion in the ischemia-reperfused myocardium in control animals (Table 2). These data indicate that hyperemia developed in the subendocardial region,

Table 2. Regional myocardial blood flow data

<table>
<thead>
<tr>
<th></th>
<th>Epi</th>
<th>Mid</th>
<th>Endo</th>
<th>Nonischemic Region (LCX)</th>
<th>Epi</th>
<th>Mid</th>
<th>Endo</th>
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<tbody>
<tr>
<td>30-min Occlusion</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.09 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.65 ± 0.09</td>
<td>0.78 ± 0.12</td>
<td>0.77 ± 0.12</td>
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</tr>
<tr>
<td>IB-MECA</td>
<td>0.12 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>1.00 ± 0.25</td>
<td>0.92 ± 0.10</td>
<td>0.81 ± 0.07</td>
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<tr>
<td>IB-MECA Rep</td>
<td>0.12 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.71 ± 0.16</td>
<td>0.63 ± 0.06</td>
<td>0.64 ± 0.09</td>
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<tr>
<td>IB-MECA Occ</td>
<td>0.12 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.86 ± 0.19</td>
<td>0.79 ± 0.16</td>
<td>0.75 ± 0.16</td>
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<tr>
<td>IB-MECA Occ-Glib</td>
<td>0.44 ± 0.08</td>
<td>0.50 ± 0.07</td>
<td>1.17 ± 0.20</td>
<td>0.54 ± 0.07</td>
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<tr>
<td>3-h Reperfusion</td>
<td></td>
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<tr>
<td>Control</td>
<td>0.42 ± 0.04</td>
<td>0.39 ± 0.07</td>
<td>1.05 ± 0.16</td>
<td>0.58 ± 0.07</td>
<td>0.85 ± 0.13</td>
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<tr>
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<td>1.17 ± 0.25</td>
<td>0.65 ± 0.11</td>
<td>0.59 ± 0.05</td>
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<tr>
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<td>0.97 ± 0.16</td>
<td>0.78 ± 0.14</td>
<td>0.87 ± 0.15</td>
<td>0.86 ± 0.15</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE (in ml·min⁻¹·g⁻¹); n = 8 control dogs, 8 IB-MECA Occ dogs, 8 IB-MECA Rep dogs, and 7 IB-MECA Occ-Glib dogs. LAD, left anterior descending coronary artery; LCX, left circumflex artery; Epi, epicardium; Mid, midmyocardium; Endo, endocardium.
which we believe is related to the short period of ischemia utilized in our protocol. Importantly, the distribution of blood flow across the myocardium in the ischemia-reperfused region was identical in the two groups of dogs treated with IB-MECA, indicating further that IB-MECA had no effect on blood flow at the dose utilized in our investigation.

The second novel aspect of this work is that we found that IB-MECA was effective when administered at the time of reperfusion. In fact, we found that IB-MECA produced an equivalent reduction in infarct size whether it was administered 10 min before the onset of ischemia or administered 5 min before the release of the occlusion. These results suggest that, in addition to exerting favorable effects during ischemia, IB-MECA produced beneficial actions that attenuated reperfusion injury. We predict that there are two potential mechanisms by which IB-MECA may have reduced reperfusion injury. The first is via an anti-inflammatory mechanism. Jordan and colleagues (20) have previously observed, using an in vitro assay, that nanomolar concentrations of Cl-IB-MECA reduced the adhesion of neutrophils to coronary artery segments via actions on the endothelium rather than on the neutrophils. This effect of Cl-IB-MECA to reduce neutrophil adhesion was attributed to the A3AR, because the A3AR antagonist MRS-1220 (but not A1AR or A2AAR antagonists) antagonized its effects completely (20).

These investigators (20) further demonstrated that treatment with Cl-IB-MECA reduced contractile dysfunction and neutrophil margination in an isolated rabbit heart model of neutrophil-mediated reperfusion injury. These observations, taken together with additional work by other investigators demonstrating that A3AR agonists reduce the expression of proinflammatory cytokines (35, 36) and reduce neutrophil function (7), provide additional support for the idea that IB-MECA reduced infarct size in the present investigation by an anti-inflammatory effect. A second complementary mechanism by which IB-MECA may have reduced infarct size, however, is by inhibiting apoptosis, because the A3AR is known to be capable of coupling to two well-known cell survival-signaling pathways including the phosphatidylinositol 3'-kinase/Akt kinase pathway (14, 37) and the Ras/Raf-1/MEK/ERK 1/2 pathway (37). This potential mechanism is supported by the results of Maddock and colleagues (27), who reported that Cl-IB-MECA reduced apoptosis of isolated rat cardiac myocytes subjected to simulated ischemia when administered during reoxygenation.

IB-MECA was originally shown to be ~50-fold selective for rat A3ARs vs. rat A1ARs and A2AARs (13). With the use of canine adenosine receptors expressed in HEK-293 cells, we have also found in the present investigation that IB-MECA may have reduced infarct size. It should be noted, however, that Linden’s group (31) has recently provided evidence that A3AR agonists including Cl-IB-MECA may bind to A2AARs with higher affinity than originally appreciated. Furthermore, this group has suggested (25) that A2AAR agonists are effective anti-inflammatory/tissue-protective agents at low, nonhypotensive doses due to efficient coupling of A2AARs in immune cells. It remains possible, therefore, that IB-MECA was effective in the present investigation via an anti-inflammatory effect mediated via the A2AAR or via a combined effect mediated through
A2AARs and A3ARs. The large size of the dog precludes the use of selective antagonists to verify the involvement of individual AR subtypes in the present investigation. In preliminary studies (15), however, we observed that CI-IB-MECA does not reduce infarct size in mice lacking A2ARs (A2AR gene “knockout” mice), supporting a role for these agents acting to modulate ischemia-reperfusion injury by interacting with the A3AR.

The effect of A2AR agonists on hemodynamics differs among species. Although we (11, 17, 45) have observed that IB-MECA (and CI-IB-MECA) has no hemodynamic effects in rabbits and dogs, CI-IB-MECA and N\(^6\)-(3-aminophenylethyl)adenosine has been reported to cause a short-lived hypotension without any change in heart rate in rats and mice. The hypertensive actions of A2AR agonists in rodents is likely an effect mediated via the release of vasoactive mediators from mast cells because 1) a concomitant increase in plasma histamine levels occurs in rodents (but not in rabbits (5)) in response to A2AR agonists (11, 45); 2) stimulatory A2ARs have been identified on rodent mast cells (34); and 3) the hypertensive actions of A2AR agonists are antagonized by mast cell stabilizers, including sodium cromoglycate and lodoxamide (17). We have previously demonstrated that A2BRs rather than the A3AR modulate degradation of canine mast cells (3). Feoktistov and Biaggioni (10) have demonstrated that A2BRs regulate human mast cell functions. Hence, differences in the adenosine receptors that regulate mast cells may underlie the variable hemodynamic actions of A2AR agonists observed between rodents and other mammalian species.

In conclusion, we demonstrated in a large animal model that the A3AR agonist IB-MECA effectively reduces infarct size when given before ischemia or when given during reperfusion. The protective actions of IB-MECA occurred without altering systemic hemodynamics or coronary blood flow. These results suggest that A3AR agonists may be useful cardioprotective agents for the treatment of acute myocardial ischemia-reperfusion injury.

The authors thank Ana Hsu for assistance with the preparation of the figures.

DISCLOSURE

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REFERENCES

21. Kodani E, Shinmura K, Xuan YT, Takano H, Auchampach JA, Tang XL, and Bolli R. Cyclooxygenase-2 does not mediate...


