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Protection of IB-MECA against myocardial stunning in conscious rabbits is not mediated by the A₁ adenosine receptor

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Abstract The goal of this study was to determine whether the protective effects of the A₃AR agonist N⁶-(3-iodobenzyl)adenosine-5'-N-methylcarboxamide (IB-MECA) against myocardial stunning are mediated by the A₁AR. Six groups of conscious rabbits underwent a sequence of six 4-minute coronary occlusion (O)/4-minute reperfusion (R) cycles for three consecutive days (days 1, 2, and 3). In vehicle-treated rabbits (group I), the recovery of systolic wall thickening (WTh) in the ischemic/reperfused region was markedly depressed on day 1, indicating the presence of severe myocardial stunning. On days 2 and 3, however, the recovery of systolic WTh was markedly accelerated, indicating the presence of late ischemic preconditioning (PC). When rabbits were pretreated with the A₁AR agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA, 100 µg/kg i.v.) or with IB-MECA (100 µg/kg i.v.) 10 min prior to the first sequence of O/R cycles on day 1 (group III and V, respectively), the recovery of systolic WTh was markedly accelerated compared to vehicle-treated animals (reflected as an ~48% decrease in the total deficit of systolic WTh). The magnitude of the protection afforded by adenosine receptor agonists was equivalent to that provided by late ischemic PC. Pre-treating rabbits with the A₁AR antagonist N-0861 completely blocked both the hemodynamic and the cardioprotective effects of CCPA (group IV). However, the same dose of N-0861 did not block the cardioprotective actions of IB-MECA (group VI). Importantly, N-0861 did not influence the degree of myocardial stunning in the absence of PC (group II) and it did not block the development of late ischemic PC. Taken together, these results provide conclusive evidence that the cardioprotective effects of IB-MECA are not mediated via the A₁AR, supporting the concept that activation of A₃ARs prior to an ischemic challenge provides protection against ischemia/reperfusion injury.

Key words Adenosine receptors – ischemia/reperfusion injury – myocardial stunning – myocardial infarction – conscious rabbits

Introduction

We (1) recently demonstrated in conscious rabbits that pretreatment with the A₃AR agonist IB-MECA produced marked protection against reversible injury induced by multiple occlusion/reperfusion cycles (myocardial

“stunning”) as well as from irreversible injury induced by a prolonged occlusion and reperfusion (myocardial infarction). The cardioprotective effects of IB-MECA were blocked by the non-selective adenosine receptor antagonist, 8-*p*-sulfophenyltheophylline (8-SPT), demonstrating that they were mediated by adenosine receptors (1). However, it remains uncertain whether the

cardioprotective actions of IB-MECA were mediated via the A₃AR. Since IB-MECA has recently been found to be only slightly more potent at binding to rabbit A₃ARs than A₁ARs (2), it is possible that IB-MECA protects by interacting with the A₁AR rather than the A₃AR. Accordingly, the goal of this study was to determine whether IB-MECA protects against ischemia/reperfusion injury in conscious rabbits during A₁AR blockade. The study involved the use of N-0861, a potent and selective (~1,400-fold) A₁AR antagonist (2–5).

Materials and methods

Experimental preparation

The experimental preparation has been described previously in detail (1, 2). Briefly, New Zealand White male rabbits (weight, 2.5 ± 0.1 kg; age, 3 – 4 months) were instrumented under sterile conditions with a balloon occluder around a major branch of the left coronary

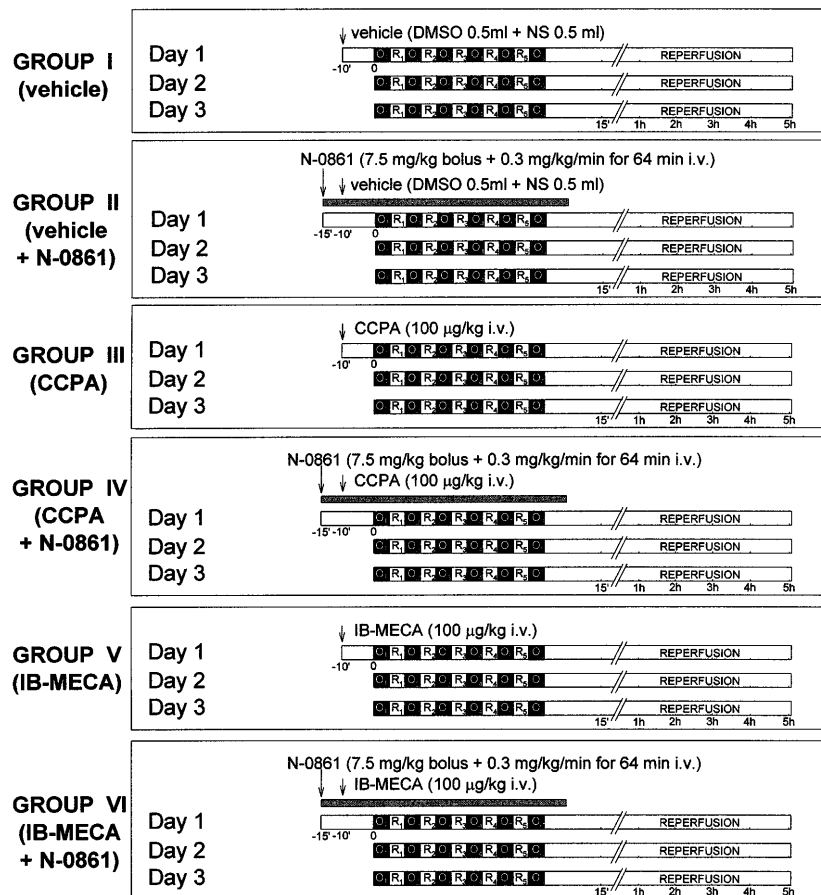
artery, a 10-MHz pulsed Doppler ultrasonic crystal in the center of the region to be rendered ischemic, and bipolar ECG leads on the chest wall. The animals were allowed to recover for a minimum of 10 days after surgery. Left ventricular systolic WTh, range gate depth, and the ECG were recorded throughout the experiments.

Experimental protocol

The experimental protocol consisted of three consecutive days of coronary artery occlusions (days 1, 2, and 3, respectively). On each day, the rabbits were subjected to a sequence of six 4-minute coronary occlusion/4-minute reperfusion cycles (Fig. 1). The performance of successful coronary occlusions was verified by observing the development of ST-segment elevation, changes in the QRS complex, and the appearance of paradoxical systolic wall thinning. No sedative or anti-arrhythmic agents were given at any time.

Rabbits were randomly assigned to six groups (Fig. 1). Groups I (vehicle group), III (CCPA group), and V (IB-MECA group) received an intravenous bolus injection

Fig. 1 Experimental protocol. NS-normal saline.



tion of vehicle (1 ml), CCPA (100 µg/kg), or IB-MECA (100 µg/kg), respectively, 10 minutes prior to the first sequence of coronary occlusion/reperfusion cycles on day 1. No drug treatments were given on subsequent days. In groups II (vehicle + N-0861 group), IV (CCPA + N-0861 group), and VI (IB-MECA + N-0861 group), rabbits received an intravenous bolus injection of N-0861 (7.5 mg/kg) 5 minutes before the bolus of vehicle, CCPA, or IB-MECA, respectively, followed by continuous infusion of this agent (0.3 mg/kg/min) for 64 minutes (total dose = 26.7 mg/kg) on day 1. Previous studies have shown that this dose and route of administration of N-0861 produces effective and long-lasting blockade of A₁ARs in rodents and pigs (2–5). The bolus injections of CCPA and IB-MECA (Research Biochemicals International, Natick, MA) were dissolved in a 50% solution (v/v) of DMSO in normal saline. The bolus injection of N-0861 (gift from Dr. Noel Cusack, Discovery Therapeutics, Richmond, VA) was dissolved in 1 ml of a 50% solu-

tion of DMSO in normal saline and the solution for infusion consisted of 0.5% solution of DMSO in normal saline (total volume injected, 11 ml). All solutions were filtered through a 0.2 µm filter prior to their administration to ensure sterility.

■ Measurement of regional myocardial function

Regional myocardial function was assessed as systolic WTh fraction using the pulsed Doppler probe (1). The total deficit of systolic WTh (an integrative assessment of the overall severity of myocardial stunning) was calculated by measuring the area comprised between the systolic WTh-versus-time line and the baseline (100% line) during the 5-hour recovery phase after the 6th reperfusion (1). In all animals, measurements from at least ten beats were averaged at baseline, and from at least five beats at all subsequent time-points.

Table 1 Heart rate during coronary occlusion and reperfusion

	Baseline	Pre-Occlusion	Third Occlusion	Reperfusion 5 min	30 min	1 h	3 h	5 h
Time (min)	0	10	30	60	90	120	240	360
Group I (vehicle)								
Day 1	238 ± 6	242 ± 7	245 ± 8	229 ± 7	236 ± 7	228 ± 8	224 ± 8	216 ± 7
Day 2	246 ± 10	–	243 ± 7	236 ± 13	231 ± 9	233 ± 7	226 ± 11	215 ± 6
Day 3	239 ± 3	–	242 ± 8	227 ± 5	225 ± 7	223 ± 6	217 ± 4	222 ± 6
Group II (vehicle + N-0861)								
Day 1	223 ± 12	220 ± 8	218 ± 9	230 ± 14	222 ± 10	219 ± 10	231 ± 14	228 ± 12
Day 2	240 ± 9	–	249 ± 16	226 ± 12	234 ± 8	227 ± 18	222 ± 11	226 ± 15
Day 3	242 ± 11	–	250 ± 17	223 ± 12	239 ± 14	232 ± 19	239 ± 16	226 ± 14
Group III (CCPA)								
Day 1	230 ± 13	171 ± 14*†	169 ± 10*†	152 ± 8*†	152 ± 11*†	187 ± 16	205 ± 10	218 ± 9
Day 2	220 ± 16	–	206 ± 18	199 ± 17	203 ± 22	209 ± 17	213 ± 11	213 ± 16
Day 3	224 ± 14	–	222 ± 9	209 ± 9	214 ± 11	224 ± 11	211 ± 9	226 ± 10
Group IV (CCPA + N-0861)								
Day 1	233 ± 12	220 ± 8	218 ± 10	238 ± 23	239 ± 19	229 ± 23	232 ± 11	238 ± 10
Day 2	253 ± 13	–	255 ± 11	234 ± 13	239 ± 5	237 ± 13	241 ± 15	242 ± 15
Day 3	250 ± 10	–	248 ± 10	238 ± 8	235 ± 10	250 ± 11	235 ± 13	238 ± 7
Group V (IB-MECA)								
Day 1	225 ± 16	220 ± 13	214 ± 9	211 ± 10	221 ± 14	218 ± 11	239 ± 10	237 ± 13
Day 2	228 ± 10	–	237 ± 7	214 ± 9	217 ± 9	209 ± 11	211 ± 11	213 ± 14
Day 3	221 ± 9	–	221 ± 10	198 ± 9	216 ± 9	214 ± 10	219 ± 12	214 ± 7
Group VI (IB-MECA + N-0861)								
Day 1	228 ± 13	213 ± 12	213 ± 14	248 ± 18	242 ± 14	238 ± 13	237 ± 12	234 ± 14
Day 2	238 ± 6	–	237 ± 9	240 ± 9	244 ± 5	244 ± 10	242 ± 8	235 ± 8
Day 3	258 ± 4	–	255 ± 10	230 ± 10	244 ± 2	241 ± 7	241 ± 10	223 ± 7

In the studies of myocardial stunning (groups I–VI), all rabbits underwent a sequence of six cycles of 4-min coronary occlusion/4-min reperfusion followed by a 5-h observation period on days 1, 2, and 3. Heart rate (beats/min) was measured before any treatment (baseline), 1 min before occlusion (pre-occlusion) 3 min into the 3rd occlusion, and at selected times after the 6th reperfusion. Time (min) shows the interval after injection of vehicle, CCPA, or IB-MECA. Data are means ± SEM. *P < 0.05 vs. baseline within the same group; †P < 0.05 vs. corresponding value in group I.

■ Measurement of region at risk

At the conclusion of the study, the rabbits were given heparin (1,000 U i.v.), after which they were anesthetized with sodium pentobarbital (50 mg/kg i.v.) and euthanized with KCl. The heart was excised and the size of the ischemic-reperfused region (region at risk) was determined by tying the coronary artery at the site of the previous occlusion and by perfusing the aortic root for 2 minutes with a 5% solution of Phthalo blue dye in normal saline at a pressure of 70 mmHg using a Langendorff apparatus. The heart was then cut into 6 to 7 transverse slices. All atrial and right ventricular tissues were excised. The region at risk (which was identified by the absence of blue dye) was separated from the rest of the left ventricle and both components were weighed.

■ Statistical analysis

Data are reported as means \pm SEM. For intragroup comparisons, hemodynamic variables and WTh were analyzed by a two-way repeated-measures ANOVA (time and day) followed by Student's *t*-tests for paired data with the Bonferroni correction. For intergroup comparisons, data were analyzed by either a one- or two-way repeated-measures (time and group) ANOVA, as appropriate, followed by unpaired Student's *t*-tests with the Bonferroni correction.

Results

■ Exclusions and postmortem analysis

Of the 37 rabbits instrumented for the studies, 6 were assigned to group I (vehicle group), 5 to group II (N-0861 group), 5 to group III (CCPA group), 8 to group IV (CCPA + N-0861 group), 8 to group V (IB-MECA group), and 5 to group VI (IB-MECA + N-0861 group). A total of five rabbits (14%) were excluded from data analysis because of ventricular fibrillation (one rabbit in group IV), an excessively large risk region (one rabbit in group IV), or technical difficulties (one rabbit each in groups IV and two rabbits in group V). All of the other rabbits completed the protocols satisfactorily and were included in the data analysis.

Postmortem analysis showed that the size of the occluded-reperfused vascular bed was similar ($P > 0.05$) in the six groups: 0.79 ± 0.21 g ($19.2 \pm 4.4\%$ of LV weight) in group I, 0.83 ± 0.19 g ($20.3 \pm 3.4\%$) in group II, 0.85 ± 0.09 g ($24.0 \pm 1.5\%$) in group III, 0.84 ± 0.15 g ($20.8 \pm 3.0\%$) in group IV, 1.02 ± 0.09 g ($24.0 \pm 2.5\%$) in group V, 0.78 ± 0.09 g ($21.3 \pm 2.6\%$) in group VI. Tissue stain-

ing with triphenyltetrazolium chloride confirmed the absence of infarction in all hearts.

■ Hemodynamic variables

On day 1, there were no significant differences in heart rate following the bolus injections of vehicle (group I) or IB-MECA (group V). In contrast, administration of CCPA (group V) produced a marked decrease in heart rate (maximal decrease of $\sim 36\%$), which persisted for ~ 90 minutes. The decrease in heart rate induced by CCPA was abrogated completely by N-0861 (group IV), demonstrating that the dose of N-0861 blocked A_1 ARs. On day 2, there were no significant differences in heart rate at any time throughout the occlusion/reperfusion cycles or the first 5 hours of the recovery period (Table 1).

■ Regional myocardial function

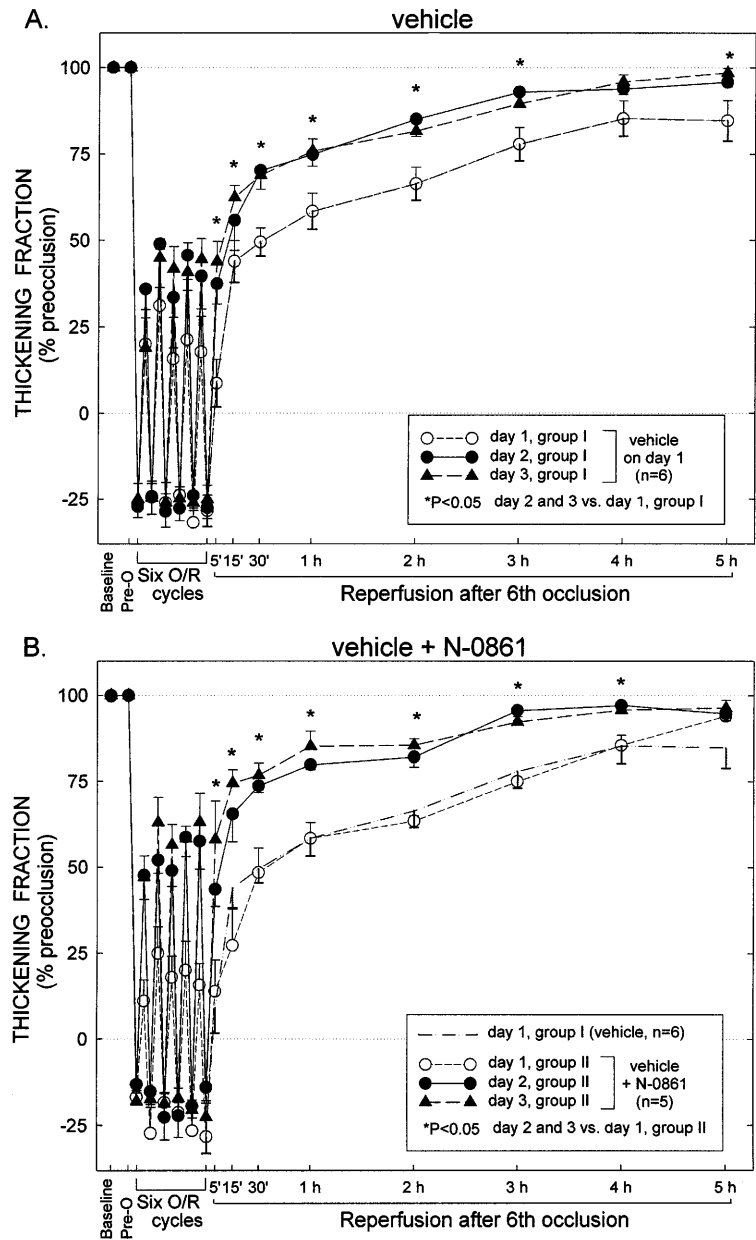
There were no significant differences among the 6 groups of rabbits in baseline systolic WTh fraction or paradoxical wall thinning during the six occlusions (Table 2 and Figs. 2–4). Furthermore, there were no significant differ-

Table 2 Thickening fraction on each experimental day

	Baseline	Pre-occlusion
Group I (vehicle)		
Day 1	31.3 \pm 2.2	31.7 \pm 1.9
Day 2	30.3 \pm 2.2	–
Day 3	32.7 \pm 1.5	–
Group II (vehicle + N-0861)		
Day 1	33.6 \pm 4.2	33.8 \pm 4.5
Day 2	34.3 \pm 4.0	–
Day 3	34.4 \pm 3.8	–
Group III (CCPA)		
Day 1	31.2 \pm 4.0	29.8 \pm 3.5
Day 2	30.5 \pm 4.0	–
Day 3	30.4 \pm 3.8	–
Group IV (CCPA + N-0861)		
Day 1	34.4 \pm 4.3	33.3 \pm 4.0
Day 2	32.8 \pm 3.2	–
Day 3	32.6 \pm 3.2	–
Group V (IB-MECA)		
Day 1	31.9 \pm 4.1	31.2 \pm 3.8
Day 2	32.5 \pm 3.5	–
Day 3	33.8 \pm 4.3	–
Group VI (IB-MECA + N-0861)		
Day 1	29.9 \pm 4.1	29.8 \pm 3.5
Day 2	31.8 \pm 3.0	–
Day 3	32.7 \pm 3.3	–

Thickening fraction (%) was measured before any treatment (baseline) and 1 min before occlusion (pre-occlusion). Data are means \pm SEM.

Fig. 2 Systolic WTh fraction in the ischemic-reperfused region in groups I (vehicle group) and II (N-0861 group) 5 minutes before the administration of vehicle or N-0861 (Baseline), 3 minutes into each coronary occlusion (O), 3 minutes into each reperfusion (R), and at selected times during the 5-hour reperfusion interval following the 6th occlusion. For purposes of comparison, the data from day 1 of group I (vehicle group) are also shown in panel B. Systolic WTh fraction is expressed as a percentage of baseline values. Data are the means \pm SEM.



ences in systolic WTh among the groups immediately before the first occlusion/reperfusion cycle (pre-occlusion; Table 2), indicating that none of the adenosine receptor ligands influenced regional contractile function.

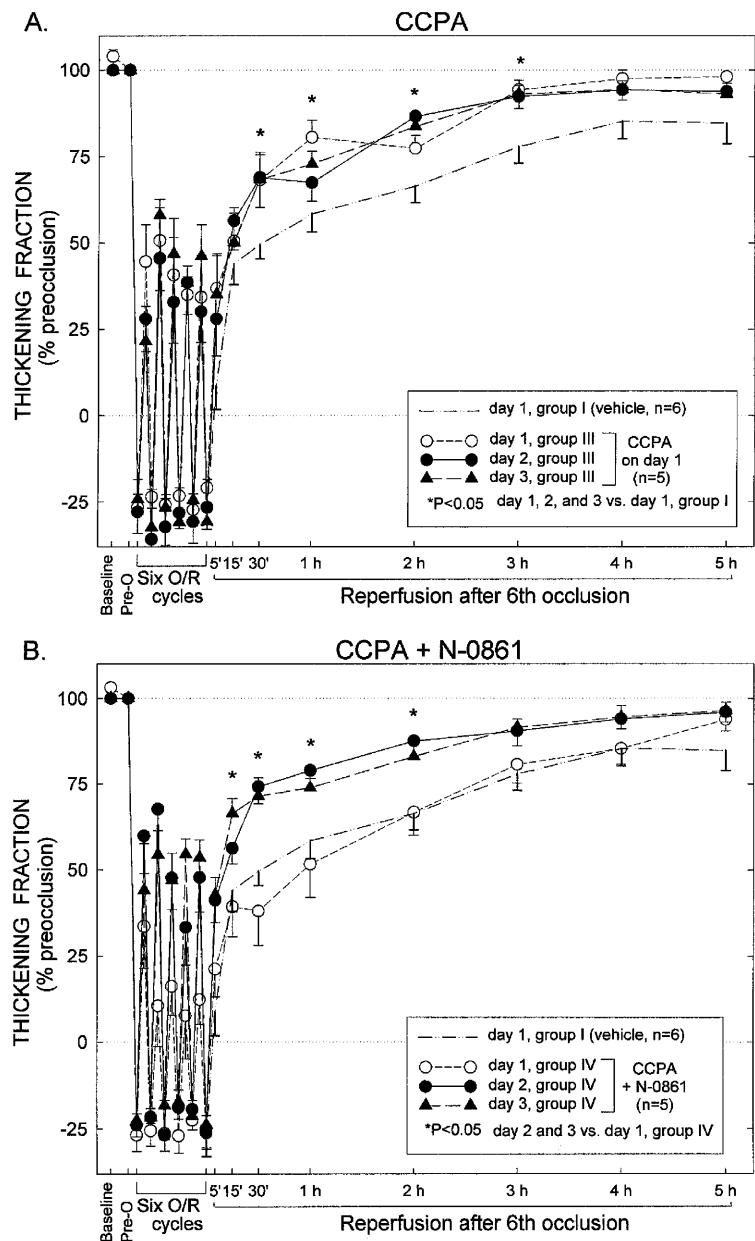
Groups I and II (vehicle groups)

On day 1, thickening fraction in group I (vehicle group) remained significantly depressed for 5 hours after the 6th reperfusion (Fig. 2), indicating that the sequence of six 4-minute occlusion/4-minute reperfusion cycles resulted in severe myocardial stunning. On days 2 and 3,

however, the recovery of WTh after six occlusion/reperfusion cycles was markedly improved compared with that observed on day 1 (Fig. 2). The total deficit of WTh after the 6th reperfusion was 48% less on day 2 and 45% less on day 3 compared with day 1 (155.5 ± 20.7 , 81.5 ± 1.8 , and 86.2 ± 11.0 on days 1, 2, and 3, respectively; $P < 0.05$; Fig. 5). Thus, the occlusion/reperfusion cycles on day 1 induced late PC, which provided protection against myocardial stunning on days 2 and 3.

The recovery of systolic WTh on days 1, 2, and 3 in group II (N-0861-treated group) was nearly identical to that of group I (vehicle group). Thickening fraction

Fig. 3 Systolic WTh fraction in the ischemic-reperfused region in groups III (CCPA group) and IV (CCPA + N-0861 group) 5 minutes before the administration of CCPA or N-0861 (Baseline), 3 minutes into each coronary occlusion (O), 3 minutes into each reperfusion (R), and at selected times during the 5-hour reperfusion interval following the 6th occlusion. For purposes of comparison, the data from day 1 on day 1 (vehicle group) are also shown. Systolic WTh fraction is expressed as a percentage of baseline values. Data are the means \pm SEM.

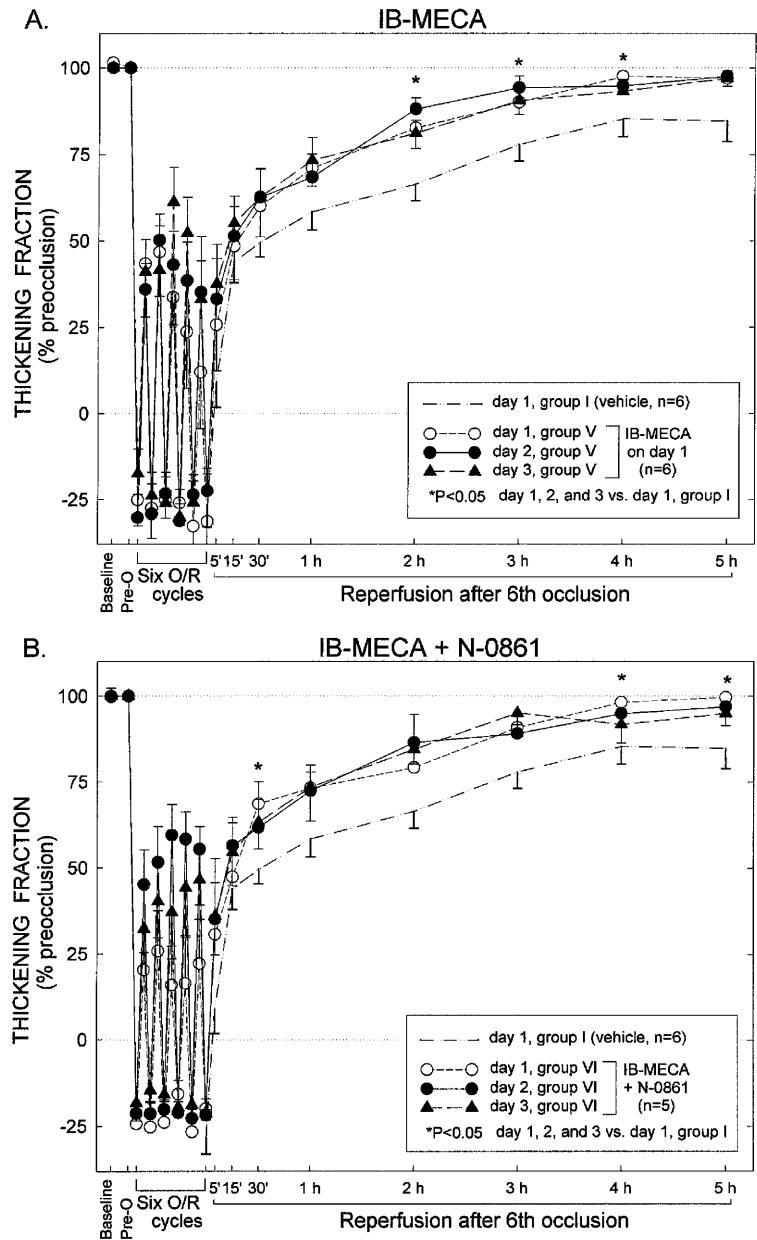


remained significantly depressed for 4 hours after the 6th reperfusion (Fig. 2), and the recovery of systolic WTh was markedly improved on days 2 and 3 compared to day 1. The total deficit of WTh was 55% less on day 2 and 60% less on day 3 compared to day 1 (159.8 ± 6.3 , 71.3 ± 5.9 , and 62.2 ± 3.6 on days 1, 2, and 3, respectively; $P < 0.05$; Fig. 5). Thus, pretreatment with N-0861 did not affect myocardial stunning on day 1 or the development of late PC on days 2 and 3.

Groups III and IV (CCPA-treated groups)

In group III (CCPA group), the recovery of systolic WTh following the occlusion/reperfusion cycles on day 1 was faster than in group I (vehicle group), and this improvement was sustained throughout the 5-hour recovery period. The total deficit of WTh on day 1 was 48% less than that observed on day 1 in vehicle-treated rabbits (80.7 ± 13.3 versus 155.5 ± 20.7 , respectively; $P < 0.05$) and was similar to that observed in vehicle-treated rabbits on days 2 and 3 (Fig. 5). Similarly, on days 2 and 3, there was no further improvement in recovery of systolic

Fig. 4 Systolic WTh fraction in the ischemic-reperfused region in groups V (IB-MECA group) and VI (IB-MECA + N-0861 group) 5 minutes before the administration of CCPA or N-0861 (Baseline), 3 minutes into each coronary occlusion (O), 3 minutes into each reperfusion (R), and at selected times during the 5-hour reperfusion interval following the 6th occlusion. For purposes of comparison, the data from day 1 on day 1 (vehicle group) are also shown. Systolic WTh fraction is expressed as a percentage of baseline values. Data are the means \pm SEM.



WTh compared with group I (total deficits of WTh were 71.3 ± 6.3 and 62.2 ± 3.6 on days 2 and 3, respectively; Fig. 5). Thus, administration of CCPA 10 minutes prior to six 4-minute occlusion/4-minute reperfusion cycles resulted in an attenuation of myocardial stunning on day 1 that was essentially equivalent in magnitude to that effected by late ischemic PC. An additive cardioprotective effect with A_1AR activation and late ischemic PC did not occur.

To determine whether blockade of A_1AR s abolishes the protective effect of CCPA, rabbits in group IV (CCPA + N-0861 group) were given N-0861 prior to the administration of CCPA. As illustrated in Figure 3, recovery of

systolic WTh in group IV was similar to that noted in vehicle-treated rabbits (group I). On day 1, thickening fraction remained significantly depressed following the 6th reperfusion. On days 2 and 3, however, the recovery of WTh after the six occlusion/reperfusion cycles was markedly improved compared with day 1 (Fig. 4). Compared to day 1, the total deficit of WTh after the 6th reperfusion was 51% less on day 2 and 48% less on day 3 (156.6 ± 18.4 , 76.1 ± 15.3 , and 81.5 ± 15.9 on days 1, 2, and 3, respectively; $P < 0.05$; Fig. 5). Thus, the improvement in the recovery of WTh provided by pretreatment with CCPA was blocked completely by N-0861, indicating that

the cardioprotective effects of CCPA are mediated via A_1 ARs.

Groups V-VI (IB-MECA-treated groups)

In group V (IB-MECA group), the recovery of systolic WTh was very similar to that noted in group III (CCPA group). The recovery of WTh following the occlusion/reperfusion cycles on day 1 was faster than in group I (vehicle group), and this improvement was sustained throughout the entire 5-hour recovery period. The total deficit of WTh was 40% less than that observed on day 1 of vehicle-treated rabbits (92.1 ± 18.0 versus 155.5 ± 20.7 , respectively; $P < 0.05$) and was similar to that observed in vehicle-treated rabbits on days 2 and 3 (Fig. 5). On days 2 and 3, there was no further improvement in recovery of systolic WTh compared with group I (total deficits of WTh were 84.2 ± 17.1 and 91.7 ± 17.3 on days 2 and 3, respectively; Fig. 5). Thus, administration of IB-MECA 10 minutes prior to six 4-minute occlusion/4-minute reperfusion cycles resulted in attenuation of myocardial stunning on day 1 that was equivalent in magnitude to that produced by either late ischemic PC or CCPA.

To determine whether the A_1 AR is necessary for IB-MECA-induced protection against myocardial stunning, rabbits in group VI (IB-MECA + N-0861) were given N-0861 prior to the administration of IB-MECA. As illustrated in Fig. 4, the recovery of systolic WTh in group VI was similar to that noted in rabbits treated with IB-MECA alone (group V). On day 1, systolic WTh was markedly faster compared to group I (vehicle group), and this improvement in regional wall function was sustained throughout the reperfusion period. The total deficit of WTh in group VI was 44% less than that observed on day 1 of vehicle-treated rabbits (87.8 ± 27.9 versus 155.5 ± 20.7 , respectively; $P < 0.05$) and was similar in magnitude to that provided by late PC in vehicle-treated rabbits (Fig. 5). On days 2 and 3, there was no further improvement in recovery of systolic WTh compared to group I (total deficits of WTh were 87.5 ± 24.0 and 86.4 ± 23.0 on

days 2 and 3, respectively; Fig. 5). Thus, blockade of A_1 ARs with N-0861 did not abrogate the cardioprotective effects of IB-MECA.

Discussion

Although we have previously demonstrated that IB-MECA is a potent cardioprotective agent against ischemia/reperfusion injury in conscious rabbits (1), the possibility remains that its cardioprotective effects are mediated via the A_1 AR rather than the A_3 AR. To address this important issue, in the present study we administered IB-MECA to conscious rabbits in the presence of the A_1 AR antagonist N-0861 (2–5). N-0861 was administered at a dose that effectively blocked the A_1 AR, evidenced by the observation that it abrogated both the anti-stunning and hemodynamic effects of the highly-selective A_1 AR agonist CCPA (Table 1 and Figs. 3 and 5). An A_3 AR antagonist could not be used in these studies, since selective antagonists for this receptor subtype in rabbits has not yet been developed (2). We observed that IB-MECA was as effective at protecting against the development of myocardial stunning in the presence of N-0861 as it was when it was administered alone (Figs. 4 and 5). These results provide conclusive evidence that the cardioprotective effects of IB-MECA are not mediated via the A_1 AR. Considering the high affinity of IB-MECA for the A_3 AR, these results support the concept that activating A_3 AR immediately prior to an ischemic challenge provides protection against myocardial ischemia/reperfusion injury.

Armstrong and Ganote (6) and Liu et al (6, 7) were the first to suggest that A_3 ARs may play a protective role in the heart during ischemia/reperfusion injury. These investigators observed in isolated rabbit cardiomyocytes and isolated rabbit hearts, respectively, that the cardioprotective effects of the mixed acting A_1/A_3 AR agonist N^6 -[2-(4-aminophenyl)ethyl]adenosine (APNEA) could not be blocked by the A_1 AR antagonist 1,3-dipropyl-8-cyclopentylxanthine (CPX), but was blocked by high concentrations of non-selective antagonists such as 8-SPT or BWA 1433 (6, 7). Subsequent studies in various other *in vitro* model systems have obtained similar results and there is evidence to suggest that the A_3 AR is also involved in the mechanism of early phase of ischemic PC (6–15). Although these *in vitro* studies provided evidence that the A_3 AR plays a cardioprotective role in the heart, the importance of the A_3 AR during ischemia/reperfusion injury *in vivo* remained to be assessed.

Accordingly, we studied the effects of IB-MECA in conscious rabbits, i.e., a physiologically relevant *in vivo* model of ischemia/reperfusion injury (1). At the time this study was performed, IB-MECA was identified as a

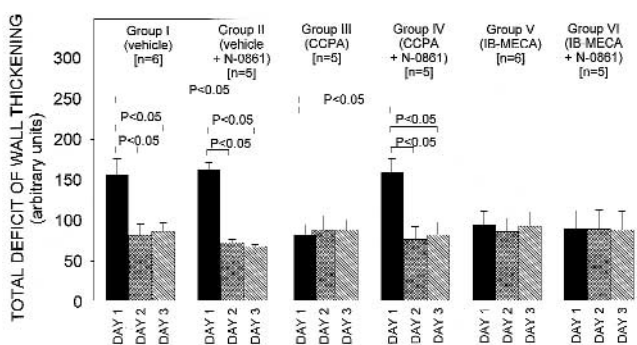


Fig. 5 Total deficit of WTh after the 6th reperfusion on days 1, 2, and 3 in groups I–VI. Data are the means \pm SEM.

potent and selective (~50-fold) agonist for rat A₃ARs (16). We observed that pre-treating (10 minutes) conscious rabbits with IB-MECA produced a profound protection against myocardial stunning induced by multiple occlusion/reperfusion cycles. This protection was blocked by the non-specific adenosine receptor antagonist 8-SPT as well as the protein kinase C inhibitor chelerythrine, suggesting that IB-MECA acted via a receptor-mediated mechanism involving kinase signaling. Pre-treatment with IB-MECA also produced a marked reduction in infarct size following prolonged coronary occlusion and reperfusion (1). Since IB-MECA was effective at a dose that produced no changes in heart rate or systemic blood pressure (1), we concluded that the A₃AR is the predominant receptor subtype responsible for the cardioprotective actions produced by IB-MECA. Two recent developments, however, weaken this line of reasoning and suggest that, theoretically, the beneficial effects of IB-MECA may be mediated via the A₁AR. First, since it has become apparent that the potency of adenosine receptor ligands in eliciting responses can differ markedly depending on coupling efficiency and receptor reserve (17–19), IB-MECA may act on A₁ARs to produce cardioprotection at doses that do not effect systemic hemodynamics. Secondly, we have determined that IB-MECA is only ~13–21-fold selective at binding to rabbit A₃ARs versus rabbit A₁ARs (2). The results of the present study, however, confirm our original interpretation (1).

The mechanisms by which A₃ARs protect the ischemic myocardium have yet to be determined. It has been suggested that A₃ARs are expressed in cardiomyocytes, which may modulate ATP-sensitive potassium channels via kinase signaling (20). This mechanism is analogous to that by which several different G-protein coupled receptors, including the A₁AR, induce the early phase of ischemic PC (21). Indeed, although improvement in the oxygen-supply balance can not be ruled out, we predict that CCPA provided cardioprotection in the present study by enhancing the function of K_{ATP} channels. The

cell types within the heart that express the A₃AR may not be limited to the cardiomyocyte, however. It has been suggested that A₃ARs are expressed in endothelial cells, vascular smooth muscle cells, and/or tissue resident leukocytes (22–24). It remains possible, therefore, that A₃ARs provide cardioprotection via actions on non-myocytic cells.

Another important observation of the present study is that blockade of A₁ARs with N-0861 in vehicle-treated animals did not abrogate the late phase of ischemic PC against myocardial stunning. This observation is consistent with previous studies from our research group in which we observed that 8-SPT did not block the development of late PC against myocardial stunning in conscious rabbits (25) and pigs (26). Interestingly, however, Baxter and colleagues (27) have demonstrated that blockade of A₁ARs with CPX abrogates the late phase of ischemic PC against infarction in rabbits, and we have observed in conscious rabbits that administration of either CCPA or IB-MECA induces late PC against infarction, but not against myocardial stunning (2, 25). Collectively, these results demonstrate that adenosine receptors play a differential role in late PC against myocardial stunning and infarction, indicating that there are important differences between these two phenomena.

In conclusion, the results of the present study provide further evidence that the potent cardioprotective effects of IB-MECA are mediated via the A₃AR, rather than through non-specific interactions with the A₁AR. Since the dose of IB-MECA used herein did not cause any appreciable changes in systemic hemodynamic parameters (Table 1 and ref. 1), the results suggest that therapies targeting A₃ARs may be useful in the clinical arena.

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