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Overexpression of A₃ adenosine receptors decreases heart rate, preserves energetics, and protects ischemic hearts

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Abstract

To determine whether A₃ adenosine receptor (A₃AR) signaling modulates myocardial function, energetics, and cardioprotection, hearts from wild-type and A₃AR-overexpressor mice were subjected to 20-min ischemia and 40-min reperfusion while ³¹P NMR spectra were acquired. Basal heart rate and left ventricular developed pressure (LVDP) were lower in A₃AR-overexpressor hearts than wild-type hearts. Ischemic ATP depletion was delayed and postischemic recoveries of contractile function, ATP, and phosphocreatine were greater in A₃AR-hearts. To determine the role of depressed heart rate and to confirm A₃AR-specific signaling, hearts were paced at 480 beats/min with or without 60 nmol/l MRS-1220 (A₃AR-specific inhibitor) and then subjected to ischemiareperfusion. LVDP was similar in paced A₃AR-overexpressor and paced wild-type hearts. Differences in ischemic ATP depletion and postischemic contractile and energetic dysfunction remained in paced A₃AR-overexpressor hearts versus paced wild-type hearts but were abolished by MRS-1220. In summary, A₃AR overexpression decreased basal heart rate and contractility, preserved ischemic ATP, and decreased postischemic dysfunction. Pacing abolished the decreased contractility but not the ATP preservation or cardioprotection. Therefore, A₃AR overexpression results in cardioprotection via a specific A₃AR effect, possibly involving preservation of ATP during ischemia.

Keywords

cardioprotection; ischemia; nuclear magnetic resonance spectroscopy

Adenosine is an endogenous metabolite that is released from the myocardium during ischemia-reperfusion. Exogenous administration of adenosine or adenosine receptor agonists has been shown to be cardioprotective (20, 26). Because of its potent cardioprotective effects, much attention has focused on the use of adenosine as a cardiotherapy. Thus far the main approach in therapy development has been to administer adenosine, adenosine receptor agonists, or modulators of adenosine degradation (15, 22). Clinical use of such approaches is complicated by the effects of these agents on noncardiac tissue and by progressive desensitization of adenosine receptors. Cardiac-specific targeting could alleviate some of these problems. Therefore, a transgenic mouse model was recently developed with cardiac-specific overexpression of the G_{i/o}-coupled A₁ adenosine receptor (A₁AR) (23). Hearts from

these mice were inherently resistant to ischemia, indicating that increasing the number of functional adenosine receptors alone can enhance endogenous adenosine signaling and lead to cardioprotection.

Although it is thought that the cardioprotective effects of adenosine are mediated primarily via the A₁AR, recent evidence suggests that signaling through the A₃ adenosine receptor (A₃AR), which also couples to G_{i/o}, could be protective (1, 28, 31). Although levels of the A₃AR are low in cardiac tissue from most species (29, 32), A₃AR overexpression could be useful as a genetic therapy. To determine whether cardiac-specific overexpression of the A₃AR protects against ischemic injury, transgenic mice were created with a gene construct consisting of the canine A₃AR cDNA under the control of the α -myosin heavy chain promoter (2). It was demonstrated that, after in vivo regional ischemia and reperfusion, infarct size was lower in low copy number A₃AR overexpressors than in wild-type mice, confirming that A₃AR signaling could protect against myocardial necrosis (2). In the present study, we determined whether cardiac-specific overexpression of the A₃AR could also protect against postischemic contractile dysfunction after short periods of ischemia. Perfused hearts from A₃AR-overexpressor and wild-type mice were subjected to 15- or 20-min global ischemia and 40-min reperfusion. Because adenosine has negative chronotropic effects (14) and cardioprotection has been related to preservation of ischemic ATP (8, 24, 27) and intracellular pH levels (5, 7, 19), contractile parameters and energetics were also studied in the A₃AR-overexpressor hearts. Furthermore, the canine A₃AR-specific inhibitor, MRS-1220, was used to confirm that any observations in the A₃AR-overexpressor hearts were indeed due to A₃AR signaling.

MATERIALS AND METHODS

Animals

Transgenic mice exhibiting cardiac-specific overexpression of the A₃AR were created by microinjection of a transgene construct, containing canine A₃AR cDNA under the control of the α -myosin heavy chain promoter, into the pronuclei of fertilized FVB/N mouse oocytes (2). After embryo implantation, the presence of the transgene in the resultant founder mice was confirmed by Southern blot analysis and expression of canine A₃AR mRNA was determined by Northern blot analysis. A₃AR levels were assessed in the transgenic lines by radioligand binding assays (2).

In the present study, 15 heterozygous low copy number transgenic mice (A₃tg.1) and 16 of their wild-type littermates were used; all animals were treated in accordance with National Institutes of Health (NIH) guidelines and the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. Binding assays revealed 12.7 fmol/mg of G protein-coupled A₃ARs in A₃tg.1 hearts and negligible levels in wild-type littermates. A₃tg.1 hearts exhibited no evidence of cardiac abnormalities, as determined from morphological and histochemical analysis and determination of expression of hypertrophic marker genes (2).

Ischemia-Reperfusion Protocol

Hearts were isolated and perfused in the Langendorff mode as described previously (4). Hearts were then subjected to 20 min of no-flow ischemia followed by 40-min reperfusion. Left ventricular developed pressure (LVDP), rate of contraction (+dP/dt), rate of relaxation (−dP/dt), and heart rate were monitored via a water-filled latex balloon in the left ventricle. Recovery of contractile function was assessed by measurement of LVDP at the end of reperfusion and expressed as a percentage of preischemic LVDP.

Control Hearts, Pacing Protocol, and MRS-1220 Treatment

A control group of untreated A₃tg.1 ($n = 5$) and wild-type ($n = 6$) hearts was allowed to beat at their intrinsic heart rate, and another group of A₃tg.1 ($n = 5$) and wild-type ($n = 5$) hearts was paced throughout preischemia, and reperfusion at 8 Hz with a 4-ms square pulse generated by a Grass stimulator (Grass-Telefactor). We found that pacing itself increased ischemic injury (unpublished observations); therefore, the ischemic period was reduced to 15 min in the paced hearts to provide a suitable level of postischemic recovery of contractile function. A further group of A₃tg.1 ($n = 5$) and wild-type ($n = 5$) hearts was paced at 8 Hz and then treated with 60 nmol/l of the canine A₃AR-specific inhibitor MRS-1220, beginning 5 min before the 15-min ischemic period and continuing throughout reperfusion.

NMR Spectroscopy

Relative changes in concentrations of phosphorus metabolites were observed during the ischemia-reperfusion protocols by acquiring consecutive ³¹P NMR spectra as described previously (6). The areas of the spectral peaks were expressed as a percentage of the peak areas of an initial, preischemic control spectrum from each heart. Intracellular pH was estimated from the chemical shift of the P_i peak relative to phosphocreatine (PCr) with previously obtained titration curves.

Statistics

Results are expressed as means $\pm$ SE. Significance ($P < 0.05$) was determined for discrete variables by one-way analysis of variance and for continuous variables by analysis of variance for repeated measures; both analyses were followed by a Fisher's post hoc test.

RESULTS

Basal Contractile Function

During the preischemic period, LVDP was lower in unpaced A₃AR-overexpressor hearts (87 cmH₂O) than in unpaced wild-type hearts (110 cmH₂O) ($P < 0.0001$; Table 1). Heart rate was also lower 319 beats/minute in unpaced A₃AR-overexpressor hearts than in unpaced wild-type hearts (390 beats/min). +dP/dt (2.8 cmH₂O/ms in A₃AR overexpressors vs. 3.9 cmH₂O/ms in wild type) and -dP/dt (-2.1 cmH₂O/ms in A₃AR overexpressors vs. -3.0 cmH₂O/ms in wild type) ($P < 0.001$; Table 1) were also lower in unpaced A₃AR-overexpressor hearts than in unpaced wild-type hearts. Thus basal myocardial contractility was decreased by overexpression of the A₃AR.

On pacing at 480 beats/min, LVDP increased in A₃AR-overexpressor hearts to 109 cmH₂O ($P < 0.0001$ vs. unpaced A₃AR overexpressors), whereas LVDP did not differ in paced versus unpaced wild-type hearts (Table 1). +dP/dt and -dP/dt were also increased by pacing in A₃AR-overexpressor hearts, to 3.8 cmH₂O/ms and -2.8 cmH₂O/ms, respectively. The increase in LVDP, +dP/dt, and -dP/dt in paced A₃AR-overexpressor hearts resulted in these values not being significantly different from those of paced or unpaced wild-type hearts (Table 1).

Pretreatment of paced hearts with 60 nmol/l of the A₃AR-specific inhibitor MRS-1220 had no effect on LVDP, +dP/dt, or -dP/dt in either A₃AR-overexpressor or wild-type hearts (Table 1).

Postischemic Contractile Recovery

Recovery of contractile function after 20-min ischemia and 40-min reperfusion was higher in unpaced A₃AR-overexpressor hearts (56% initial LVDP) than in unpaced wild-type hearts

(30% initial LVDP) ($P < 0.0001$; Fig. 1). Posts ischemic contractile function was also higher in absolute terms in unpaced A₃AR hearts (49 ± 2 cmH₂O) than in unpaced wild-type hearts (33 ± 4 cmH₂O) ($P < 0.05$).

In paced hearts, recovery of contractile function after 15-min ischemia and 40-min reperfusion was also higher in A₃AR overexpressors (60% initial LVDP) than in wild type (35% initial LVDP) ($P < 0.001$; Fig. 1). Posts ischemic contractile function was also higher in absolute terms in paced A₃AR hearts (65 ± 4 cmH₂O) than in paced wild-type hearts (39 ± 6 cmH₂O) ($P < 0.001$).

Pretreatment with 60 nmol/l MRS-1220 lowered functional recovery after 15-min ischemia and 40-min reperfusion in paced A₃AR-overexpressor hearts to 27% initial LVDP ($P < 0.0001$ vs. untreated paced A₃AR overexpressors), which was not significantly different from that observed in untreated paced wild-type hearts (35% initial LVDP) or MRS-1220-treated paced wild-type hearts (28% initial LVDP) (Fig. 1). Posts ischemic LVDP was also lower, at 40 ± 6 cmH₂O, in MRS-1220-treated versus untreated paced A₃AR-overexpressor hearts ($P < 0.0001$). Pretreatment with MRS 1220 had no significant effect on percent recovery of contractile function (28% initial LVDP) or absolute LVDP (29 ± 6 cmH₂O) in paced wild-type hearts.

Phosphate Metabolites and Intracellular pH

Phosphate metabolites and intracellular pH were measured in the transgenic and wild-type hearts to determine whether overexpression of the A₃AR altered myocardial energetics and pH regulation.

ATP levels—During ischemia, ATP depletion was delayed in the unpaced A₃AR-overexpressor hearts compared with wild-type hearts (Fig. 2A), but by the end of 20-min ischemia, ATP levels were the same in unpaced A₃AR-overexpressor and wild-type hearts, both at ~20% initial ATP. By the end of reperfusion, ATP had increased to a greater extent in unpaced A₃AR-overexpressor hearts, which reached 50% initial ATP, than in unpaced wild-type hearts, which reached 24% initial ATP ($P < 0.01$; Fig. 2A).

In hearts paced at 480 beats/min, similar to the unpaced hearts, ischemic ATP depletion was delayed in the A₃AR overexpressors compared with wild type. However, the shorter 15-min ischemic period used in the paced heart experiments resulted in ATP levels being significantly higher at the end of ischemia in paced A₃AR-overexpressor hearts (41% initial ATP) than in paced wild-type hearts (20% initial ATP) ($P < 0.05$; Fig. 3A). During reperfusion, ATP increased to a greater extent in paced A₃AR-overexpressor hearts, which reached 63% initial ATP by the end of 40-min reperfusion, than in paced wild-type hearts, which reached 35% initial ATP ($P < 0.01$; Fig. 3A).

Pretreatment with 60 nmol/l MRS-1220 abolished the delay in ATP depletion during ischemia in the paced A₃AR-overexpressor hearts (Fig. 3A). Correspondingly, ATP levels were 22% of initial ATP by the end of 15-min ischemia in the paced A₃AR-overexpressor hearts ($P < 0.05$ vs. untreated paced A₃AR overexpressors), which was not significantly different from that observed in untreated paced wild-type hearts (20% initial ATP) or MRS-1220-treated paced wild-type hearts (23% initial ATP). Pretreatment with MRS-1220 had no significant effect on ischemic ATP levels in paced wild-type hearts. Pretreatment with MRS-1220 also abolished the increased ATP recovery during reperfusion in paced A₃AR-overexpressor hearts, end-reperfusion ATP levels being 28% of initial ATP in MRS-1220-treated paced A₃AR-overexpressor hearts ($P < 0.001$ vs. untreated paced A₃AR overexpressors), which was not significantly different from that observed in untreated paced wild-type hearts (35% initial ATP) or MRS-1220-treated paced wild-type hearts (30% initial

ATP). Pretreatment with MRS-1220 had no significant effect on reperfusion ATP levels in paced wild-type hearts.

To further analyze the delay in ATP depletion in paced and unpaced A₃AR-overexpressor hearts compared with wild-type hearts, the rates of ATP depletion during the first 15 min of ischemia were calculated. Pacing increased the rate of ATP depletion in both A₃AR-overexpressor and wild-type hearts from $-3.6 \pm 0.8\%$ initial ATP/min in unpaced A₃AR-overexpressor hearts to $-5.4 \pm 0.4\%$ initial ATP/min in paced A₃AR-overexpressor hearts ($P < 0.05$) and from $-5.1 \pm 0.2\%$ initial ATP/min in unpaced wild-type hearts to $-7.1 \pm 0.4\%$ initial ATP/min in paced wild-type hearts ($P < 0.05$). Confirming the delay in ATP depletion in A₃AR-overexpressor versus wild-type hearts, the rate of ATP depletion was significantly lower in both unpaced A₃AR-overexpressor versus unpaced wild-type hearts ($P < 0.05$) and paced A₃AR-overexpressor versus paced wild-type hearts ($P < 0.05$).

PCr levels—During ischemia, PCr levels fell in all hearts. At the end of ischemia, there were no differences in PCr levels ($\sim 5\%$ initial PCr) between unpaced A₃AR-overexpressor and wild-type hearts (Fig. 2B) or between any of the paced groups, all also at $\sim 5\%$ of initial PCr (Fig. 3B). Although PCr levels were not different during early reperfusion, by the end of reperfusion PCr levels had increased to a greater extent in unpaced A₃AR-overexpressor hearts, which reached 66% of initial PCr, than in unpaced wild-type hearts, which reached 42% of initial PCr ($P < 0.05$; Fig. 2B).

In paced hearts, reperfusion PCr levels recovered to a greater extent in A₃AR-overexpressor hearts, which reached 107% of initial PCr by the end of reperfusion, than in wild-type hearts, which reached 74% of initial PCr ($P < 0.001$; Fig. 3B).

Pretreatment with MRS-1220 abolished the increased PCr recovery during reperfusion in paced A₃AR-overexpressor hearts, end-reperfusion PCr levels being 67% of initial PCr in MRS-1220-treated paced A₃AR-overexpressor hearts ($P < 0.001$ vs. untreated paced A₃AR overexpressors), which was not significantly different from that observed in untreated paced wild-type hearts (74% initial PCr) or MRS-1220-treated paced wild-type hearts (62% initial PCr). Pre-treatment with MRS-1220 had no significant effect on reperfusion ATP levels in paced wild-type hearts.

Intracellular pH—During ischemia, pH decreased in all hearts. At the end of ischemia, there were no differences in pH levels, at pH ~ 5.80 , between unpaced A₃AR-overexpressor and wild-type hearts (Fig. 2C) or between any of the paced groups, all also at pH ~ 5.80 (Fig. 3C). During reperfusion, pH increased in all hearts back to preischemic values. At the end of reperfusion, there were also no differences in pH levels, at pH ~ 7.10 , between unpaced A₃AR-overexpressor and wild-type hearts (Fig. 2C) or between any of the paced groups, all at pH ~ 7.10 (Fig. 3C).

DISCUSSION

Effect of A₃AR Overexpression on Heart Rate, Basal Contractility, Energetics, and Postischemic Dysfunction

In the present study, preischemic heart rate, peak contraction (LVDP), $+dP/dt$, and $-dP/dt$ were lower in perfused hearts from A₃AR-overexpressor mice than wild-type mice (Table 1). This observation of A₃AR-induced negative chronotropy and inotropy is reminiscent of that induced by exogenous adenosine or by overexpression of the A₁AR (14, 23). The A₃AR-overexpressor and wild-type hearts were then subjected to 20-min ischemia and 40-min reperfusion. During ischemia, the decrease in PCr and intracellular pH were similar in A₃AR-overexpressor and wild-type hearts (Fig. 2). However, ischemic ATP depletion was

delayed in the A₃AR-overexpressor hearts compared with wild-type hearts, the rate of fall of ATP being -3.6% initial ATP/min in A₃AR-overexpressor hearts compared with -5.1% initial ATP/min in wild-type hearts during the first 15 min of ischemia. During reperfusion, recovery of the energy metabolites ATP and PCr was faster in A₃AR-overexpressor hearts than wild-type hearts (Fig. 2). In addition, postischemic contractile dysfunction was lower in A₃AR-overexpressor hearts than wild-type hearts (Fig. 1). It appears, therefore, that overexpression of the A₃AR results in short-term preservation of ATP levels during ischemia and protects against postischemic contractile and energetic dysfunction.

Effect of Pacing on Contractility, Energetics, and Postischemic Dysfunction in A₃AR-Overexpressor Versus Wild-Type Hearts

Depressed preischemic heart rate and contractility can lead to decreased ATP utilization and increased postischemic recovery (17). To determine whether depressed heart rate was responsible for the ischemic ATP preservation and cardioprotection in the A₃AR overexpressors, hearts were paced at 480 beats/min throughout the protocol. Pacing increased LVDP, +dP/dt, and -dP/dt in A₃AR-overexpressor hearts but, surprisingly, had no effect on these parameters in wild-type hearts (Table 1). Although surprising, this finding is consistent with recent studies indicating that the force-frequency response is biphasic in mouse hearts. It has been shown that increasing heart rate in the range of 300–400 beats/min results in increased developed pressure, whereas increasing heart rate from 400 beats/min upward either has no effect or decreases developed pressure (9, 16). The peak of the force-frequency curve in mice varies slightly with different experimental models (9, 16). Our results would suggest a positive force-frequency relationship in the 300- to 400 beats/min range and a plateau in the 400- to 500 beats/min range in isolated mouse hearts, consistent with a recent study by Headrick et al. (13).

Pacing itself increased the rate of ischemic ATP depletion from -3.6 to -5.4% initial ATP/min in A₃AR-overexpressor hearts and from -5.1 to -7.1% initial ATP/min in wild-type hearts over the 15-min ischemic period. As can be seen from these values, despite the equivalent contractility in paced A₃AR-overexpressor hearts compared with paced wild-type hearts, ATP depletion remained slower in the A₃AR-overexpressor hearts at -5.4% initial ATP/min in paced A₃AR-over-expressor hearts compared with -7.1% initial ATP/min in paced wild-type hearts. Likewise, the postischemic recovery of ATP, PCr, and contractile function remained greater in the paced A₃AR-overexpressor hearts compared with paced wild-type hearts (Figs. 1 and 3). From these results, we can conclude that the preservation of ischemic ATP and the reduced postischemic contractile and energetic dysfunction in the A₃AR-overexpressor hearts were not due to the decreased basal heart rate and contractility induced by A₃AR overexpression.

Effect of A₃AR Inhibition on Contractility, Energetics, and Postischemic Dysfunction in A₃AR-Overexpressor Versus Wild-Type Hearts

To determine whether the ischemic ATP preservation and cardioprotection observed in A₃AR-overexpressor hearts was due specifically to A₃AR signaling, and not to secondary alterations induced by the presence of the transgene, paced hearts were pretreated with 60 nmol/l of a canine A₃AR-specific inhibitor, MRS-1220. The concentration of MRS-1220 was chosen on the basis of the MRS-1220 inhibitor constant for the canine A₃AR of 6 nmol/l (18) and on preliminary dose-response studies in isolated hearts. The 60 nmol/l concentration had no effect on contractile function; LVDP, +dP/dt, and -dP/dt being the same before and after infusion of 60 nmol/l MRS-1220 in both paced wild-type and paced A₃AR-overexpressor hearts (Table 1).

Pretreatment with MRS-1220 completely abolished the ischemic ATP preservation and the greater postischemic recovery of ATP, PCr, and contractile function in the A₃AR-overexpressor compared with wild-type hearts (Figs. 1 and 3). It appears, therefore, that the preservation of ischemic ATP levels and the protection from postischemic contractile and energetic dysfunction observed in the A₃AR-overexpressor hearts were mediated specifically by the A₃AR and were not due to adaptive alterations induced by long-term A₃AR over-expression.

In summary, we have demonstrated that cardiac-specific overexpression of the A₃AR results in decreased basal heart rate and contractility, short-term ischemic ATP preservation, and protection from postischemic contractile and energetic dysfunction. Depressed preischemic contractility can itself be protective. However, by pacing hearts at a rate that resulted in similar contractile function in the A₃AR-overexpressor hearts as in wild-type hearts, we demonstrated that the depressed contractility had no role in the ischemic ATP preservation and cardioprotection in the A₃AR-overexpressor hearts. By pretreating hearts with the A₃AR-specific inhibitor MRS-1220, we also demonstrated that the energetic and functional effects observed in the A₃AR-overexpressor hearts were mediated specifically by the A₃AR. Together, these results imply that myocardial A₃AR signaling is cardioprotective and leads to preservation of ischemic ATP levels. Because cardioprotection was coincident with decreased ATP depletion in all our experiments, the decreased ATP depletion may be the mechanism by which A₃AR signaling leads to cardioprotection. Interestingly, decreased ATP depletion during ischemia was also found to be coincident with protection in A₁AR-overexpressor hearts (12), suggesting that the A₃AR and A₁AR may mediate protection via a common mechanism of energy preservation.

To our knowledge, this is the first study to assess the energetic consequences of cardiac-specific overexpression of the A₃AR and is also the first study to determine the effects of A₃AR overexpression on postischemic contractile dysfunction after short-term ischemia. Our results support the use of cardiac-specific expression techniques. Previous studies showed that, although A₃AR agonists can have direct beneficial effects on isolated hearts (11), A₃AR signaling in mast cells may be detrimental to the myocardium via proinflammatory mechanisms (21, 25, 30), such that systemic A₃AR inhibition can actually be protective (3, 10). These studies highlight the necessity for cardiac-specific A₃AR targeting in future therapeutic approaches and also highlight the possible complications of using systemic A₃AR agonists as cardioprotective agents.

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Fig. 1.

Recovery of contractile function after 15-min (paced groups) or 20-min (unpaced groups) ischemia and 40-min reperfusion in wild-type (WT) and A₃AR-overexpressor (A3) mouse hearts. WT, unpaced WT hearts; WT paced, WT hearts paced at 480 beats/min; A3, unpaced A3 hearts; A3 paced, A3 hearts paced at 480 beats/min; WT paced+MRS 1220, WT paced hearts pretreated with A₃AR inhibitor MRS-1220; A3 paced+MRS 1220, A3 paced hearts pretreated with MRS-1220; LVDP, left ventricular developed pressure. Data are means $\pm$ SE. *Significant difference from corresponding WT group ($P < 0.001$).

Fig. 2. Myocardial intracellular levels of ATP (A) and phosphocreatine (PCr) (B) and intracellular pH (C) during 20-min ischemia and 40-min reperfusion in unpaced WT and A3 mouse hearts. Points are means $\pm$ SE. *Significant difference from corresponding WT group ($P < 0.05$).

Fig. 3. Myocardial intracellular levels of ATP (A) and PCr (B) and intracellular pH (C) during 15-min ischemia and 40-min reperfusion in paced WT and A3 mouse hearts with and without pretreatment with the A₃AR inhibitor MRS-1220. Points are means $\pm$ SE. *Significant difference from corresponding WT group ($P < 0.05$).

Table 1
Effect of A₃AR overexpression and A₃AR inhibition on basal myocardial contractile function

Group	Preinfusion				Postinfusion			
	Heart rate, beats/min	LVDP, cmH ₂ O	+dP/dt, cmH ₂ O/ms	-dP/dt, cmH ₂ O/ms	Heart rate, beats/min	LVDP, cmH ₂ O	+dP/dt, cmH ₂ O/ms	-dP/dt, cmH ₂ O/ms
Unpaced								
Wild type	390 ± 11	110 ± 6	3.9 ± 0.2	-3.0 ± 0.1				
A ₃	319 ± 16 [*]	87 ± 1 [*]	2.8 ± 0.1 [*]	-2.1 ± 0.1 [*]				
Paced								
Wild type	480 ± 0	113 ± 2	3.9 ± 0.1	-3.2 ± 0.1				
A ₃	480 ± 0	109 ± 2	3.8 ± 0.1	-2.8 ± 0.3				
Paced + MRS 1220								
Wild type	480 ± 0	103 ± 1	3.7 ± 0.2	-2.7 ± 0.3	480 ± 0	105 ± 3	3.5 ± 0.3	-2.7 ± 0.2
A ₃	480 ± 0	109 ± 3	3.6 ± 0.1	-2.8 ± 0.1	480 ± 0	109 ± 1	3.7 ± 0.1	-2.9 ± 0.1

Data are means ± SE, A₃AR, A₃ adenosine receptor; A₃, A₃AR overexpressor; LVDP, left ventricular developed pressure; +dP/dt, rate of contraction; -dP/dt, rate of relaxation.

[†]Significant difference between postinfusion and corresponding preinfusion value ($P < 0.05$).

^{*}Significant difference from corresponding wild-type group ($P < 0.001$)