Reduced oxygen supply explains the negative force-frequency relation and the positive inotropic effect of adenosine in buffer-perfused hearts

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Reduced oxygen supply explains the negative force-frequency relation and the positive inotropic effect of adenosine in buffer-perfused hearts. In the present study, we compared the effects of changes in heart rate and adenosine on the left ventricular pressure of isolated rat hearts perfused with HEPEES and red blood cell-enriched buffers. We examined changes in left ventricular pressure induced by increases in heart rate or infusion of adenosine to investigate whether the negative force-frequency relation and the positive inotropic effect of adenosine are related to an inadequate oxygen supply provided by crystalloid perfusates. Hearts perfused with HEPEES buffer at a constant flow demonstrated a negative force-frequency relation, whereas hearts perfused with red blood cell-enriched buffer exhibited a positive force-frequency relation. In contrast, HEPEES buffer-perfused hearts showed a concentration-dependent increase in left ventricular systolic pressure [EC50 = 7.0 ± 1.2 nM, maximal effect (Emax) = 104 ± 2 and 84 ± 2 mmHg at 0.1 μM and baseline, respectively] in response to adenosine, whereas hearts perfused with red blood cell-enriched buffer showed no change in left ventricular pressure. The positive inotropic effect of adenosine correlated with the simultaneous reduction in heart rate (r = 0.67, P < 0.01; EC50 = 3.8 ± 1.4 nM, baseline 228 ± 21 beats/min to a minimum of 183 ± 22 beats/min at 0.1 μM) and was abolished in isolated hearts paced to suppress the adenosine-induced bradycardia. In conclusion, these results indicate that the negative force-frequency relation and the positive inotropic effect of adenosine in the isolated rat heart are related to myocardial hypoxia, rather than functional peculiarities of the rat heart.

A contradictory finding reported in studies using isolated hearts of small rodents is the negative force-frequency relation. Factors such as metabolic support (12, 31), calcium concentration (10, 22), and frequency range (4) have been shown to influence the nature of the force-frequency relation in isolated hearts of such animals. In addition, the demonstration that adequately oxygenated cardiac muscle shows a positive, instead of negative, force-frequency relation (4, 11, 17) raises the possibility that inadequate oxygen supply may be an important factor in the negative force-frequency relation of rat heart. However, the contribution of myocardial hypoxia to this phenomenon has not been elucidated.

Another conflicting finding in isolated rodent hearts is the positive inotropic effect of adenosine (23, 24, 27). Adenosine is known to exert direct and indirect effects on cardiac function through specific receptor subtypes (33, 35). Activation of the A1 receptor decreases heart rate, conduction rate, and adrenergic responsiveness and mediates cardioprotection (5, 6, 15, 25), whereas the A2 receptor mediates coronary vasodilation (2, 16) and activation of the A3 receptor results in cardioprotection (19, 20). In addition, it has been shown (23) that activation of A2A receptors elicits a positive inotropic effect accompanied by increases in adenylyl cyclase activity, cAMP accumulation, and myocyte twitch amplitude (7, 18, 34, 37). Conflicting data, however, also show that adenosine or A2 receptor agonists do not increase the contractility of isolated ventricular myocytes, ventricular strips, or isolated hearts from small rodents (14, 26, 32, 36). Moreover, it has recently been shown that the maximal increase in developed pressure, induced by adenosine as well as by the A2A agonist 5′-(N-ethylcarboxamido)adenosine, is preserved in isolated hearts from A2A receptor knockout mice (24), suggesting that the increased contractile performance induced by adenosine might be caused by a mechanism other than direct A2A-receptor activation. Conceivably, the adenosine-induced positive inotropic effect might be related to a reduction in myocardial oxygen consumption and consequent improvement of myocardial energetic status that accompanies the reduction in heart rate evoked by adenosine. However, the influence of myocardial hypoxia or bradycardia on the contractile effect of adenosine in isolated hearts remains unknown.

In the present study, we compared the effects of changes in heart rate and adenosine on the left ventricular pressure of isolated rat hearts perfused with HEPEES and red blood cell-enriched buffers. Our findings indicate that addition of red blood cells to perfusates resulted in a positive force-frequency relation and abolished the positive inotropic effect of adenosine.
in the isolated rat heart. These findings imply that an inadequate oxygen supply may be the underlying cause of the negative force-frequency relation and the positive inotropic effect of adenosine in isolated rat heart perfused with crystalloid buffers.

**METHODS**

**Reagents**

Adenosine (Calbiochem, La Jolla, CA) was dissolved in HEPES buffer to achieve final perfusate concentrations of 1 pM–100 μM. HEPES and other chemicals were obtained from Merck (Darmstadt, Germany).

**Perfusate Preparation**

Isolated rat hearts were perfused with HEPES buffer (140 mM NaCl, 6 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM dextrose, 5 U/l insulin, and 20 mM HEPES, pH 7.4) or a red blood cell-enriched HEPES buffer that was modified from a previously published method (30). Briefly, HEPES buffer (140 mM NaCl, 6 mM KCl, 1.2 mM MgSO₄, 1.75 mM CaCl₂, 10 mM dextrose, 2.0 mM pyruvate, 5 U/l insulin, and 20 mM HEPES, pH 7.4) was prepared. Blood was withdrawn from a donor rabbit and centrifuged to remove plasma. The red blood cells were washed with physiological saline solution (pH 7.4) and centrifuged again. The cells were washed twice more and then resuspended in a protein solution (HEPES buffer with 5% bovine serum albumin). The resultant red blood cell suspension was mixed with additional buffer to achieve a final hematocrit of 5%.

**Isolated Heart Preparation**

All animals received care in compliance with the principles of laboratory animal care formulated by the university’s Animal Care and Use Committee. Male Wistar rats weighing 200–250 g were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a temperature-controlled surgical table. After injection of heparin sodium (500 U/kg iv), hearts were removed (8), and the aorta was cannulated with a 20-gauge catheter and positioned 2 mm above the coronary ostia and perfused with HEPES buffer or red blood cell-enriched HEPES buffer bubbled with 100% oxygen. Hearts were perfused at a constant flow rate (10 ml/min). Left ventricular diastolic pressure was maintained constant at 5 mmHg and continuously monitored (WINDAQ) through a water-filled latex balloon inserted into the lumen of the left ventricle via the left atrium. The distal end of the balloon-attached catheter was connected to a pressure transducer for intraventricular pressure monitoring. Ventricular function was determined from the left ventricular systolic pressure and the maximal first derivative of left ventricular pressure (dP/dt max). For cardiac pacing, stainless steel leads were placed in the aorta and right ventricle and connected to an electric stimulator, which was turned on as indicated in protocols. Adenosine was added to the HEPES buffer and infused via retrograde perfusion of the coronary artery.

![Fig. 1. Force-frequency relation of isolated rat heart perfused with red blood cell-enriched and HEPES buffers. A and B: left ventricular pressure (LVP) recording and average left ventricular systolic pressure (LVSP) of paced isolated hearts perfused with red blood cell-enriched buffer. C and D: representative example of LVP and average LVSP of paced isolated hearts perfused with HEPES buffer. *P < 0.05 vs. 180 beats/min.](http://ajpheart.physiology.org/ by 10.220.33.1 on April 10, 2017)
Experimental Protocols

**Group 1:** effect of changes in heart rate on left ventricular pressure of isolated rat hearts perfused with HEPES or red blood cell-enriched buffer. To test the influence of myocardial hypoxia on the force-frequency relation, isolated rat hearts were perfused at constant flow with HEPES \((n = 4)\) or red blood cell-enriched buffer \((n = 5)\). The right atria and vena cava were crushed to allow the heart rate to be controlled by external pacing. During the stabilization period (30 min), the isolated hearts were paced to maintain the heart rate at 240 beats/min. Then the heart rate was sequentially changed to 300, 240, and 180 beats/min while the left ventricular pressure was continuously recorded. Each stimulatory step lasted for 5 min.

**Group 2:** effect of adenosine on isolated hearts perfused with HEPES buffer. After stabilization, spontaneously beating isolated rat hearts \((n = 5)\) perfused with HEPES buffer at a constant flow rate were continuously infused with adenosine \((0.1 \mu M/min)\) via a parallel infusion line connected to an infusion pump. Left ventricular pressure was recorded for the next 20 min after the beginning of adenosine infusion. For the adenosine concentration-response protocol, after the stabilization period, the isolated rat hearts \((n = 4)\), perfused at constant flow, were infused with adenosine at 10 pM/min to 100 \(\mu M/min\) for 5 min at each dose.

**Group 3:** effect of pacing on the inotropic effect of adenosine in HEPES buffer-perfused hearts. To test the influence of changes in heart rate on the positive inotropic effect of adenosine, HEPES-perfused isolated rat hearts \((n = 5)\) were continuously infused with adenosine \((0.1 \mu M/min)\). After 10 min of adenosine infusion, the hearts were paced at 240 beats/min, which was close to the average baseline heart rate of isolated hearts. In the other subgroup, after the stabilization period, hearts \((n = 5)\) were paced at 240 beats/min before adenosine infusion was started. After 10 min of adenosine infusion, the hearts were unpaced and allowed to beat spontaneously for the next 10 min during adenosine infusion.

**Group 4:** effect of perfusion with red blood cell-enriched buffer on the inotropic effect of adenosine in isolated rat heart. To test the influence of perfusion on the inotropic effect of adenosine, isolated rat hearts perfused with red blood cell-enriched buffer were subjected to experimental protocols identical to those described for groups 2 and 3.

**Statistical Analysis**

Values are means ± SE. Two-way ANOVA with repeated measures was performed to test possible differences in the within-group and between-group pre- and postinterventions. ANOVA was performed to compare the absolute changes (postintervention vs. preintervention) between groups. Two-way ANOVA with repeated measures was performed to test differences (preintervention vs. postintervention) within each group. When significance was found, Bonferroni’s corrected t-test post hoc comparison was performed. \(P \leq 0.05\) was considered statistically significant.

**RESULTS**

Perfusion With Red Blood Cell-Enriched Buffer Abolished the Negative Force-Frequency Relation of Isolated Rat Heart

Because the negative force-frequency relation of the Langendorff buffer-perfused rat heart might be related to hypoxia, we examined the effects of changes in heart rate on the left ventricular systolic pressure in hearts perfused with red blood cell-enriched buffer compared with those perfused with HEPES. Although the baseline heart rate was similar in both groups, baseline left ventricular systolic pressure was higher in hearts perfused with red blood cell-enriched buffer (14%) than

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**Fig. 2.** Representative example of positive inotropic effect induced by adenosine, as shown by actual LVP recording of spontaneously beating isolated rat heart perfused with HEPES buffer before and after infusion of adenosine at 0.1 \(\mu M/min\). Corresponding heart rates are indicated above pressure waves.

**Fig. 3.** A and B: adenosine concentration effects on LVSP and heart rate (HR) of isolated rat heart perfused at constant coronary flow and left ventricular diastolic pressure. Perfused adenosine concentrations ([ADO]) varied from 10 pM to 100 \(\mu M\). \(E_{\text{max}},\) maximal effect. C: relation between LVSP and HR at increasing adenosine concentration. Linear regression analysis indicated a negative correlation.

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AJP-Heart Circ Physiol • VOL 289 • JULY 2005 • www.ajpheart.org
in hearts perfused with HEPES buffer (Table 1). As demonstrated in the representative examples and average values in Fig. 1, isolated rat hearts perfused with red blood cell-enriched buffer (Fig. 1, A and B) showed a positive relation between left ventricular systolic pressure and heart rate, instead of the negative relation in hearts perfused with HEPES buffer (Fig. 1, C and D).

Adenosine Reduces Heart Rate and Enhances Contractile Performance of Isolated Rat Heart

Figure 2 illustrates the effect of adenosine on left ventricular pressure and heart rate of spontaneously beating isolated rat hearts perfused with HEPES buffer at constant flow. Adenosine infusion increased left ventricular systolic pressure and reduced heart rate. The average values of left ventricular systolic pressure, dP/dt max, and heart rate of hearts perfused with HEPES buffer under the influence of adenosine are shown in Table 1. Adenosine concentration-response curves for left ventricular systolic pressure and heart rate are shown in Fig. 3, A and B, respectively. EC50 values for left ventricular systolic pressure and heart rate were similar, whereas the maximal effect occurred at 0.1 µM adenosine. A significant negative correlation (r = 0.62, P < 0.01) was found between left ventricular systolic pressure and heart rate in response to 10 pM–100 µM adenosine, indicating the interdependence of the increases in left ventricular systolic pressure and the progressive bradycardia induced by increasing concentrations of adenosine (Fig. 3C).

Positive Inotropic Effect of Adenosine is Abolished by Suppression of Bradycardia

Experiments were performed to directly test the influence of changes in heart rate on the positive inotropic effect of adenosine. In hearts perfused with adenosine at constant flow, restoration of the heart rate to a value similar to that in controls by external pacing reduced left ventricular systolic pressure to levels close to those seen at baseline (Fig. 4A). In contrast, in hearts paced at 240 beats/min, adenosine had no significant effect on left ventricular systolic pressure (Fig. 4B). Consistent increases in left ventricular systolic pressure (from 83 ± 3.1 to 102 ± 3.6 mmHg, P < 0.05) occurred, however, after these hearts were sequentially allowed to beat spontaneously.

Perfusion With Red Blood Cell-Enriched Buffer Abolished the Adenosine Positive Inotropic Effect

Because the bradycardia-induced positive inotropic effect may be related to an improvement in cardiac mechanical performance due to a reduction in the myocardial oxygen demand, we next examined whether perfusion with red blood cell-enriched buffer modified adenosine positive inotropic and negative chronotropic effects. Adenosine reduced the heart rate to comparable levels in hearts perfused with HEPES and red blood cell-enriched buffers (Table 1). However, adenosine produced no significant change in left ventricular systolic pressure in hearts perfused with red blood cell-enriched buffer. Restoration of heart rate to control levels increased left ventricular systolic pressure by ∼13% in hearts perfused with red blood cell-enriched buffer. Furthermore, in hearts perfused with red blood cell-enriched buffer and paced to maintain the heart rate at 240 beats/min, adenosine had no significant effect on left ventricular systolic pressure (Fig. 5B). After these hearts were allowed to beat spontaneously, however, left ventricular systolic pressure was reduced by ∼10%.

Fig. 4. Influence of changes in heart rate on positive inotropic effect of adenosine in isolated hearts perfused with HEPES buffer. A: average LVSP and HR before and during infusion of adenosine in sequentially unpaced and paced hearts. CT, control. B: average LVSP and HR before and during infusion of adenosine in sequentially paced and unpaced hearts. *P < 0.05 vs. baseline. †P < 0.05 vs. after adenosine infusion.

Fig. 5. Influence of perfusion with red blood cell-enriched buffer on inotropic effect of adenosine in isolated hearts. A: average LVSP and HR before and during infusion of adenosine in sequentially unpaced and paced hearts. B: average LVSP and HR before and during infusion of adenosine in sequentially paced and unpaced hearts. *P < 0.05 vs. baseline. †P < 0.05 vs. after adenosine infusion.
DISCUSSION

In the present study, the force-frequency relation and the effect of adenosine on contractile activity were comprehensively examined in isolated rat hearts perfused with HEPES and red blood cell-enriched buffers. Our data show increased left ventricular pressure with higher heart rate and abolition of the positive inotropic effect of adenosine in hearts perfused with red blood cell-enriched buffer in contrast to the reduced left ventricular pressure with higher heart rate and positive inotropic effect of adenosine in hearts perfused with HEPES buffer. These results are consistent with the notion that the negative force-frequency relation and the positive inotropic effect of adenosine in isolated rat hearts perfused with crystalloid buffer are related to the reduced myocardial oxygen supply, rather than to distinct features of the rat heart.

Force or pressure has been shown to decrease with higher stimulation frequency in isolated myocardial strips and hearts from small rodents (10, 12). This seems contradictory to the physiological requirement of such species, because the frequency at which cardiac muscle produces maximum force would be expected to fall within the physiological range of their heart rates. Nevertheless, the negative force-frequency relation has been suggested (3) to be related to peculiarities of intracellular calcium kinetics of small rodents. In contrast, our present data indicate that, under conditions of adequate oxygen supply, the force-frequency relation in the rat heart is indeed positive, as demonstrated by the differences in the force-frequency relation between hearts perfused with HEPES buffer and those perfused with red blood cell-enriched buffer. Although the myocardial oxygen concentration and distribution were not directly assessed in the present study, previous evidence indicates that the oxygen concentration of isolated hearts from small rodents perfused with crystalloid buffer is inadequate or just above the threshold for impaired myocardial function (1, 24, 29, 30). In addition, it has been shown that perfusion with red blood cell-enriched buffer (30) may improve myocardial distribution and mean myoglobin saturation as well as resting cardiac performance. Accordingly, our finding that left ventricular pressure is higher in hearts perfused with red blood cell-enriched buffer than in those perfused with HEPES buffer implies an improved baseline mechanical performance, presumably due to an improved oxygen supply. Thus our present data indicate that an inadequate myocardial oxygen supply of crystalloid perfusates conceals a true positive force-frequency relation in the rat heart, which is fully manifested when the myocardial oxygen supply is improved by perfusion with red blood cell-enriched buffer.

The results of the present study were extended to show that the positive inotropic effect of adenosine in isolated rat heart is also related to an inadequate myocardial oxygen supply provided by crystalloid buffers. This was indicated by our demonstration that such an effect was absent in hearts perfused with red blood cell-enriched buffer. Further support for this assumption was provided by the demonstration that the adenosine-induced increase of left ventricular pressure was abolished by suppression of the bradycardia by external pacing of isolated rat hearts perfused with HEPES buffer. Given that the adenosine-induced increases in left ventricular pressure were paralleled by reductions in heart rate, one may argue that the positive inotropic effect of adenosine in isolated hearts perfused with crystalloid buffers is related to better contractile performance as a result of an improvement in myocardial energetic status linked to the reduction in heart rate. However, one cannot exclude, on the basis of the experimental design of this study, the possibility that adenosine has a direct, albeit minor, positive inotropic effect. Indeed, it has been previously shown (7, 18, 34, 37) that the positive inotropic effect of adenosine and A_2A receptor agonists in isolated papillary muscle is characterized by small, which contrasts with the moderate positive inotropic effect in isolated hearts.

In conclusion, the present results demonstrate that addition of red blood cells to the perfusate improves the function of the isolated rat heart, changing the force-frequency relation to positive and considerably attenuating the positive inotropic effect of adenosine. Taken together, these results indicate that the reduced oxygen-carrying capacity of crystalloid buffers may result in an inadequate energetic status, which may affect the contractile performance and influence the responses of isolated rat heart to physiological and pharmacological challenges.

GRANTS

This study was sponsored by grants from Fundação de Auxílio à Pesquisa do Estado de São Paulo (Proc. 01/11698-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Proc. 521098/97-1).

REFERENCES

14. Kilpatrick EL, Narayan P, Mentzer RM Jr, and Lasley RD. Cardiac myocyte adenosine A_2a receptor activation fails to alter CAMP or contrac-


