Free Rad. Res., Vol. 24, No. 1, pp. 31–38
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The Effect of Exogenous Adenosine on Functional Injury Caused by Hydrogen Peroxide in the Isolated Rat Heart

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Accepted by Professor Dr H. Esterbauer

(Received November 24th, 1994; in revised form, May 19th, 1995)

Adenosine is an endogenous cardioprotective substance. The present study examines whether exogenous adenosine attenuates cardiac injury induced by oxidative stress. Rat hearts (Langendorff model) were perfused with H_2O_2 (180 μM) for 10 min, then recovered for 60 min (n = 10). In other groups adenosine 55 μM, 11 0 μM, or 220 μM (n = 10 in each) was given in addition to H_2O_2 throughout perfusion. Control perfusion with Krebs Henseleit only (n = 7), adenosine 110 μM throughout perfusion (n = 7), and adenosine 110 μM as an intervention (n = 7) was performed. The hearts were paced at 320 beats/min. Left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures were measured together with coronary flow (CF), and left ventricular developed pressure (LVDP = LVSP − LVEDP) was calculated. H_2O_2 decreased LVSP from 105 ± 8 to 60 ± 5 mmHg (mean ± SEM) after 10 min infusion ($p<0.008$). Adenosine did not attenuate the decrease of LVSP. LVEDP increased from 0 to 59 ± 10 mmHg ($p<0.004$) and 62 ± 11 mmHg 5 and 15 min after end of infusion of H_2O_2, respectively. Neither 55 μM nor 220 μM adenosine inhibited the H_2O_2-induced increase of LVEDP. Adenosine 110 μM attenuated the increase after 15 (15 ± 4 mmHg, $p<0.004$) and 25 min observation (26 ± 7 mmHg, $p<0.012$). Adenosine did not attenuate the reduction of LVDP. CF initially increased during infusion of H_2O_2, thereafter decreased. Hearts given adenosine had higher basal CF, and CF did not increase after H_2O_2. Control perfusion with adenosine, given throughout perfusion or as an intervention, increased CF and tended to increase LVSP. In summary, adenosine did not inhibit H_2O_2-induced depression of contractility or reduction of CF. One concentration of adenosine (110 μM) attenuated H_2O_2-induced impairment of relaxation. Exogenous adenosine does not have an important influence on functional injury caused by exogenous oxidants.

Key words: Adenosine, antioxidant, hydrogen peroxide, ischaemia-reperfusion, toxic oxygen metabolites

INTRODUCTION

Ischaemia-reperfusion of the heart induces an inflammatory response with a cascade of injurious mediators, of which toxic oxygen metabolites (TOM) may be the most important.[1–5] Cardioprotective substances, such as adenosine, are also released from the ischaemic-reperfused heart.[6,7] Adenosine attenuates myocardial

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stunning,[8] is suggested to initiate and mediate preconditioning,[7] reduces infarct size,[7] and inhibits microvascular stunning.[8–10] Adenosine has several mechanisms of action: Induces coronary vasodilation, reduces myocardial oxygen demand, increases uptake of glucose, and replenishes depleted ATP-stores.[8,11,12] Furthermore, adenosine protects the endothelium by inhibiting platelet activation, leukocyte adherence and extravasation, and inhibits formation and release of TOM from activated leukocytes.[8, 12–15] Adenosine has also been suggested to inhibit the cardiotoxic effects of TOM by adenosine A_1 receptor activation.[16]

At present adenosine receptor agonists are not clinically available. Adenosine levels in patients can be increased by infusion,[17] or by pharmacologically increasing the contents of adenosine in ischaemic tissue.[18] Consequently, it is important to investigate if adenosine directly attenuates oxidant injury.

This study investigates the effects of adenosine on cardiac dysfunction induced by exogenous TOM in isolated rat hearts. Hearts were perfused with H_2O_2, and three different concentrations of adenosine were added to the perfusate.

METHODS

Heart Perfusion

The study was approved of by the Ethical Committee for Animal Research at the University of Tromsø, Tromsø, Norway. Male Wistar rats (250–300 g) were anaesthetized with diethyl ether. Heparin (200 IU) was injected into the femoral vein. The hearts were excised through a median sternotomy and placed in ice-cold buffer during preparation for aortic cannulation. The average time of ischaemia before start of perfusion was 80 seconds. Modified Langendorff perfusion was performed with Krebs Henseleit buffer (NaCl 118.5 mM, $NaHCO_3$ 25.0 mM, KCl 4.7 mM, KH_2PO_4 1.2 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM, Glucose $\cdot H_2O$ 11.1 mM, $CaCl_2 \cdot 2H_2O$ 2.4 mM). Perfusion pressure was kept constant at 100 cm H_2O, and the perfusate was continuously gassed with 5% CO_2 and 95% O_2. Heated water jackets around the perfusate reservoirs and heart chamber maintained the temperature at 37°C. Two perfusate reservoirs were employed to rapidly change perfusates at intervention. Isovolumetric recordings of left ventricular peak systolic (LVSP) and end-diastolic (LVEDP) pressures were made via a balloon in the left ventricle, introduced through the left atrium. The volume of the balloon (approximately 0.1 ml H_2O) was adjusted in the height of the left ventricle before insertion into each heart to obtain a pressure of 0 mmHg. Hearts that did not have LVEDP of 0 mmHg after 15 min stabilization were discarded. Left ventricular developed pressure (LVDP = LVSP – LVEDP) was calculated. Coronary flow (CF) was measured by timed collections of the coronary effluent. The hearts were paced via an electrode in the left ventricular epicardium at a frequency of 320 beats/min to overcome the negative chronotropic effects of adenosine. Adenosine (crystalline, Sigma Chemical Company, MO, USA) was dissolved in buffer and administered via one perfusate reservoir throughout perfusion unless otherwise stated. H_2O_2 (3% solution) was added to buffer alone or buffer with adenosine and administered via the second perfusate reservoir.

Experimental Protocol

After start of perfusion the hearts were stabilized for 20 min before intervention (defined as time 0). Only hearts with LVSP between 60–160 mmHg, LVEDP 0 mmHg and CF over 6 ml/min after 15 min stabilization were included in one of the following groups:

1) (n = 10) H_2O_2 (180 μM) was given from time 0 for 10 min, followed by a 60 min recovery period.
2) (n = 10) H_2O_2 given as described above, but with adenosine (55 μM) added to the buffer throughout perfusion.

3) (n = 10) H_2O_2 as in group 1, with addition of adenosine 110 μM.
4) (n = 10) H_2O_2 as group 1, with adenosine 220 μM.
5) (n = 7) Control perfusion for 70 min with buffer only.
6) (n = 7) Control perfusion with adenosine 110 μM throughout perfusion.
7) (n = 7) Control perfusion with adenosine 110 μM added from time 0 and onwards.

Measurements of LVSP, LVDP, LVEDP, and CF were made after 15 min stabilization (time −5), at time 0, and after 5, 10, 15, 25, 40, and 70 min observation. Only values after 0, 10, 25, and 70 min are presented in the tables for didactic reasons. Some of the hearts perfused with H_2O_2 had severe arrhythmias (asystolia or ventricular fibrillation) during recovery. Severe arrhythmias were evaluated from the pressure curve as a pressure generation <4 mmHg. Values from hearts with severe arrhythmias were excluded from the haemodynamic comparisons. The number of hearts being compared at each time point is thus n = 10 − number of arrhythmic hearts at that time shown in Table 1.

Statistics

A Mann-Whitney test for comparison of independent samples was used to evaluate differences between groups, and Wilcoxon's Signed Rank test was used for evaluation within groups at different time points. The p-values were adjusted according to the Bonferroni method,[19] and $p<0.0125$ was considered significant at a $p<0.05$ level in comparison between groups 1–4. For comparison between groups 5–7, $p<0.017$ was considered significant. In evaluation within groups at different time points $p<0.008$ was considered significant. Values are presented as mean ± SEM.

TABLE 1 The number of Langendorff-perfused rat hearts with ventricular fibrillation or asystolia after infusion of H_2O_2 (180 μM) for 10 minutes from time 0, followed by recovery for 60 min. The effects of H_2O_2 alone (group 1, n = 10), or H_2O_2 in addition to adenosine 55 μM (group 2, n = 10), adenosine 110 μM (group 3, n = 10), or adenosine 220 μM (group 4, n = 10) are shown.

Time (min)	0	10	25	70
Group 1	0	2	2	4
Group 2	0	0	2	7
Group 3	0	0	1	0
Group 4	0	1	3	3

There were no statistical differences in comparison between group 1 and the other groups.

RESULTS

Arrhythmias

Severe arrhythmias were observed in few hearts during administration of H_2O_2. After return to buffer perfusion, severe arrhythmias which were not modified by adenosine occurred. The number of hearts in groups 1–4 with severe arrhythmias at the various time points are shown in Table 1. There were no significant differences between groups.

Left Ventricular Systolic Pressure

At time 0 LVSP was similar in groups 1–4 (Table 2), and in groups 5–7 (Table 3). H_2O_2 decreased LVSP in group 1 ($p<0.008$ after 10 min), and LVSP improved during recovery (Table 2). Addition of adenosine (55 μM, 110 μM, or 220 μM, groups 2–4) did not attenuate the H_2O_2-induced depression of LVSP (Table 2).

There was a gradual reduction of LVSP during perfusion in group 5 (Table 3). Control perfusion with adenosine (group 6) tended to increase LVSP compared to group 5, significantly so after 25 min ($p<0.016$) (Table 3). When adenosine was given as an intervention (group 7), LVSP did not increase (Table 3).

Left Ventricular End-diastolic Pressure

LVEDP was 0 mmHg in all groups at time 0. H_2O_2 increased LVEDP to 28 ± 10 mmHg after 10 min. After end of H_2O_2-perfusion LVEDP continued to

TABLE 2 Left ventricular systolic pressure (LVSP, mmHg), left ventricular developed pressure (LVDP, mmHg) and coronary flow (CF, ml/min) in Langendorff-perfused rat hearts exposed to H_2O_2 for 10 min (group 1), and H_2O_2 with addition of adenosine 55 μM (group 2), 110 μM (group 3), or 220 μM (group 4). n = 10 in each group initially, but values from hearts with severe arrhythmias are excluded (see Table 1 for number).

	0 min	10 min	25 min	70 min
LVSP				
Group 1	105 ± 8	60 ± 5*	84 ± 7	73 ± 6
Group 2	105 ± 5	51 ± 6	93 ± 7	81 ± 7
Group 3	104 ± 4	50 ± 4	77 ± 4	68 ± 5
Group 4	124 ± 8	73 ± 5	99 ± 6	73 ± 7
LVDP				
Group 1	105 ± 8	43 ± 5*	35 ± 3*	39 ± 2
Group 2	105 ± 5	32 ± 3	42 ± 6	51 ± 6
Group 3	104 ± 4	46 ± 5	49 ± 5	43 ± 5
Group 4	124 ± 8	25 ± 4**	41 ± 9	36 ± 9
CF				
Group 1	14 ± 11	15 ± 1	6 ± 0.3*	5 ± 0.5
Group 2	21 ± 1**	18 ± 1	8 ± 1	10 ± 2
Group 3	17 ± 1**	16 ± 1	8 ± 1	6 ± 1
Group 4	20 ± 1**	11 ± 1**	7 ± 1	5 ± 1

Values are mean ± SEM. * denotes $p<0.05$ compared to initial value, ** denotes $p<0.05$ compared to group 1.

increase, to 59 ± 10 mmHg 5 min after end of H_2O_2 (15 min observation, $p<0.004$), and to 62 ± 11 mmHg after 25 min observation (Figure 1). Addition of adenosine 55 μM and 220 μM (groups 2 and 4) did not inhibit the H_2O_2-induced increase of LVEDP (Figure 1). However, adenosine 110 μM attenuated the increase of LVEDP after 15 (15 ± 4 mmHg, $p<0.004$ compared to group 1) and 25 min observation (26 ± 7 mmHg, $p<0.012$). There was no difference between groups after 70 min observation (Figure 1). LVEDP remained 0 mmHg throughout perfusion in groups 5–7 (data not shown).

Left Ventricular Developed Pressure

Before start of intervention (time 0) LVDP was equal to LVSP. H_2O_2 decreased LVDP after 10 min infusion ($p<0.008$), and LVDP was still decreased after 25 min observation ($p<0.008$)

TABLE 3 Left ventricular systolic pressure (LVSP, mmHg) and coronary flow (CF, ml/min) in Langendorff-perfused rat hearts. The effects of control perfusion with buffer only (group 5, n = 7), with adenosine 110 μM throughout perfusion (group 6, n = 7), and adenosine 110 μM given from time 0 (group 7, n = 7) are shown.

	0 min	10 min	25 min	70 min
LVSP				
Group 5	93 ± 8	88 ± 7	87 ± 5	78 ± 5
Group 6	109 ± 5	112 ± 5	106 ± 4**	83 ± 4
Group 7	96 ± 7	115 ± 7	112 ± 7	85 ± 5
CF				
Group 5	12 ± 1	10 ± 0.5	9 ± 0.3	8 ± 0.4
Group 6	21 ± 1**	21 ± 1**	21 ± 2**	21 ± 2**
Group 7	12 ± 1	17 ± 1**	16 ± 1**	11 ± 2

Values are mean ± SEM. ** denotes $p<0.05$ compared to group 5.

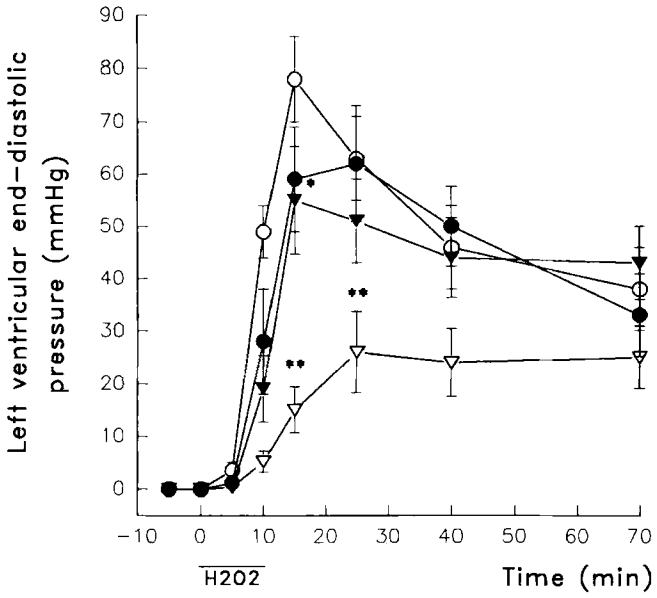

FIGURE 1 Left ventricular end-diastolic pressure in isolated rat hearts perfused with H_2O_2 (180 μM) for 10 min followed by recovery for 60 min (●—●), or H_2O_2 with addition of adenosine 55 μM (▼—▼), 110 μM (▽—▽), or 220 μM (○—○) given throughout perfusion. n = 10 initially in all groups, but values from hearts with severe arrhythmias are excluded (see Table 1 for numbers) * denotes $p<0.05$ compared to initial value, ** denotes $p<0.05$ compared to H_2O_2 alone.

(Table 2). There was no attenuation of the H_2O_2-induced depression of LVDP in groups 2 and 3 (Table 2). Adenosine 220 μM (group 4) tended to decrease LVDP further, significant after 10 min ($p<0.01$) (Table 2).

In control perfused hearts (groups 5–7) LVDP remained the same as LVSP throughout perfusion (Table 3).

Coronary Flow

Adenosine given from start of perfusion increased CF compared to perfusion with Krebs Henseleit only at time 0. CF was higher in group 2 ($p<0.003$), group 3 ($p<0.002$), and group 4 ($p<0.002$) than in group 1 (Table 2). H_2O_2 increased CF to 22 ± 1 ml/min after 5 min infusion ($p<0.004$ compared to initial value). CF then decreased compared to initial value after 25 min observation ($p<0.008$) (Table 2). CF did not increase in groups 2–4 after infusion of H_2O_2. CF was actually reduced after 10 min of H_2O_2 in group 4 ($p<0.001$ compared to group 1) (Table 2).

There was a gradual reduction of CF in group 5 (Table 3). Adenosine 110 μM throughout perfusion (group 6) increased CF compared to controls at time 0 ($p<0.003$), and at all other times of observation ($p<0.002$ at all time points) (Table 3). Addition of adenosine at time 0 (group 7) increased CF after 5, 10, 15, 25, and 40 min ($p<0.002$, $p<0.002$, $p<0.002$, $p<0.002$ and $p<0.016$, respectively) (Table 3).

DISCUSSION

To investigate if exogenous adenosine had direct effects on oxidant stress, infusion of H_2O_2 for 10 minutes was employed as a convenient and reproducible model of TOM-induced injury. H_2O_2 has been measured in cardiac tissue after ischaemia-reperfusion injury to isolated rat and rabbit

hearts,[20,21] the level corresponding to loss of ventricular function during reperfusion.[20] H_2O_2 may be the main injurious TOM released by activated leukocytes.[22,23] Exogenous H_2O_2 dose-dependently influences cardiac function, ranging from only coronary vasodilation to severe impairment of left ventricular function (unpublished results). In the present study a high concentration of H_2O_2 was chosen, in order to detect a possible attenuation of oxidant injury by adenosine.

The exact mechanism of injury in this model is presently unknown. H_2O_2 may have direct toxic effects, or other TOM may be generated. We have previously inhibited all functional impairment of isolated rat hearts caused by H_2O_2 with catalase,[24] a scavenger of H_2O_2,[1] and with thiourea,[25] a hydroxyl radical scavenger with some effect on H_2O_2.[26,27] Recently production of the hydroxyl radical was measured in isolated rat hearts perfused with H_2O_2.[28] Inhibition of the hydroxyl radical reduced biochemical and ultrastructural injury, while catalase additionally inhibited functional depression.[28] It is possible that H_2O_2 is the main injurious oxygen metabolite in the present study.

Three different concentrations of adenosine were given from start of stabilization in order to saturate the hearts, and throughout perfusion in addition to H_2O_2. The concentrations have previously protected against ischaemia-reperfusion injury in buffer-perfused, isolated hearts.[29,30] The hearts were paced in order to eliminate the inhibitory effects of adenosine on the conduction system.[8,11] Adenosine did not modify the H_2O_2-induced reductions of LVSP or LVDP. However, 110 μM adenosine attenuated the increase of LVEDP. Adenosine has previously delayed the onset of ischaemic contracture in models of ischaemia-reperfusion injury.[10,30,31] Our finding is in accordance with this. However, as only one of three relatively close concentrations of adenosine influenced the increase of LVEDP, the interpretation of this finding is difficult. Adenosine may have a bell-shaped dose-response curve in oxidant injury of the heart, explaining why the highest dose (220 μM adenosine) had no beneficial effect. Indeed the highest dose tended to exacerbate oxidant injury, since it decreased both LVDP and CF after perfusion with H_2O_2 for 10 min.

H_2O_2 increased CF after 5 min infusion. This increase was not seen when adenosine was given in addition to H_2O_2. H_2O_2 increases CF by release of endothelial derived relaxing factor (EDRF),[32] while vasodilation induced by adenosine is independent of EDRF.[33] The lack of H_2O_2-induced vasodilation in hearts pretreated with adenosine could be due to the hearts being maximally dilated before intervention, and not by inhibition of the H_2O_2-induced vasodilation per se. Adenosine did not influence reduction of CF during recovery.

Control perfusion with adenosine (110 μM) increased CF and tended to increase LVSP. This is in accordance with well known inotropic and vasodilatory properties of adenosine.[8,11]

Severe arrhythmias were observed after end of, but not during, infusion of H_2O_2. TOM may cause reperfusion arrhythmias.[34] In the present study either other TOM than H_2O_2, or intracellular injury secondary to H_2O_2 (membrane function deficiency, lipid peroxidation, disturbed calcium homeostasis) most likely mediate arrhythmias. Adenosine protects against ischaemia-reperfusion induced arrhythmias in other models.[35] There were no differences in occurrence of severe arrhythmias in hearts receiving H_2O_2 alone or in combination with adenosine in the present study.

Few works have attempted to elucidate if adenosine has direct antioxidant effects. A recent work by Karmazyn & Cook,[16] infusing 100 μM H_2O_2 for 30 min in isolated rat hearts with addition of two adenosine A_1 receptor agonists, concluded that adenosine A_1 receptor stimulation reduced functional injury induced by H_2O_2, possible by preservation of high-energy phosphates and adenine nucleotide contents.[16] However, Karmazyn & Cook employed small experimental groups ($n = 5$ or less) which were evaluated with parametric statistics.[16] A severe model of H_2O_2-induced functional injury with stop of pressure-generating beating was employed.[16] Possibly the effects of

selective adenosine A_1 receptor activation and administration of exogenous adenosine are unlike.

In conclusion, three different concentrations of adenosine did not inhibit the H_2O_2-induced depression of contractility or occurrence of severe arrhythmias. One concentration of adenosine attenuated the impairment of relaxation induced by H_2O_2, but the other two did not. Adenosine did not influence H_2O_2-induced changes in coronary flow. An antagonistic effect against oxidant injury does not appear to be a main component of the cardioprotective actions of adenosine.

Acknowledgements

G.V. has been a research fellow of the Norwegian Research Council. Further financial support was provided by the University of Tromsø.

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