Stimulation of endothelial adenosine A_1 receptors enhances adhesion of neutrophils in the intact guinea pig coronary system

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Objective: The primary aim was to determine the action of pathophysiologically relevant adenosine concentrations (0.1-1 μM) on adhesion of neutrophils to coronary endothelium. Further aims were to evaluate the nature and localisation of the adenosine receptor involved, and to assess the effect of endogenous adenosine. **Methods:** Adhesion was studied in isolated perfused guinea pig hearts by determining the number of cells emerging in the coronary effluent after intracoronary bolus injections of 600 000 neutrophils prepared from guinea pig or human blood. The system was characterised by the use of the proadhesive stimulus thrombin. **Results:** A 5 min infusion of adenosine (0.1-0.3 μM) or the A_1 receptor agonist N^6-cyclopentyladenosine (CPA, 0.01 μM) significantly increased adhesion from about 20% (control) to 30%. This effect was prevented by the A_1 receptor antagonist dipropyl-8-cyclopentylxanthine (DPCPX, 0.1 μM). It was not diminished by cessation of adenosine infusion 90 s prior to neutrophil injection. At a higher concentration of adenosine (1 μM), adhesion did not seem to be enhanced. However, coinfusion of the A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX, 0.1 μM) with 1 μM adenosine unmasked the A_1 action, adhesion rising to 39%. Adenosine had a quantitatively identical effect on adhesion of human neutrophils. Total ischaemia of 15 min duration raised adhesion of subsequently applied neutrophils to 35%. This effect was completely blocked by DPCPX, as well as by ischaemic preconditioning (3 × 3 min). Preconditioning raised initial postischaemic coronary effluent adenosine from about 0.8 μM to 1.5 μM. **Conclusions:** The findings suggest a bimodal participation of adenosine in the development of postischaemic dysfunction by an endothelium dependent modulation of neutrophil adhesion. Stimulation occurs via endothelial A_1 receptors at submicromolar adenosine levels, whereas cardioprotection by adenosine may in part relate to the use of pharmacologically high concentrations of adenosine or enhanced endogenous production after preconditioning.
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Adhesion of polymorphonuclear neutrophils to the vascular endothelium is a crucial event in inflammation and reperfusion injury.[1 2] The nature of the mediators involved in the case of cardiac reperfusion remains uncertain. Adenosine, which is produced by ischaemic tissues, is reported to have anti-inflammatory and protective effects after ischaemia when applied at concentrations of more than 1 μM.[3 4] However, the concentrations detected in coronary venous blood under circumstances such as coronary insufficiency or percutaneous transluminal coronary angioplasty (PTCA) range between 0.1 and 1 μM.[5 6] Moreover, in a recent study by our group, adenosine (0.1 to 0.2 μM) was found to be essential for inducing reperfusion damage by neutrophils in an isolated working heart preparation.[7]

In an attempt to resolve this discrepancy, the role of adenosine in neutrophil adhesion to intact coronary endothelium was examined at concentrations of 0.1 to 1 μM. Since a strong influence of shear stress on adhesive cell – cell interactions has become evident,[2 8] the approach chosen was to apply suspensions of guinea pig and human neutrophils to the complete coronary bed of isolated guinea pig hearts, perfused at physiological flow rates. This model also circumvents possible artefacts arising from alterations of endothelial cells during culture.[2] Another advantage concerns elaborating the site of action of adenosine. To date, only the effects of adenosine on neutrophils have been investigated,[9] neglecting the possibility of endothelium mediated responses. This is deemed to be of interest, because not only neutrophils[9] but also coronary endothelial cells possess both A_1 and A_2 adenosine receptors.[10 11] Selective prestimulation of the coronary vascular endothelium can be readily achieved in the perfusion model, avoiding contact of the infused neutrophils with adenosine.

The primary objectives of this study were: (1) to determine what effect adenosine has on neutrophil adhesion to coronary endothelium under shear stress conditions, with special consideration of the (patho)physiologically relevant concentrations of adenosine; (2) to characterise pharmacologically and to localise the adenosine receptor (A_1 *v* A_2 receptors; endothelium *v* neutrophil) responsible for a specific action; and (3) to investigate the consequences of ischaemia and preconditioning on neutrophil adhesion, defining the possible role of adenosine in both phenomena. The direct comparison of responses of guinea pig and human neutrophils served to elaborate the potential of the outlined perfusion model for investigating species differences in adhesion phenomena.

Methods

Materials

Human thrombin was purchased from Sigma. CPA (N^6-cyclopentyladenosine), DMPX (3,7-dimethyl-1-propargylxanthine), and DPCPX (dipropyl-8-cyclopentylxanthine) were obtained from Research Biochemicals. Iloprost was a gift from Schering AG, Berlin, Germany,

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and adenosine came from Boehringer Mannheim. Percoll was purchased from Pharmacia. Hydroxy-ethyl starch (HES) was from Fresenius, and [8-^{14}C]adenosine from Amersham Buchler. All other chemicals, including the salts for the buffer solutions, were obtained from Merck.

Isolation of neutrophils

Human neutrophils were isolated from venous blood of healthy donors, guinea pig neutrophils from fresh arterial blood, drawn, in both cases, into polypropylene syringes containing 0.1% EDTA (disodium ethylenediamine tetra-acetate) for anticoagulation. The blood was centrifuged for 15 min at 350 *g* and the platelet-rich plasma removed. The remaining cells were sedimented in 6% hydroxy-ethyl starch for 75 min and the supernatant was centrifuged at 350 *g* for 10 min. The pellet was resuspended in phosphate buffered saline (PBS) containing 0.1% EDTA and layered over Percoll (density 1.077 g·ml^{-1} for human cells and 1.082 for guinea pig neutrophils). After centrifugation at 400 *g* for 25 min and washing, the residual erythrocytes were destroyed by hypotonic lysis with 5 ml distilled water at 4°C (1 and 2 min for human and guinea pig neutrophils, respectively). The neutrophils were washed in PBS, centrifuged, and finally resuspended in tris buffered Tyrode solution. Purity (>95%) and viability (>95%) of the cell preparation was routinely controlled by light microscopy (Pappenheim stain and Trypan blue exclusion test). The cell count was determined and adjusted to a number of 600 000 neutrophils·ml^{-1} buffer. This cell suspension was drawn into a polypropylene syringe (10 ml) immediately before the experiment. To determine neutrophil numbers in Tyrode solution and in samples of venous effluent (see below), cells were immediately counted in triplicate with a Coulter counter ZM.

Though the cells obtained by this procedure cannot be regarded as completely unperturbed, they still presented uniformly excellent responses (chemiluminescence, aggregation, and chemotaxis) to stimulation with a chemotactic peptide.[12]

Heart preparation and perfusion

Hearts of male guinea pigs (body weight 200-300 g, stunned by neck dislocation) were isolated and perfused as "Langendorff" preparations at 37°C with a modified Krebs-Henseleit bicarbonate buffer, equilibrated with 94.4% O_2 and 5.6% CO_2 [pH 7.4 (SEM 0.02)]. The experimental details have been reported previously.[13] The veins entering the right atrium were ligated, ensuring that the perfusate emerging from the coronary sinus passed through the pulmonary artery. The latter was cannulated to enable the collection of the coronary effluent. The perfusion pressure was continuously registered with a pressure transducer.

Experimental protocol

Experiments conformed with the *Guide for the care and use of laboratory animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

A flow chart of the standard protocol is shown in fig 1. After the preparation and an initial period of 5 min of constant pressure perfusion at 60 mm Hg, the hearts were perfused at constant flow (5 ml·min^{-1}) for 30 min. In experiments with DPCPX and DMPX, the substances were present in the perfusate from the onset of perfusion. For the treatment of hearts with thrombin, adenosine, CPA, or iloprost, respective stock solutions in isotonic saline were infused after the 30 min into the aortic cannula at rates calculated to yield the desired end concentration in the coronary perfusate. These volumes never exceeded 50 µl·min^{-1}. After a further equilibration period of 5 min, a 1 ml bolus of the Tyrode solution containing the neutrophils was applied evenly over 1 min into the coronary system via the aortic cannula with an infusion pump, resulting in a flow of 6 ml·min^{-1} during the bolus. Thus about 100 000 neutrophils·ml^{-1} perfusate, or 600 000 neutrophils in total were infused. Coronary effluent was collected during the bolus and for the following 40 s to quantify the number of neutrophils leaving the coronary system (neutrophil output). Pilot studies had shown that only a negligible number of cells (< 1%) ever emerged in the following 5 min of perfusion. However, we have no knowledge as to whether the remaining cells were irreversibly retained or just severely retarded in washout.

In each case, a test bolus of equal volume and duration (1 ml, 1 min) was sampled without coronary passage immediately before the intracoronary bolus to determine the number of cells actually leaving the syringe (neutrophil input). The percentage of neutrophils remaining adherent to the endothelium was then calculated as:

$$[1 - (\text{output/input})] \times 100$$

To test the validity of our model, we assessed neutrophil adhesion in response to thrombin, a well characterised, rapidly acting (within 5 min) proadhesive agent.[14] We carried out these experiments with different neutrophil numbers (6×10^5 to 7×10^6 neutrophils per bolus) to establish whether percentual adhesion was influenced by the cell density. As this was not the case (n = 9, data not shown), we used the relatively low cell count of 6×10^5 per neutrophil bolus throughout our study.

In one particular group of experiments, the adenosine infusion was stopped after 5 min and the adenosine was washed out for 1.5 min before the injection of neutrophils. Since the half time of adenosine washout was about 10 s (fig 2), this procedure avoided contact between infused cells and infused adenosine.

In a further experimental set, neutrophils were incubated with 1 µM adenosine for 15 min prior to injection. The hearts to which these particular cells were applied received 0.1 µM adenosine starting 5 min before. The effective perfusate adenosine concentration during the neutrophil bolus therefore amounted to approximately 0.26 µM.

Myocardial ischaemia (global) was induced by interrupting perfusion for 15 min (zero flow) after the initial 30 min equilibration period. The temperature of the hearts was kept constant at 37°C during ischaemia by suspending them in warm Tyrode buffer. After 1 min of reperfusion (at 5 ml·min^{-1}), the neutrophil bolus was injected. Effluent samples for adenosine measurement were taken in 1 min intervals during the first 5 min of reperfusion.

Ischaemic preconditioning of the hearts was performed by interrupting coronary perfusion three times for 3 min, each ischaemia being followed by a reperfusion period of 5 min. After the third reperfusion phase, the heart were subjected to a final global ischaemia of 15 min duration. Again, the temperature of the hearts was kept constant throughout the experiment. Application of the neutrophil bolus and adenosine sampling were carried out as described above. The time course of the preconditioning experiments is evident from fig 5.

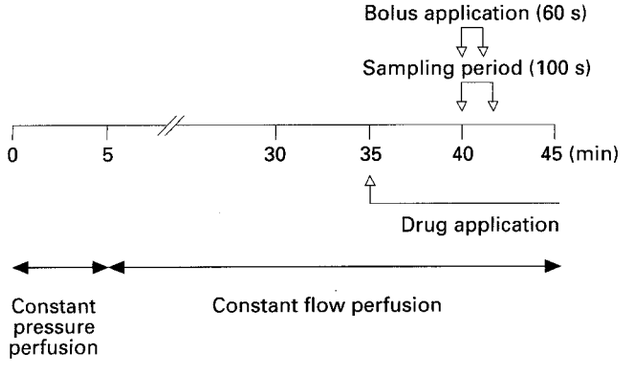

Figure 1 *Flow chart of a standard experimental protocol.*

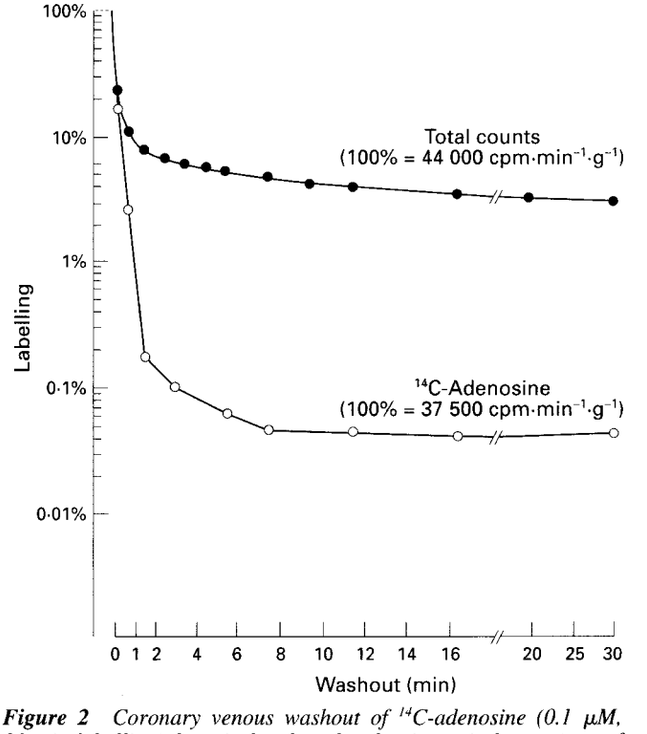

Figure 2 *Coronary venous washout of ^{14}C-adenosine (0.1 µM, 30 min labelling) from isolated perfused guinea pig hearts (one of three similar experiments).*

Adenosine measurement
Quantification of adenosine in coronary effluent and in the supernatant of neutrophil suspensions was performed by high performance liquid chromatography (HPLC). As described previously,[15] acidified samples (200 µl) were applied directly to a C-18 nucleosil column (Macherey-Nagel, Düren, Germany) and eluted with $HClO_4$ (pH 2) using a methanol (60 vol % in H_2O) gradient (0 to 24% in 20 min). Retention time for adenosine was 20.4 min.

Kinetics of adenosine washout
To determine the half time of adenosine washout from the isolated hearts, [8-^{14}C]adenosine (specific activity 189×10^{10} Bq·mol^{-1}) was infused into the aortic cannula for 30 min at a concentration of 0.1 µM. After cessation of infusion, coronary effluent was continuously sampled in timed aliquots (see fig 2). Radioactivity in the coronary venous effluent samples was measured in a scintillation spectrometer using Rialuma (Baker, Deventer, Holland) as scintillcation fluid. The radioactivity of the adenosine fraction obtained through HPLC (see above) was similarly counted. The relatively constant rate of ^{14}C-adenosine release observed after 5 min of the washout protocol reflects release of adenosine from prelabelled adenine nucleotides of the coronary endothelium.[16 17]

Statistical methods
Each group consisted of five experiments, if not otherwise stated. The results are given as mean(SEM). Statistical analysis was performed with one way analysis of variance (ANOVA). Whenever a significant effect with ANOVA was obtained, we performed multiple comparison tests between the groups using Student-Newman-Keul's test. Differences between groups were considered significant when $p \leq 0.05$.

Results

To test the ability of our model to detect changes in adhesivity, we first assessed neutrophil adhesion under control conditions and in response to a 5 min application of thrombin (table I). Under control conditions, about 20% of the injected guinea pig neutrophils did not emerge from the perfused coronary system at the chosen rate of coronary flow. Thrombin caused a significant rise in adhesion of the homologous neutrophils of about 50% versus control. As shown in fig 3 and table II, adenosine (0.1 µM), infused into the coronary system for 5 min prior to and maintained during neutrophil infusion, enhanced adhesion of guinea pig neutrophils to about the same extent as thrombin.

To characterise the adenosine receptor involved in this effect pharmacologically, we applied the A_1 receptor agonist CPA (0.01 µM), also for 5 min, and, in another series of experiments, adenosine (0.1 µM) together with the A_1 receptor antagonist DPCPX. The data in fig 3 show that CPA is able to simulate the adenosine effect, whereas DPCPX completely blocks it. DPCPX had no effect on adhesion [22(SEM 2)% retention] in the absence of exogenous adenosine. Thus the proadhesive effect of adenosine must clearly be mediated via the A_1 receptor subtype.

Coronary effluent adenosine levels dropped to baseline within 1 min after cessation of arterial infusion (fig 2). Washout experiments, in which the neutrophils were injected 1.5 min after the end of a 5 min infusion of adenosine (0.1 µM), showed fully sustained increases in retention (fig 3). Previous studies have shown that the intact endothelium is an efficient barrier for 0.1 µM adenosine.[16 18] Consequently, A_1 receptors on the endothelial cells, and not those on the neutrophils or cardiac myocytes and vascular smooth muscle, must play the decisive role.

Although thrombin is capable of inducing endothelial production of prostacyclin,[19] the prostaglandin I_2 analogue iloprost did not alter neutrophil adhesion (table I). Since both iloprost and adenosine – via the A_2 receptor[20] – can enhance endothelial cell cAMP, this is further evidence favouring the involvement of A_1 adenosine receptors. The result obtained with iloprost also helps to rule out an indirect adenosine effect on adhesion by way of a dilatation induced reduction of intravascular shear stress or by an increase in coronary surface area. At the concentration applied (0.01 µM), iloprost reduced perfusion pressure to at least the same extent as adenosine (data not shown). This lowering of shear stress elicited no increase of neutrophil adhesion (table I). Furthermore, CPA enhanced adhesion without causing any drop in perfusion pressure.

Table I *Effects of thrombin and iloprost on adhesion of guinea pig polymorphonuclear neutrophils to homologous coronary endothelium. Both agents were infused into the guinea pig hearts for 5 min before and during application of the cells. Values are means(SEM), n = 5.*

Perfusion condition	*Neutrophil adhesion (%)*
Control	21(3.4)
Thrombin (0.3 U·ml^{-1})	34(2.4)*
Iloprost (10 nM)	21(2.3)

*p < 0.05 v control.

Table II *Efffects of different adenosine concentrations and of the A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) on adherence of guinea pig neutrophils to homologous coronary endothelium. Values are means(SEM), n = 10 in control group, n = 5 in the other groups.*

Perfusion condition	*Neutrophil adhesion (%)*
Control[a]	22(2.0)
0.1 µM Adenosine	30(1.4)*
0.3 µM Adenosine	31(0.8)*
1.0 µM Adenosine	19(2.2)
1.0 µM Adenosine + 0.1 µM DMPX	39(3.0)*
0.1 µM Adenosine/1 µM adenosine (neutrophils)	32(2.0)*

Adenosine was infused into the coronary perfusate for 5 min before and during the neutrophil bolus, whereas DMPX was present in the perfusate from the onset of the heart preparation. In the last set of experiments, neutrophils were preincubated with 1 µM adenosine, and the hearts received only 0.1 µM adenosine.
[a] < 0.01 µM effluent adenosine.
*p < 0.05 v control.

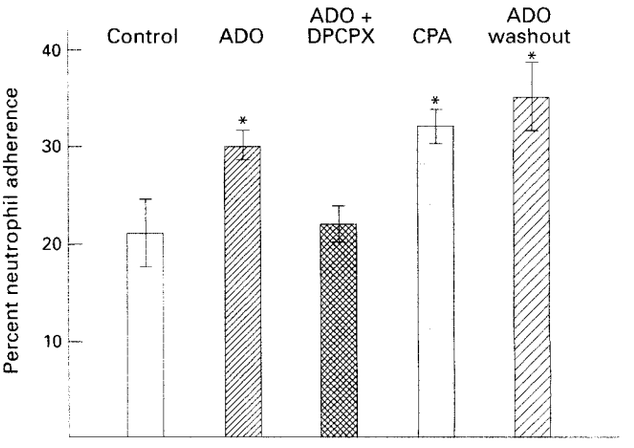

Figure 3 *A_1 receptor mediated action of adenosine on adhesion of guinea pig neutrophils to coronary endothelium. Adhesion is shown under the influence of adenosine (0.1 µM), of adenosine in the presence of the A_1 receptor antagonist dipropyl-8-cyclopentylxanthine (DPCPX, 0.1 µM), and of the A_1 receptor agonist N^6-cyclopentyladenosine (CPA, 0.01 µM). A_1 mediated effects on the neutrophils were excluded by the washout protocol: neutrophils were injected 1.5 min after cessation of adenosine infusion. Values are means of five experiments each; error bars = SEM.*
**p < 0.05 v control.*

Interestingly, the proadhesive effect of adenosine showed a bell shaped concentration dependence with a maximum in the range of 0.1-0.3 μM (table II). At 1 μM adenosine, adhesion was again reduced to the control level. However, if the A_2 receptor antagonist DMPX (0.1 μM) was co-infused with 1 μM adenosine, adhesion was further stimulated, increasing to 39(3)% (table II). DMPX alone did not influence neutrophil adhesion (results not shown). When neutrophils were incubated with 1 μM adenosine for 15 min prior to infusion into hearts that had received 0.1 μM adenosine according to the standard protocol, adhesion was not reduced in comparison to untreated neutrophils (table II). Therefore, the adhesion reducing A_2 receptors have to be located on the endothelium.

Subjecting the isolated hearts to global normothermic ischaemia of 15 min duration augmented subsequent adhesion of neutrophils, an effect that could be fully blocked by the A_1 receptor antagonist DPCPX (fig 4). Preconditioning the hearts with three short ischaemic periods of 3 min each had quantitatively the same protective effect as A_1 receptor blockade (fig 4).

In the experiments without preconditioning, postischaemic adenosine levels in the venous effluent were initially 0.8 μM and, at the time of neutrophil injection, about 0.45 μM (fig 5). After preconditioning, the adenosine values were notably higher, initially about 1.5 μM and still in the order of 0.6 μM during the bolus (fig 5).

To test the hypothesis that repetitive stimulation of A_1 receptors by preconditioning might lead to short term tachyphylaxis and therefore to cessation of the proadhesive effect, adenosine (0.1 μM) was continuously infused over 30 min. Since adhesion remained enhanced [30(7)%], this possibility was ruled out.

Finally, fig 6 shows that when human neutrophils were injected, control adhesion, as well as thrombin or adenosine stimulated adhesion, was nearly identical to the respective values obtained for guinea pig neutrophils.

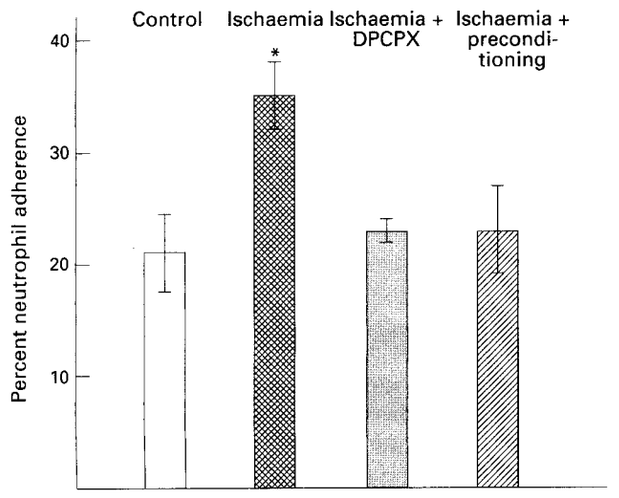

Figure 4 *Effect of 15 min of normothermic global ischaemia on the adhesion of guinea pig neutrophils to homologous coronary endothelium. The enhanced adhesion after ischaemia was fully prevented by applying the A_1 receptor antagonist dipropyl-8-cyclopentylxanthine (DPCPX, 0.1 μM) throughout the experiment or by preconditioning with three short (3 min) ischaemic periods, each followed by 5 min of reperfusion. Values are means of five experiments each; error bars = SEM.*
**$p < 0.05$ v control.*

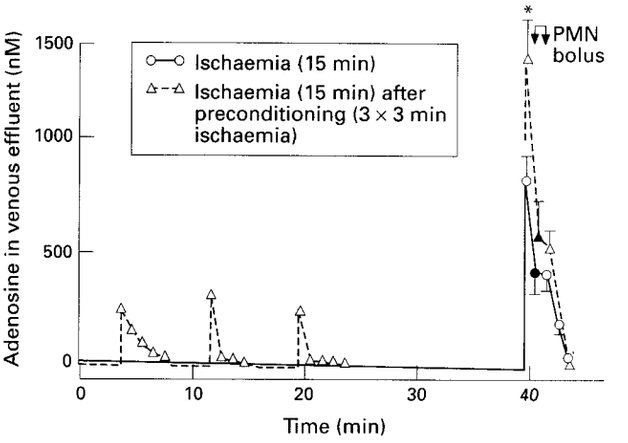

Figure 5 *Adenosine concentrations in the venous coronary effluent of isolated guinea pig hearts subjected to 15 min of global normothermic ischaemia. The postischaemic adenosine release was significantly enhanced by preconditioning with three ischaemic intervals, each of 3 min duration. The arrows indicate the time of injection (second minute of reperfusion) of the neutrophils (600 000 cells in a bolus). Error bars indicate SEM where graphical resolution is possible. Filled triangle: n = 3, no injection of neutrophils, filled circle: n = 7, no injection of neutrophils; all other experiments n = 5.*
**$p < 0.05$ v ischaemia.*

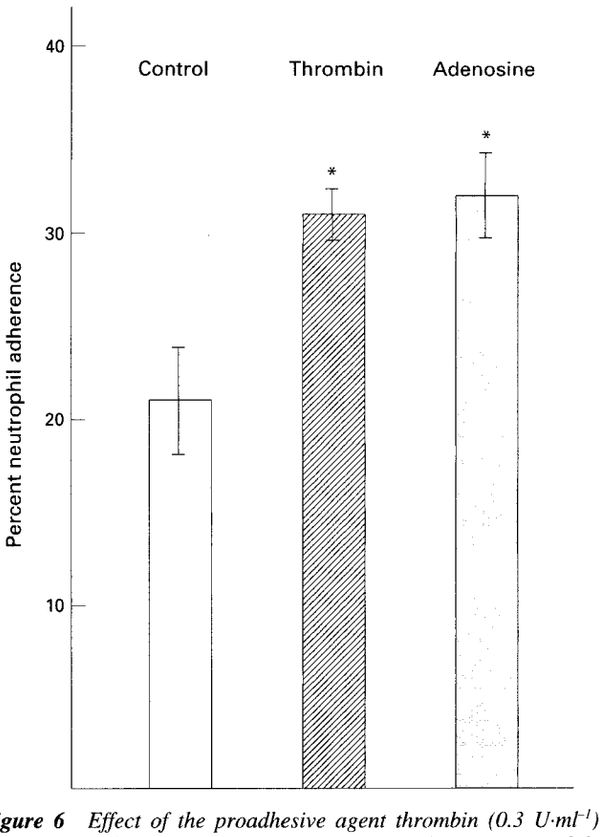

Figure 6 *Effect of the proadhesive agent thrombin (0.3 U·ml^{-1}) and of adenosine (0.1 μM) on the adhesion of human neutrophils to guinea pig coronary vasculature. Neutrophils were infused as a 1 ml bolus of 600 000 cells into the coronary perfusate of isolated guinea pig hearts. Non-adherent neutrophils were subsequently determined in the coronary effluent. Adhesion is expressed as percentage of cells not leaving the coronary system within 100 s after bolus onset. Values are means, error bars = SEM, of five experiments each.*
**$p < 0.05$ v control.*

Discussion

Several papers have been published concerning beneficial effects of adenosine on inflammatory processes in general,[4] on the extent of reperfusion injury,[3] and especially on a participation, via A_1 receptors, in the phenomenon of ischaemic preconditioning.[21 22] On the other hand, we have previously shown that endogenous adenosine (0.1-0.2 μM) contributes to postischaemic reperfusion injury mediated by neutrophils in an isolated guinea pig heart model, and that this too depends on adenosine A_1 receptors.[7] According to the results obtained in the present study, adenosine (0.1-0.3 μM) induces adhesion of guinea pig as well as of human neutrophils to the intact coronary system of guinea pig hearts by acting on endothelial A_1 receptors. This proadhesive effect provides a basis for a deleterious action of adenosine, although we do not know the ultimate fate of the cells, that is, whether they emigrate into the tissue or are protractedly washed out.

The finding that adenosine can enhance neutrophil adhesion via A_1 receptors corresponds to some results of Cronstein and coworkers.[9] However, there seem to be differences concerning the underlying mechanisms. First, Cronstein's group relates its findings to an effect of adenosine on the neutrophil, whereas we demonstrate an effect elicited on the endothelium. Second, Cronstein's studies pertain to effects occurring when neutrophils were stimulated by a chemotactic peptide, in contrast to our investigation using relatively unperturbed neutrophils. Third, we examined neutrophil adhesion under physiological shear stress, and not in quiescent culture dishes. Although species differences could also be involved in causing divergent results, there may well be several distinct ways of modulating neutrophil attachment to endothelial cells with adenosine. This could occur either via the previously described A_1 and A_2 receptors on neutrophils,[23] or via endothelial A_1 and A_2 receptors, guinea pig coronary endothelial cells being known to possess both types.[10 11]

In our model, the proadhesive effect vanished at a high adenosine concentration (1 μM). However, 1 μM adenosine further enhanced adhesion if applied in the presence of the A_2 antagonist DMPX (table II). Although DMPX is not absolutely selective for A_2 receptors, a submaximal concentration (0.1 μM) was applied, that is, one which significantly but not completely reduced coronary dilatation elicited by 1 μM adenosine via A_2 receptor mechanisms (drop in coronary perfusion pressure 14(1) and 25(2) mm Hg, with and without DMPX, respectively). At such a low concentration, receptor specificity of DMPX should be largely guaranteed. The suppression of adhesion by 1 μM adenosine thus reflects the onset of inhibitory A_2 actions[3 23] and complies with the fact that A_2 receptors have a lower affinity for adenosine than A_1 receptors.[24] At first we assumed the responsible A_2 mechanisms to be those familiar in the neutrophil but, again, the endothelium proved to be the principal site of action: experiments involving pre-exposure of the neutrophil to micromolar concentrations of adenosine, while the endothelium was only confronted with low levels (0.26 μM), showed retention of the pro-adhesive action. Thus endothelial A_1 and A_2 receptors exert mutually antagonistic effects on neutrophil adhesion. Although the adhesion of neutrophils can clearly be selectively regulated by adenosine A_1 and A_2 receptors, the receptor subtype specific mechanisms of the endothelium still remain to be characterised. Synergism with the respective A_1 and A_2 receptor actions on the neutrophil seems possible. The concept of a concentration dependent bimodal action of adenosine is additionally supported by the finding that the proadhesive effect of 15 minutes of global ischaemia, caused via the A_1 receptor and associated with coronary effluent adenosine levels in the submicromolar range, was notably attenuated by preconditioning (fig 4), an intervention which lead to even higher postischaemic adenosine concentrations (initially > 1 μM). Because the A_2 receptor mediated inhibition of adhesion was evoked at the endothelium in our model, it should be these initial values which set the stage for responsiveness towards subsequently applied neutrophils. Pertinantly, enhancement of adenosine release from ischaemic hearts after preconditioning has already been described in a different model.[25] An alternative explanation for the effect of preconditioning on adhesion, namely desensitisation of the A_1 receptor, seems unlikely during short term ischaemia and preconditioning, there being no evidence for tachyphylaxis of the A_1 response following sustained (30 min) exposure of the endothelium to moderately elevated adenosine (0.1 μM).

The discrepant results concerning the role of adenosine in adhesion and in reperfusion injury (see, for example, references 3, 9, 18, and 19 as opposed to 7 and 9) most likely arise from the range of adenosine concentrations employed, and therefore the receptor subtype engaged. Though adenosine at high concentrations inhibits various functions of neutrophils (aggregation,[26] radical production[27]), these responses remain unaffected in both guinea pig and human neutrophils at adenosine concentrations below 1 μM.[12] Similarly, in studies reporting inhibition of adhesion of neutrophils by adenosine, either adenosine analogues of higher potency and lower metabolic turnover than the physiological agent were applied at extreme levels of 10 to 200 μM,[4 28] or adenosine itself was used in concentrations > 2 μM.[3] In vivo, however, adenosine concentrations in arterial and coronary sinus blood under basal conditions have been found to be about 0.08 μM.[29] In blood leaving hearts subjected to PTCA or pacing induced ischaemia, adenosine levels are in the order of 0.1 to 1 μM.[5 6] In the context of concentration dependency, it is also interesting to note that, in our present study, the adenosine concentrations in the postischaemic venous effluent of individual hearts at the onset of reperfusion (0.6-2.0 μM) and the respective degree of neutrophil adhesion correlated negatively (adhesion= 35–0.0065 × [adenosine], regression coefficient 0.5).

There is another reason why most in vitro studies which have examined adenosine effects on neutrophils to date reflect more a scenario of pharmacological intervention than of postischaemic reperfusion. This is because the neutrophils were consistently incubated with high concentrations of adenosine.[4 9 27] After an ischaemic insult, however, the adenosine concentration is only locally elevated, not systemically, due to rapid elimination of adenosine from plasma by vascular endothelium and red blood cells. Therefore, the endothelial cells and the few resident neutrophils within an ischaemic vessel may be "incubated" with raised adenosine concentrations, but not the circulating neutrophils that reach the infarcted area only during reperfusion. The same objection pertains to in vivo models in which adenosine was systemically applied.[30]

Certain characteristics of the adhesion model described here bear mentioning. The basal adhesion of 20% seems rather high and may reflect a certain degree of prestimulation of both the endothelial cells and the neutrophils. However, lower values were obtainable if shear stress was raised by increasing the coronary flow (unpublished results). Moreover, approximately similar values of basal adhesion[31 32] as well as rate of onset and extent of stimulation with

thrombin[14] have been obtained in studies with cultured endothelium. Furthermore, we have no knowledge, so far, concerning the ultimate fate of the retained or retarded cells: the counting procedure gives a relatively rapid resolution, but does not directly yield long term insights.

The nature of both the endothelial and neutrophil localised adhesion molecules involved in the A_1 receptor effect reported here remains to be clarified. Thrombin has been shown to cause rapid induction (within five minutes) of shear stress resistant adhesion to cultured endothelial cells via externalisation of endothelial P selectin.[33] However, we have previously shown that guinea pig P selectin does not interact with human neutrophils.[34] The characteristics of the adhesion induced by adenosine (rapid, resistant to shear stress), indicate that in this case too a preformed adhesion molecule other than P selectin (perhaps a ligand for L selectin[35]) might be externalised to the endothelial cell surface. As intracoronary adhesion of human and guinea pig neutrophils responded to adenosine in exactly the same way, a species difference regarding the respective adhesion partner on the granulocytes seems unlikely.

In the face of the present results, it is difficult to explain why preconditioning in some animal models depends on A_1 receptor activation.[21 22] Of course, the proadhesive effect of adenosine may be unique to guinea pig coronary endothelium. However, the benefit of A_1 receptor stimulation in the pertinant studies was determined without allowing for possible systemic actions of the applied agonists or antagonists with respect to neutrophil adhesion. Thus simulation of cardiac preconditioning by intravenous application of A_1 receptor agonists[22] could evolve from acute neutropenia resulting from systemically stimulated neutrophil adhesion.

Although it is not yet clear whether human coronary endothelial cells also possess A_1 receptors, it is, nevertheless, appealing to relate the present findings to reperfusion of the human heart. At submicromolar concentrations (presumably relevant in reperfused myocardial tissue in man), the A_1 effect described here would cause adhesion of neutrophils to increase, thereby setting the stage for further neutrophil mediated tissue damage. Therefore, adenosine perhaps should not be expected to act as a cardioprotective agent[7] under all circumstances, and A_1 antagonists may well exert beneficial effects in the reperfused heart in vivo, as already shown in vitro.[7] Conversely, it may be speculated that the beneficial use of high adenosine concentrations or substances that enhance endogenous adenosine levels sufficiently is in part due to the antiadhesive effect of endothelial A_2 receptor stimulation. In this case A_2 receptor blocking drugs would be potentially deleterious. Finally, the protective effect of preconditioning may be partly caused by increasing adenosine levels into the cardioprotective range.[25]

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Key terms: adenosine receptor; endothelium; ischaemia; neutrophil adhesion; preconditioning; reperfusion damage.

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