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Effect of adenosine on the formation of prostacyclin in the rabbit isolated heart

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1 The effect of adenosine on cardiac biosynthesis of prostacyclin (PGI_2) was investigated. Rabbit hearts were perfused according to Langendorff at controlled pressure (with or without theophylline), or at controlled flow. The content of 6-keto-prostaglandin$_{1\alpha}$ (6-keto-$PGF_{1\alpha}$, metabolite of PGI_2) in the coronary effluent under basal conditions and during infusion of adenosine was determined using a highly specific radioimmunoassay.

2 In other experiments, rings of rabbit aorta were incubated with or without adenosine and the production of 6-keto-$PGF_{1\alpha}$ was analysed as above.

3 Administration of adenosine (10 μM) to hearts perfused at controlled pressure increased the coronary flow by up to 38%. The peak concentration of 6-keto-$PGF_{1\alpha}$ in the effluent exceeded the control by 177% ($P < 0.01$), and the total efflux of 6-keto-$PGF_{1\alpha}$ exceeded the control by 179% ($P < 0.001$). Theophylline (50 μM) reduced these effects of adenosine by 23%, 43% and 51%, respectively, without influencing the uptake of adenosine into the heart.

4 When adenosine (1–10 μM) was administered to hearts perfused at controlled flow, a dose-dependent decrease in the perfusion pressure, by 27% and 44% respectively, was observed. In parallel, the resulting increase in 6-keto-$PGF_{1\alpha}$ efflux was considerably lower (49% ($P < 0.05$) and 43% (NS), respectively). A similar decrease in perfusion pressure induced in the absence of adenosine decreased the efflux of 6-keto-$PGF_{1\alpha}$, by 15% ($P < 0.01$) and 32% ($P < 0.001$), respectively.

5 Addition of adenosine (1–10 μM) to incubates of rabbit aortic rings did not significantly affect the concentration of 6-keto-$PGF_{1\alpha}$ in the incubation medium in comparison with control.

6 We conclude that adenosine stimulates rabbit heart PGI_2 formation, mainly by an action related to the vasodilator effect of the nucleoside.

Introduction

Adenosine is a degradation product of adenosine triphosphate (ATP) that is continuously formed in the working heart. In the coronary vessels, adenosine is a vasodilator. Cardiac formation of adenosine is enhanced during hypoxia or ischaemia (Gerlach *et al.*, 1963; Imai *et al.*, 1964), supporting the hypothesis of a role of the nucleoside in the physiological regulation of coronary tone (Berne, 1961).

We showed previously (Ciabattoni & Wennmalm, 1985) that adenosine stimulates the formation of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), the stable degradation product of prostacyclin (PGI_2), in the rabbit heart. PGI_2 is a powerful vasodilator and platelet anti-aggregatory compound (Gryglewski *et al.*, 1976). A stimulatory effect of adenosine on the cardiac formation of PGI_2 may be of significance during hypoxia or ischaemia *in vivo*, since the two compounds may then act synergistically in promoting coronary flow and the anti-aggregation of platelets.

In the above-mentioned study, utilizing rabbit hearts perfused at controlled pressure, adenosine promoted both the coronary flow and efflux of 6-keto-$PGF_{1\alpha}$ (Ciabattoni & Wennmalm, 1985). Increased shear stress has been found to augment endothelial formation of PGI_2 (Grabowski *et al.*,

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1982; Frangos *et al.*, 1985). If such an effect of shear stress on PGI_2 formation by the coronary endothelium also occurs in the intact heart, the effect of adenosine in augmenting PGI_2 formation might be, at least partly, due to the flow-promoting effect of adenosine in a controlled pressure system.

To test this hypothesis, we compared the effect of adenosine on the efflux of 6-keto-$PGF_{1\alpha}$ from rabbit hearts perfused at controlled pressure with that from hearts perfused at controlled flow. In addition, we studied the effect of adenosine on vascular PGI_2 formation in the absence of flow.

Methods

Perfusion of rabbit hearts

Rabbits of mixed strains and either sex, weighing 1.4 to 2.5 kg, were used for the study. They were stunned by cervical dislocation and subsequently exsanguinated via the left carotid artery. A catheter was rapidly inserted into the root of the aorta, allowing coronary perfusion to be quickly re-established. The heart was then excised and transferred to the perfusion apparatus, in which it was perfused at controlled pressure (70 cmH_2O) with a modified Tyrode solution of the following composition (in mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, NaH_2PO_4 0.8 and glucose 5.6. The solution was saturated with a gas mixture consisting of 97% O_2 plus 3% CO_2. The pH of the bubbled solution was 7.4 and the perfusate temperature was 38°C. For perfusion at controlled flow rate, a roller pump (Ismatec SA, Zürich, Switzerland) was introduced between the perfusate reservoir and the aortic cannula, which allowed continuous adjustment of the flow rate.

The coronary flow rate was continuously followed by timed collection of the effluent in pre-tared glass beakers, which were weighed on an ordinary laboratory scale.

Perfusion pressure was monitored via an open column system connected to the perfusion system via a side branch.

Preparation of aortic rings

After excision of the heart, the aorta was removed and put into a dissection beaker containing gassed Tyrode solution. After removal of surrounding tissue, the cleaned aorta was cut into 2 mm wide rings which were transferred to the incubation tubes containing Tyrode solution.

Protocol

Three different series of experiments were performed:

Controlled pressure perfusion Seven hearts were included in the series. After the heart had equilibrated with the perfusion system for 20 min, a 10 min infusion of adenosine (Sigma, final concentration 10 μM) was commenced. Subsequently, the perfusate was changed to drug-free Tyrode solution, and the heart was allowed to recover for 30 min. Theophylline (Theophyllamine ACO, final concentration 50 μM) was then added to the perfusate. After 5 min of perfusion with theophylline, administration of adenosine for 10 min was repeated as described above.

Samples for analysis of basal purine and 6-keto-$PGF_{1\alpha}$ levels in the effluent were drawn 5 min before the beginning of each adenosine infusion. Samples were also taken in four 15 s periods during the first minute of infusion of adenosine, in two 30 s periods during the following 2 min, and at the beginning of min 4–10, i.e. until the end of administration of adenosine.

Controlled flow perfusion This series comprised 17 animals. After a 20 min period of equilibration at controlled pressure, perfusion was changed to controlled flow, set at the rate prevailing during the preceding controlled pressure perfusion. After a 10 min period at this flow rate, eleven hearts were randomly subjected either to an infusion of adenosine (final concentration 10 μM) followed by a 50% decrease in flow rate (resulting in approximately the same decrease in perfusion pressure as observed during the previous infusion of adenosine) or to the same procedures in the reverse order. The remaining six hearts in this series were randomly subjected to an infusion of adenosine (final concentration 1 μM) followed by a 25% reduction of the coronary flow rate, or the reverse procedures. All interventions were maintained for 10 min. In each experiment, the two intervention periods were separated by a 20 min recovery interval, during which controlled perfusion at the basal perfusion rate was maintained.

Samples for analysis of purines and of 6-keto-$PGF_{1\alpha}$ in the cardiac effluent were withdrawn during the basal periods 5 min before the intervention, and during 6–10 min of the intervention periods.

Aortic rings The rings from each aorta were divided into three equally large groups. They were incubated with 4 ml of one of the following solutions: Tyrode without drug (control), Tyrode + adenosine (1 μM), and Tyrode + adenosine (10 μM). The incubation was maintained for 20 min. Samples

for analysis of 6-keto-$PGF_{1\alpha}$ were taken from each incubation at 5, 10, and 20 min.

Analyses

The coronary effluent was collected on ice during the course of the experiments. After volume determination of the samples, separate portions for analysis of purines and of 6-keto-$PGF_{1\alpha}$ were frozen and kept at −80°C until analysis. Purines were analysed in unextracted samples as adenosine, inosine and hypoxanthine using high performance liquid chromatography with absorbance detection (Fredholm & Sollevi, 1981). Since the proportion of adenosine, inosine and hypoxanthine varied between the samples, the sum of these three nucleotide metabolites is presented.

Analysis of 6-keto-$PGF_{1\alpha}$ was performed in unextracted samples in duplicate using radioimmunoassay in 1 : 30 to 1 : 300 dilution (Patrono *et al.*, 1982). The antibody used was raised in the Department of Pharmacology, Catholic University, Rome. The lowest concentration measurable with 95% confidence (i.e. 2 s.d. at zero) was 0.5 pg ml^{-1} in undiluted heart effluent. The intra-assay variability averaged 4% and the inter-assay variability averaged 8% over a range of 6-keto-$PGF_{1\alpha}$ concentrations from 15 to 200 pg ml^{-1}.

Statistical analysis

Figures in the text and tables are presented as mean ± s.e. Student's *t* test (two-tailed) for paired or unpaired means was used for calculation of statistical differences, when applicable.

Results

Controlled pressure perfusion

The basal coronary flow was 26 ± 3 ml min^{-1}. When the perfusate was changed to a solution containing adenosine (10 μM), there was an immediate increase in coronary flow. The elevation was significant ($P < 0.005$) after only 30 s of infusion, the flow reaching a level of 31 ± 4 ml min^{-1}. Subsequently, the rate of increase became slower; at the end of the 10 min exposure to adenosine, the coronary flow had increased to about 36 ± 4 ml min^{-1} (Figure 1a).

The basal concentration of purines in the perfusate was 0.5 ± 0.2 nmol ml^{-1}. Perfusion with adenosine-containing solution caused an immediate increase in the effluent concentration of purines. The effluent concentration of purines reached a peak of around 11 nmol ml^{-1} during the second min of

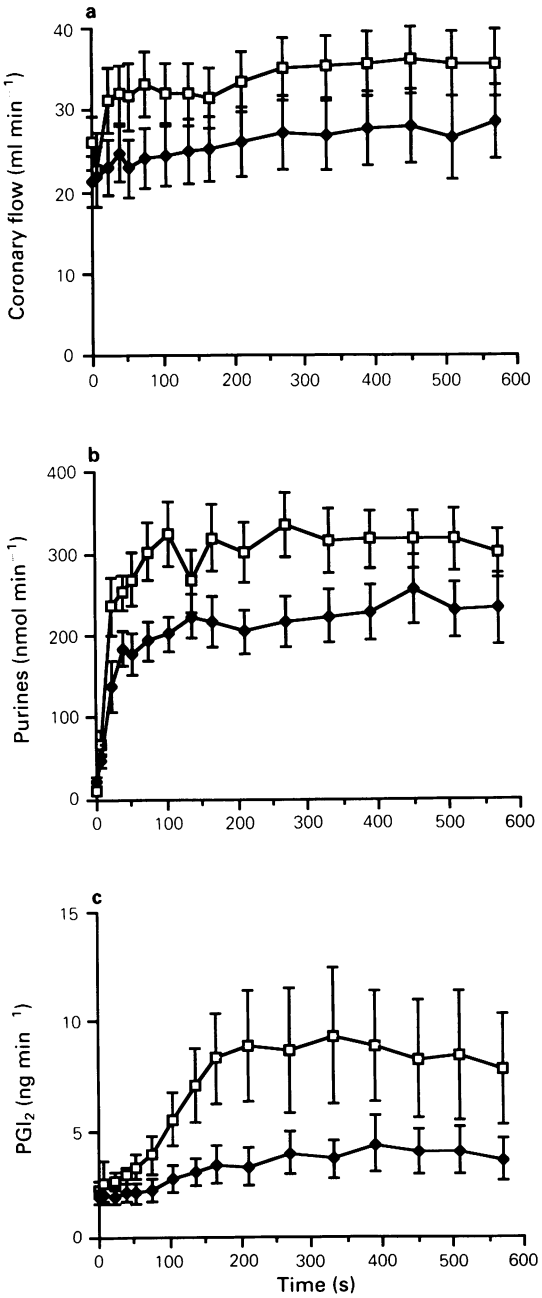

Figure 1 Rabbit hearts perfused at controlled pressure. Effect of an infusion of adenosine (10 μM) (maintained between 0 and 600 s) on: (a) coronary flow without (□) and with (◆) theophylline (50 μM); (b) efflux of purines without (□) and with (◆) theophylline (50 μM); (c) efflux of prostacyclin (PGI_2) without (□) and with (◆) theophylline (50 μM). Each point represents the mean ($n = 7$); vertical lines indicate s.e. mean.

Table 1 Effect of a 10 min infusion of adenosine (10 μM), alone or with theophylline (50 μM), on the efflux of purines and 6-keto-prostaglandin $F_{1\alpha}$ from the rabbit isolated heart perfused at controlled pressure

	Total purine efflux (nmol)			*Total 6-keto-$PGF_{1\alpha}$ efflux* (ng)		
	0–5 min	*6–10 min*	*0–10 min*	*0–5 min*	*6–10 min*	*0–10 min*
A Control ($n = 7$)	54.1 ± 12.2	54.1 ± 12.2	108 ± 24.4	9.96 ± 1.43	9.96 ± 1.43	19.9 ± 2.86
B Adenosine 10 μM ($n = 7$)	987*** ± 115	1570*** ± 168	2560*** ± 280	25.6*** ± 4.92	29.9*** ± 5.99	55.5*** ± 10.7
C Adenosine 10 μM + theophylline 50 μM ($n = 7$)	632** ± 92.0	1170*** ± 168	1800*** ± 254	12.3** ± 2.69	16.0** ± 4.23	28.3** ± 6.21

Values are expressed as mean ± s.e. mean. n = number of experiments.
Efflux of purines was measured as the sum of the effluxes of hypoxanthine, inosine and adenosine. Data represent cumulative effluxes during the first 5 min (0–5 min), the next 5 min (6–10 min) and the whole 10 min (0–10 min) period of adenosine infusion. Control data represent a 10 min period preceding infusion of adenosine.
** $P < 0.005$; (B) vs (C). *** $P < 0.001$; (B) vs (A), (C) vs (B).

exposure to adenosine. Subsequently, the effluent concentration of adenosine stabilized at around 9 nmol ml^{-1} (Figure 2). The total amount of purines leaving the heart during the 10 min perfusion with adenosine-containing solution amounted to about 80% of the perfusate content. Assuming that the basal production of purines in the heart was unaffected by the infusion of adenosine, this effluent concentration implies that the net retention of adenosine in the heart was about 25%. The efflux of adenosine was significantly ($P < 0.001$) lower during the initial part (first 5 min) of the exposure to adenosine (average gross retention 39%) compared to the last 5 min of exposure (average gross retention about 3%, Tables 1 and 2).

The basal concentration of 6-keto-$PGF_{1\alpha}$ in the coronary effluent was 83 ± 10 pg ml^{-1}. During exposure to adenosine (10 μM) there was a progressive increase in this concentration. During the latter part of the second min of adenosine exposure, the effluent concentration of 6-keto-$PGF_{1\alpha}$ had risen to 154 ± 22 pg ml^{-1} ($P < 0.01$). The peak value (230 ± 49 pg ml^{-1}) was reached 1 min later. The effluent concentration of 6-keto-$PGF_{1\alpha}$ subsequently decreased somewhat, but was significantly elevated throughout the exposure to adenosine (Figure 2).

The timed efflux of 6-keto-$PGF_{1\alpha}$ displayed the same tendency as the effluent concentration of this compound. The basal efflux was 2.3 ± 0.4 ng min^{-1}. The peak efflux, 9.3 ± 3.1 ng min^{-1} ($P < 0.001$), was reached after 5–6 min of infusion. The efflux of 6-keto-$PGF_{1\alpha}$ did not display any definite tendency towards a decrease during the latter part of the exposure to adenosine (Figure 1c).

Effect of theophylline Theophylline (50 μM) reduced the basal coronary flow from 26 ± 3 to 21 ± 3 ml min^{-1} ($P < 0.001$, Figure 1a). When the perfusate was changed to a solution containing adenosine (10 μM), the flow response was considerably less pronounced than before theophylline (Figure 1a). The average reduction by theophylline of the coronary flow during exposure to adenosine was

Table 2 Uptake of adenosine by the rabbit isolated heart perfused with 10 μM adenosine or with 10 μM adenosine + 50 μM theophylline

	Adenosine infused	*Purine efflux* (nmol 10 min^{-1})	*Adenosine uptake*
A Adenosine 10 μM ($n = 7$)	3250 ± 348	2560 ± 280	689 ± 144
B Adenosine 10 μM + theophylline 50 μM ($n = 7$)	2500* ± 356	1800*** ± 254	704 ± 100

Values are expressed as mean ± s.e. mean. n = number of experiments.
* $P < 0.02$; (B) vs (A). *** $P < 0.001$; (B) vs (A).

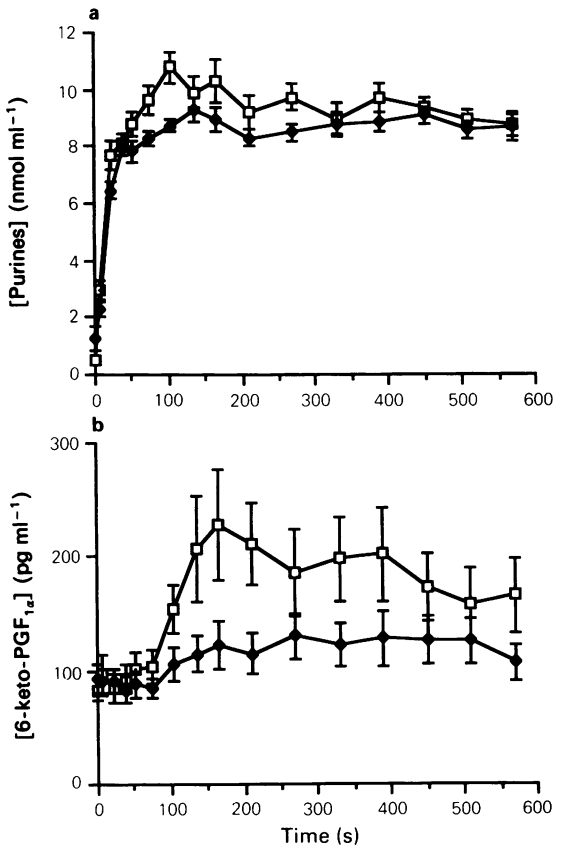

Figure 2 Rabbit hearts perfused at controlled pressure. Effect of an infusion of adenosine (10 μM) (maintained between 0 and 600 s) on (a) the effluent concentration of purines (sum of adenosine, inosine and hypoxanthine) without (□) and with (◆) theophylline (50 μM), and (b) on the effluent concentration of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) without (□) and with (◆) theophylline (50 μM). Each point represents the mean ($n = 7$); vertical lines indicate s.e. mean.

about 23%. Although the total amount of adenosine infused during the 10 min exposure was significantly ($P < 0.02$) lower in the presence than in the absence of theophylline (due to the lower flow), the total amount of adenosine retained did not differ from that retained by hearts not given theophylline (Table 2). The concentration of purines in the coronary effluent did not differ between these groups either (Figure 2).

The basal efflux of 6-keto-$PGF_{1\alpha}$ was significantly ($P < 0.05$) reduced by theophylline. The response to adenosine (10 μM) was also reduced: the peak level of 6-keto-$PGF_{1\alpha}$ in the coronary effluent was 130 ± 20 pg ml^{-1} ($P < 0.05$), and it was not reached until after 5 min of exposure to adenosine (Figure 2).

The total efflux of 6-keto-$PGF_{1\alpha}$ during exposure to adenosine was reduced by 51% by theophylline ($P < 0.005$, Table 1).

Controlled flow perfusion

The mean basal coronary flow in this series was 17 ± 1 ml min^{-1} ($n = 17$). When the perfusate was changed to a solution containing adenosine (1–10 μM), a significant ($P < 0.001$) and dose-dependent decrease in perfusion pressure developed (Figure 3). Adenosine (10 μM) also increased the efflux of purines from the heart, indicating an incomplete retention of nucleotide within the organ (Figure 3). The estimated net uptake of adenosine in the heart was 54 ± 9% at 1 μM and 57 ± 2% at 10 μM.

Adenosine (1 μM) elicited a significant ($P < 0.05$) increase in the efflux of 6-keto-$PGF_{1\alpha}$ into the coronary effluent. However, the higher concentration of adenosine (10 μM) only elicited an insignificant increase in coronary efflux of 6-keto-$PGF_{1\alpha}$ (Figure 3).

Reduction of the coronary flow rate by 25 and 50% reduced the perfusion pressure to roughly the same extent as adenosine 1 μM and 10 μM, respectively. In response to such flow reduction-induced decreases of perfusion pressure, the efflux rates of 6-keto-$PGF_{1\alpha}$ were significantly reduced, by about 15% ($P < 0.01$) and 32% ($P < 0.001$), respectively. The efflux of purines, however, was unchanged during the flow reduction-induced decrease of perfusion pressure, indicating that the washout capacity of the perfusion was still intact (Figure 3).

Incubation of aortic rings

Incubation of aortic rings in Tyrode solution elicited a time-dependent accumulation of 6-keto-$PGF_{1\alpha}$ in the incubation medium. The average production of 6-keto-$PGF_{1\alpha}$ during the second 5 min period of incubation was 0.06 ± 0.08 ng ml^{-1} incubate and the average formation rate between 5 and 20 min of incubation was 0.64 ± 0.18 ng ml^{-1}. Addition of adenosine (1 μM) to the incubation medium did not induce any significant effect on the rate of formation of 6-keto-$PGF_{1\alpha}$. By analogy, adenosine (10 μM) failed to induce any significant effect on the spontaneous formation of 6-keto-$PGF_{1\alpha}$ in the incubation medium (Figure 4).

Discussion

In these experiments, infusion of adenosine (10 μM) in rabbit isolated hearts perfused at controlled pressure induced a considerable increase in the efflux of 6-keto-$PGF_{1\alpha}$, in accordance with results pre-

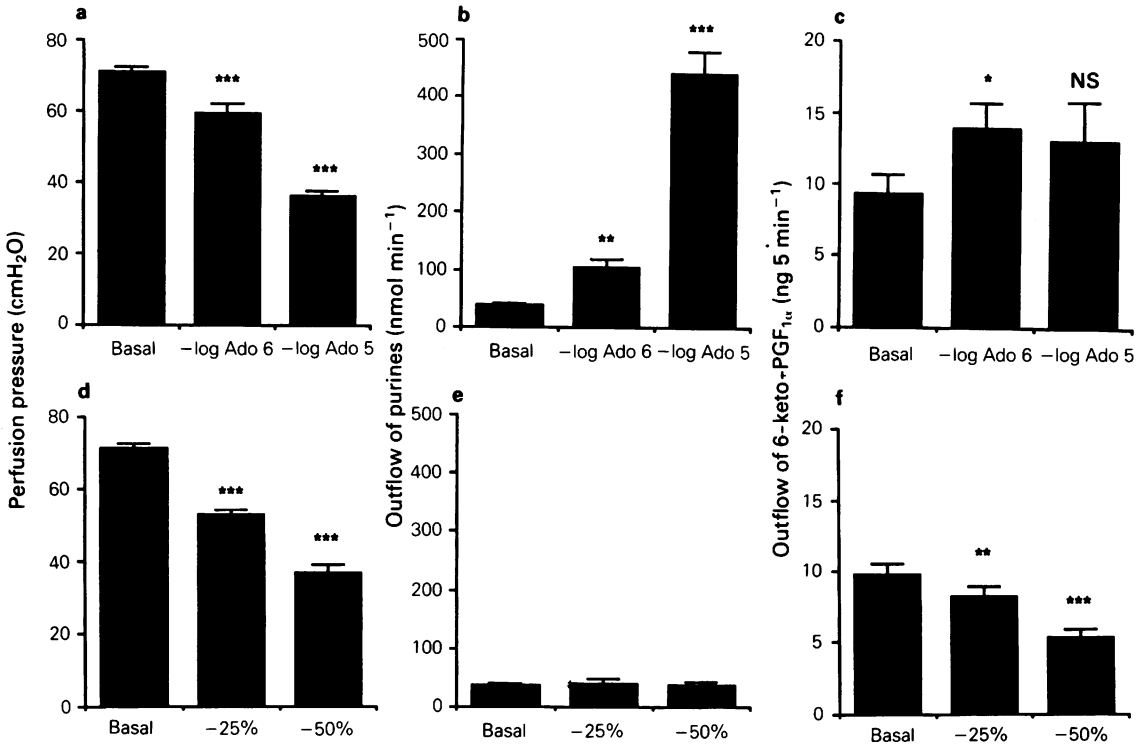

Figure 3 Rabbit hearts perfused at controlled flow. (a–c) Show the effect of infusion of adenosine (Ado, 1 or 10 μM) on (a) perfusion pressure, (b) efflux of purines (sum of adenosine, inosine and hypoxanthine), and (c) efflux of prostacyclin metabolite (6-keto-prostaglandin $F_{1\alpha}$). (d–f) The effect of reducing the perfusion pressure (by lowering the coronary flow rate by 25% or 50%), to approximately the same levels as those induced by adenosine, on (d) the perfusion pressure, (e) the efflux of purines and (f) the efflux of the prostacyclin metabolite. Columns and bars represent means and s.e. means, respectively ($n = 6$–11). Note that the reduction of the perfusion pressure with adenosine increased the efflux of prostacyclin metabolite, while a corresponding drop in pressure elicited by a lowered coronary flow was followed by a decreased efflux of prostacyclin metabolite. *, **, and *** indicate that the values differ significantly (by $P < 0.05$, 0.01, and 0.001, respectively) from the corresponding basal values; NS indicates not significant.

viously described by us (Ciabattoni & Wennmalm, 1985). Parallel to the increased efflux of 6-keto-$PGF_{1\alpha}$, adenosine increased the coronary flow rate. Experimental data on isolated endothelial cells (cf. Introduction) clearly demonstrate that shear can augment the formation of prostacyclin. The current increase in PGI_2 formation may consequently be mainly due to the promotion of flow rate. However, our data indicate that the stimulant effect of adenosine on the coronary formation of PGI_2 was more pronounced than the increase in flow. Thus, the coronary flow increased by less than 40% in response to adenosine (10 μM), while the efflux of 6-keto-$PGF_{1\alpha}$ was augmented by almost 180%. Furthermore, the purinoceptor antagonist theophylline, at a concentration known to inhibit adenosine receptors, reduced the coronary flow by 23% but decreased the efflux of 6-keto-$PGF_{1\alpha}$ by more than 50%. These results indicate that the increased flow induced by adenosine in a certain vascular circuit is less pronounced than the nucleotide's facilitation of PGI_2 formation. Alternatively, adenosine may recruit new vessels in the heart; such recruitment should elicit a similar pattern of 6-keto-$PGF_{1\alpha}$ efflux, provided that opening up of a vessel as such adds to the common cardiac formation of PGI_2. The data do not, however, exclude the possibility that the effect of adenosine on the bioformation of PGI_2 in the coronary vessels was, to some extent, dissociated from the vasodilator property of the nucleotide.

When adenosine was give under conditions that did not allow an increase in flow rate to develop, i.e. during controlled flow, the efflux of 6-keto-$PGF_{1\alpha}$ was much lower. This supports the assumption that

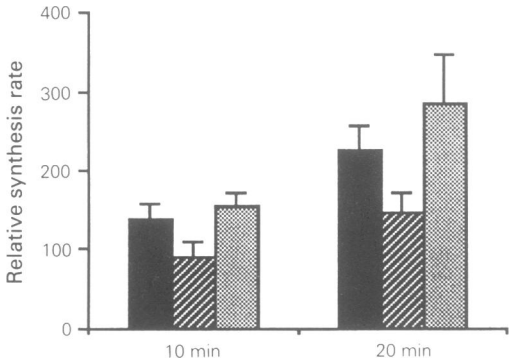

Figure 4 Incubation of aortic rings. Effect of adenosine, 1 (hatched columns) and 10 (stippled columns) μM, on the spontaneous formation of prostacyclin metabolite. The values at 10 min and 20 min express the amounts formed as a percentage of the amounts formed after 5 min of incubation (= 100%). Columns and bars represent means and s.e. means, respectively ($n = 4$). The formation rates in the presence of adenosine (1 or 10 μM) are not significantly different from their corresponding controls (solid columns), either at 10 min or at 20 min.

the major part of the effect of adenosine on PGI_2 synthesis was flow-dependent. Adenosine given at constant flow elicited a drop in perfusion pressure. Hence, vasodilatation did also occur when the heart was perfused at constant flow. Since this vasodilatation was parallelled by an increased efflux of 6-keto-$PGF_{1\alpha}$, the proposal that adenosine opens up new vessels is given some support. The drop in pressure may to some extent have masked the true effect of adenosine on the formation of 6-keto-$PGF_{1\alpha}$; when a similar drop in perfusion pressure was induced by decreasing the flow rate, the efflux of 6-keto-$PGF_{1\alpha}$ was, in fact, lowered. This may be due to a more efficient tissue uptake and elimination of PGI_2/6-keto-$PGF_{1\alpha}$ when the coronary pressure was lowered. If so, the net effect of adenosine in promoting PGI_2 formation would have been somewhat larger than that seen in these controlled flow experiments.

As to the question of the source of the increased efflux of 6-keto-$PGF_{1\alpha}$, a comment may be justified. Although vascular smooth muscle and cardiomyocytes have been shown to possess the ability to synthesize PGI_2, the coronary vascular endothelium is generally considered to be the main cardiac source of this eicosanoid (MacIntyre *et al.*, 1978). In a previous study, we demonstrated that adenosine promoted the efflux of 6-keto-$PGF_{1\alpha}$ into the venous effluent, representing mainly coronary endothelial PGI_2 formation, and not into the interstitial effluent, which reflects formation in the cardiomyocytes (De Deckere & Ten Hoor, 1977). These findings led us to suggest the coronary endothelium as the target for the administered adenosine. This assumption is supported by the above-mentioned data on the effect of shear in isolated endothelial cells (Grabowski *et al.*, 1982; Frangos *et al.*, 1985).

In contrast to the stimulant action of adenosine on the formation of 6-keto-$PGF_{1\alpha}$ in the perfused hearts, the nucleoside was completely inefficient in eliciting a corresponding action in the rabbit aortic rings. This finding is in agreement with the observation that adenosine lacks effect on the formation of PGI_2 in cultured pig endothelial monolayers (Pearson *et al.*, 1983). We cannot exclude the possibility that the doses of adenosine applied in the aortic ring incubations were insufficient to stimulate endothelial purinoceptors. Yet, the aortic ring experiments support the assumption from the heart perfusion experiments that the facilitating effect of adenosine on PGI_2 synthesis is critically flow-dependent.

In summary adenosine, which is produced continuously in the beating heart in amounts depending on the energy state of the organ, may stimulate cardiac – probably coronary – formation of prostacyclin. This effect of adenosine appears to be mediated by activation of the same coronary purinoceptors as those eliciting vascular smooth muscle relaxation. Furthermore, the stimulant effect of adenosine seems to be closely related to the extent of flow promotion induced by the relaxation of the vascular smooth muscle.

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References

BERNE, R.M. (1961). Nucleotide degradation in the hypoxic heart and its possible relation to regulation of coronary blood flow. *Fed. Proc.*, **20**, 101.

CIABATTONI, G. & WENNMALM, Å. (1985). Adenosine-induced coronary release of prostacyclin at normal and low pH in isolated heart of rabbit. *Br. J. Pharmacol.*, **85**, 557–563.

DE DECKERE, E.A.M. & TEN HOOR, P. (1977). A modified Langendorff technique for metabolic investigations. *Pflügers Arch.*, **370**, 103–105.

FRANGOS, J.A., ESKIN, S.G., McINTIRE, L.V. & IVES, C.L. (1985). Flow effects on prostacyclin production by cultured human endothelial cells. *Science*, **227**, 1477–1479.

FREDHOLM, B.B. & SOLLEVI, A. (1981). The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. *J. Physiol.*, **313**, 351–367.

GERLACH, E., DEUTICKE, B. & DREISBACH, R.H. (1963). Der Nucleotid-Abbau im Herzmuskel bei Sauerstoffmangel und seine mögliche Bedeutung für die Coronardurchblutung. *Naturwissenschaften*, **50**, 228–229.

GRABOWSKI, E.F., WEKSLER, B.B., JAFFE, E.A. & KLEIN, M.A. (1982). Effects of shear stress on prostaglandin I_2 (prostacyclin) production by cultured bovine aortic endothelial cells. *Circulation*, **66**, Suppl. 2, 53.

GRYGLEWSKI, R.J., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

IMAI, S., RILEY, A.L. & BERNE, R.M. (1964). Effect of ischemia on adenine nucleotides in cardiac and skeletal muscle. *Circ. Res.*, **15**, 443–450.

MACINTYRE, D.E., PEARSON, J.D. & GORDON, J.L. (1978). Localisation and stimulation of prostacyclin production in vascular cells. *Nature*, **271**, 549–551.

PATRONO, C., PUGLIESE, F., CIABATTONI, G., PATRIGNANI, P., MASERI, A., CHIERCHIA, S., PESKAR, B.A., CINOTTI, G.A., SIMONETTI, B.M. & PIERUCCI, A. (1982). Evidence for a direct stimulatory effect of prostacyclin on renin release in man. *J. Clin. Invest.*, **9**, 231–241.

PEARSON, J.D., SLAKEY, L.L. & GORDON, I.L. (1983). Stimulation of prostaglandin production through purinoceptors on cultured porcine endothelial cells. *Biochem. J.*, **214**, 273–276.

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