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Adenosine-induced coronary release of prostacyclin at normal and low pH in isolated heart of rabbit

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1 Rabbit hearts were perfused by the Langendorff method with drug-free perfusion medium or with a medium containing adenosine (10^{-7}M–10^{-4}M) and the coronary and transmyocardial efflux rates of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) were measured. Perfusion was performed both at pH 7.4 and 6.9.

2 In other experiments the hearts were pre-labelled with [^{14}C]-arachidonic acid and the coronary efflux of radioactivity and of labelled lipids and 6-keto-$PGF_{1\alpha}$ were determined.

3 The basal coronary flow was elevated by almost 70% during tissue acidosis, in comparison to control. Adenosine induced a dose-dependent increase in the coronary flow, amounting to about 75% at normal pH and a drug concentration of 10^{-5}M. The adenosine-induced increase in coronary flow was not facilitated by low pH.

4 The base coronary efflux of 6-keto-$PGF_{1\alpha}$ from the hearts was 2.5–3.6 ng min^{-1}. Adenosine (10^{-6}–10^{-5}M) significantly facilitated this efflux, up to 6.5 ng min^{-1}. The efflux of 6-keto-$PGF_{1\alpha}$ was not changed by perfusion with acidic medium, either in the basal state or during perfusion with adenosine.

5 The basal interstitial efflux of 6-keto-$PGF_{1\alpha}$ was 4.5–5.5 ng 3 min^{-1}. This efflux was not affected by perfusion of the heart with adenosine-containing medium. In hearts pre-labelled with [^{14}C]-arachidonic acid, adenosine (10 μM) induced a specific liberation of labelled lipid-extractable substances, including 6-keto-$PGF_{1\alpha}$.

6 From these data we conclude that adenosine stimulates the liberation of 6-keto-$PGF_{1\alpha}$ from the rabbit heart by increasing precursor availability and subsequent formation of prostacyclin in the coronary vessels. Furthermore, the increase in coronary flow induced by tissue acidosis is not related to an augmented formation of prostacyclin.

Introduction

Adenosine is an intermediate in the metabolism of adenine nucleotides. Intracellularly, adenosine triphosphate (ATP) is the most readily available form of energy. During cardiac work ATP stores are subject to a continuous turnover, yielding a small net release of adenosine. This cardiac formation of adenosine is considerably increased during ischaemia (Gerlach *et al.*, 1963) and hypoxia (Imai *et al.*, 1964, Edlund *et al.*, 1983). The coronary vasodilator action of adenosine has led to the suggestion that it may be the physiological agent that regulates the coronary flow rate to the substrate requirements of the myocardium (Berne, 1961). Of particular interest is the observation that the coronary flow and its response to adenosine is enhanced during acidosis (Merrill *et al.*, 1978).

The mechanism behind the relaxant action of adenosine on coronary vascular smooth muscle has not been definitely established. A direct inhibitory action on transmembrane calcium transport has been proposed (Herlihy *et al.*, 1976; Harder *et al.*, 1979), and also an augmenting effect on the intracellular cyclic AMP levels (Kukovetz *et al.*, 1978). A promoting action by adenosine on the formation or liberation of other vasoactive compounds has, however, not been proposed.

Prostacyclin (PGI_2) is formed in vascular tissue in a number of species (Moncada *et al.*, 1976; 1977). It is a potent vasodilator and platelet anti-aggregatory agent, active in very low concentrations. The observation that cardiac formation of prostacyclin is increased during hypoxia (DeDeckere *et al.*, 1977; Wennmalm, 1980; Edlund *et al.*, 1983) has raised the question

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whether the agent is physiologically released during tissue ischaemia, in analogy with the increased formation of adenosine under similar conditions.

In the present study we have infused adenosine in rabbit isolated hearts and analysed the efflux of prostacyclin. The object of the study was to find out whether adenosine is capable of stimulating prostacyclin formation. Adenosine was also infused during tissue acidosis, in order to establish whether the augmented coronary flow response to adenosine during lowered reactivity is based on facilitation of PGI_2 formation.

Methods

Perfusion of rabbit hearts

Rabbits of mixed strains and either sex, weighing 1.4–2.6 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 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 bubbled with a gas mixture consisting of 3% CO_2 in O_2. The pH of the bubbled solution was 7.38–7.45. The perfusion pressure was 5.9 kPa and the temperature was maintained at 38°C. In some experiments an alternative preparation method was applied, allowing separate collection of coronary and interstitial effluent. After the aortic catheter had been inserted, another catheter was introduced into the pulmonary artery. The inferior and superior caval veins, as well as the pulmonary veins, were ligated. This modified Langendorff preparation (DeDeckere & Ten Hoor, 1977) separates the coronary flow from the interstitial (transmyocardial) efflux. Thus, the coronary effluent leaves the heart via the pulmonary artery, while the interstitial effluent reaches the surface of the heart through the interstitial space and via the lymphatics. The flow rate of the interstitial effluent is usually 2–4% of the coronary effluent, both in the basal state and during infusion of coronary vasodilators. The coronary effluent is collected from the pulmonary catheter, while the interstitial effluent can be sampled as it drips from the apex of the heart. The coronary efflux was collected in pre-tared glass beakers and weighed minute by minute on an ordinary laboratory balance.

For perfusion of the heart at acidic pH the $NaHCO_3$ concentration in the perfusion medium was lowered to 11.9 mM and the NaH_2PO_4 concentration to 0.4 mM. Furthermore, the solution was bubbled with a gas mixture consisting of 5% CO_2 in O_2. This yielded a pH in the venous effluent of 6.87–6.95.

Procedure

Three different series of experiments were performed. In the first series the effect of adenosine on the coronary flow and efflux of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) at normal and acidic pH was studied. After an initial equilibration period of about 20 min, coronary flow measurements were started. After a basal period of 5 min perfusion was changed to a medium containing adenosine (10^{-7}M) for 3 min. Subsequently, perfusion was changed back to adenosine-free medium again. Twenty min later the procedure was repeated with adenosine (10^{-6}M) in the medium, and 20 min later again, with adenosine (10^{-5}M) in the medium. The coronary flow was measured continuously and pooled samples from the basal and adenosine infusion periods were taken for analysis of 6-keto-$PGF_{1\alpha}$. After the three periods of adenosine infusions, perfusion was changed to a different reactivity and the entire sequence of perfusion with basal and adenosine-containing media was repeated. In one half of the experiments perfusion was started with a pH 7.4 medium, and changed to a pH 6.9 medium during the latter part, and in the other half of the experiments the reversed scheme was applied.

The second series was designed for separate collection of coronary and interstitial effluent. In these experiments adenosine (final concentration 10^{-6}–10^{-4}M) was infused by means of a pump connected to a cannula which was applied close to the aortic root in the perfusion system. Adenosine was infused in 3 min periods separated by 20 min drug-free intervals. Coronary and interstitial effluents were collected in the two 3 min periods preceding and during the infusion of adenosine. Samples of these effluents were taken for analysis of 6-keto-$PGF_{1\alpha}$.

In the third series the hearts were pretreated with labelled prostaglandin precursor in the following way: 20 μCi (4 μmol) of [^{14}C]-arachidonic acid (New England Nuclear, sp. act. 50–60 mCi mmol^{-1}) was dissolved in 100 μl of ethanol and converted to its sodium salt by addition of 250 μl of 0.1 M NaOH. After further dilution with Tyrode solution to 10 ml the Na-[^{14}C]-arachidonate was infused into the heart via the aortic cannula for 50 min. Thirty min after the end of the infusion of isotope, coronary effluent collection in 5 min periods was started. Effluent was collected in the basal state and during one 5 min period of infusion of adenosine (final concentration 10–12 μM).

Analyses

Coronary and interstitial effluents were collected on ice during the experiments. After volume determination of the samples, portions were frozen and kept at −80°C until analysis. 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 lowest concentration measurable with 95% confidence (i.e. 2 s.d. at zero) was 0.5 pg ml^{-1} in undiluted heart effluent. The antibody used was raised in the Department of Pharmacology, Catholic University, Rome. The intra-assay variability averaged 4%, and the interassay variability averaged 8% over a range of 6-keto-$PGF_{1\alpha}$ concentrations from 15 to 200 pg ml^{-1}.

For analysis of labelled 6-keto-$PGF_{1\alpha}$ in the coronary effluent from hearts previously infused with [^{14}C]-Na-arachidonate, lipids in the effluent were adsorbed on C-18 columns (Sep-Pak, Waters) and eluted with methyl formate. After evaporation to dryness the residue was dissolved in chloroform and subjected to thin layer chromatography on silica plates (Plastikfolien, Kieselgel 60, Merck) in ethyl acetate/isooctane/acetic acid/water (110/50/20/100, v/v, water phase discarded). The plates were run twice against standards of 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, TxB_2, and PGD_2. The radiopeaks co-chromatographing with the standards were localized by radioscanning (Berthold Dunnschicht-Scanner), scraped off and quantified in a liquid scintillation spectrometer (LKB Wallac).

Statistical analysis

Figures in the text and tables are presented as mean ± s.e.mean. Student's *t* test for paired means or Wilcoxon's ranked sign test have been used for calculation of statistical differences, when applicable. Statistical differences presented by 2*P* are calculated with Student's *t* test for paired means and statistical differences presented by *P* are calculated with Wilcoxon's ranked sign test.

Results

Effects of adenosine on coronary flow and efflux of 6-keto-prostaglandin $F_{1\alpha}$ at normal and lowered pH

The beating rate of the rabbit hearts after equilibration with the perfusion system was 150–240 min^{-1}. A slight tendency to a decrease in beating frequency with time was apparent in most experiments.

The basal coronary flow at pH 7.4 was 16.1 ± 1.3 ml min^{-1} at the beginning of the experiment and 13.8 ± 0.9 ml min^{-1} at the end of the experiment. The corresponding values at pH 6.9 were 27.1 ± 3.6 and 23.7 ± 2.8 ml min^{-1}, respectively. The difference between the basal coronary flow values at pH 7.4 and 6.9 is significant ($2P < 0.01$). Adenosine (10^{-7}M) did not induce any increase in coronary flow, either at pH 7.4 or 6.9. At a concentration of 10^{-6}M, adenosine increased the coronary flow by about 30%, both at pH 7.4 ($2P < 0.005$) and 6.9 ($2P < 0.001$). At the highest concentration administered, (10^{-5}M) adenosine increased the coronary flow by about 75% at pH 7.4 ($2P < 0.005$), and by about 35% at pH 6.9 ($2P < 0.005$). It should be noted in this connection that the coronary vascular bed was probably maximally dilated already by an adenosine concentration of 10^{-6}M at pH 6.9, implying that a further increase in the concentration of adenosine could not be met by a corresponding increase in coronary flow for physical reasons (Table 1). No evidence was obtained that the effect of adenosine on the coronary flow was different at pH 7.4 compared to 6.9.

The basal coronary efflux of 6-keto-$PGF_{1\alpha}$ was 3.1 ± 0.5 ng min^{-1} in the beginning of the experiments, and 2.8 ± 0.4 ng min^{-1} at the end of the perfusion at pH 7.4. The corresponding outflow figures for 6-keto-$PGF_{1\alpha}$ at pH 6.9 were 3.7 ± 0.6 and 3.6 ± 0.8 ng min^{-1} respectively. Infusion of adenosine (10^{-7}M) did not affect the coronary efflux of 6-keto-$PGF_{1\alpha}$, either at normal or acidic pH. When adenosine was infused at a concentration of 10^{-6}M the coronary efflux of 6-keto-$PGF_{1\alpha}$ was significantly ($2P < 0.005$)

Table 1 Perfused rabbit heart; the basal coronary flow is shown and the effect of different concentrations of adenosine on the flow

	Adenosine (M)					
	10^{-7}		10^{-6}		10^{-5}	
	Before	During	Before	During	Before	During
at pH 7.4	16.1 ± 1.3	16.0 ± 1.1	14.2 ± 1.1	18.7 ± 1.8*	13.8 ± 0.9	23.9 ± 3.2*
at pH 6.9	27.1 ± 3.6	26.9 ± 3.6	24.5 ± 3.2	31.2 ± 4.1**	23.7 ± 2.8	31.9 ± 4.2*

Values are mean ± s.e.mean; $n = 11$.
* indicates that the value differs significantly ($2P < 0.005$) from the corresponding value before adenosine;
** indicates that the value differs significantly ($2P < 0.001$) from the corresponding value before adenosine.

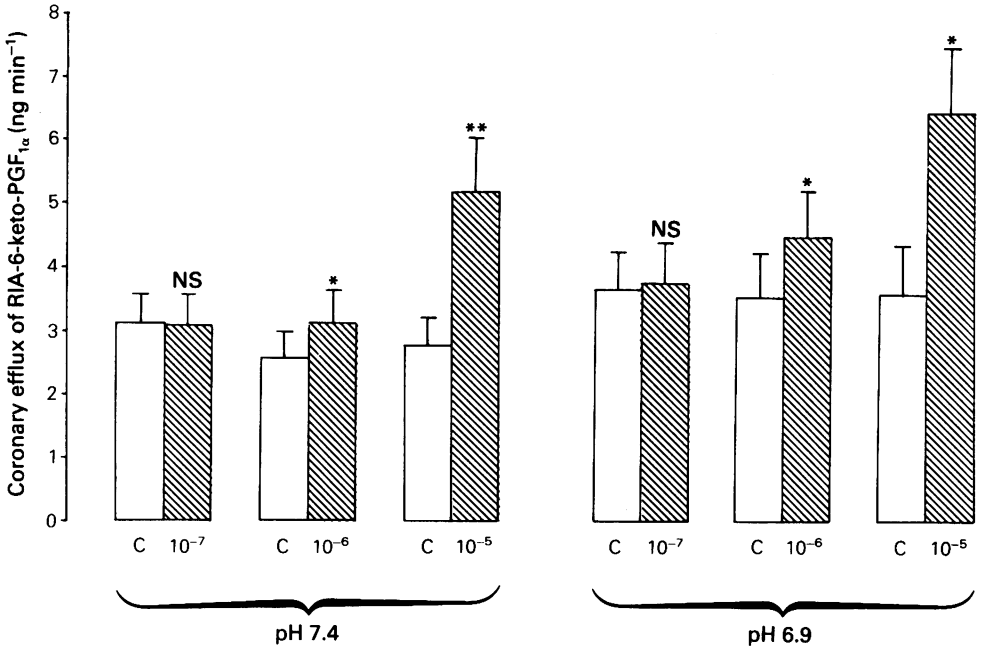

Figure 1 Cardiac production of prostacyclin, as reflected by the coronary efflux of its metabolite 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), in the basal state and during infusion of adenosine, at normal and lowered pH in the perfusion medium. The columns indicate the mean efflux of 6-keto-$PGF_{1\alpha}$ in ng min^{-1} ($n = 11$) during control periods (C, open columns) immediately preceding the periods of adenosine infusion, and during the infusions of adenosine (concentrations given below hatched columns); s.e.means shown by vertical lines. * and ** indicate that the values differ significantly ($2P < 0.005$ and $2P < 0.001$, respectively) from the corresponding values before infusion of adenosine. NS: not significant.

increased (Figure 1), by 22–27%. Administration of the highest concentration of adenosine (10^{-5}M) elicited a more marked increase ($2P < 0.001$) in the coronary efflux of 6-keto-$PGF_{1\alpha}$, by 80–87%. No definite differences between the outflow patterns of 6-keto-$PGF_{1\alpha}$ at pH 7.4 and 6.9 were observed, either during basal perfusion or during infusion of adenosine.

Coronary versus interstitial efflux of 6-keto-prostaglandin $F_{1\alpha}$ induced by adenosine

The basal coronary efflux of 6-keto-$PGF_{1\alpha}$ in this series was 6.5 ± 1.8 ng $3\,min^{-1}$. The corresponding figure for the efflux of 6-keto-$PGF_{1\alpha}$ in the interstitial fluid was 5.3 ± 0.8 ng $3\,min^{-1}$. Adenosine (10 μM) more than doubled ($P < 0.05$) the efflux of 6-keto-

Table 2 Perfused rabbit hearts were pre-labelled with [^{14}C]-arachidonic acid (0.4 μCi min^{-1} infused for 50 min); 30 min later the coronary effluent from the heart was collected in the basal state and during infusion of adenosine (12 μM)

	Total efflux of activity	*Lipid extractable activity*	*[^{14}C]-6-keto-$PGF_{1\alpha}$*
Basal	82253 ± 14782	4171 ± 1237	249 ± 79
During infusion of adenosine	156942 ± 10083	25686 ± 5178	1176 ± 429
	$2P < 0.005$	$2P < 0.02$	$P < 0.01$

Values expressed in c.p.m. are mean ± s.e.mean; $n = 6$

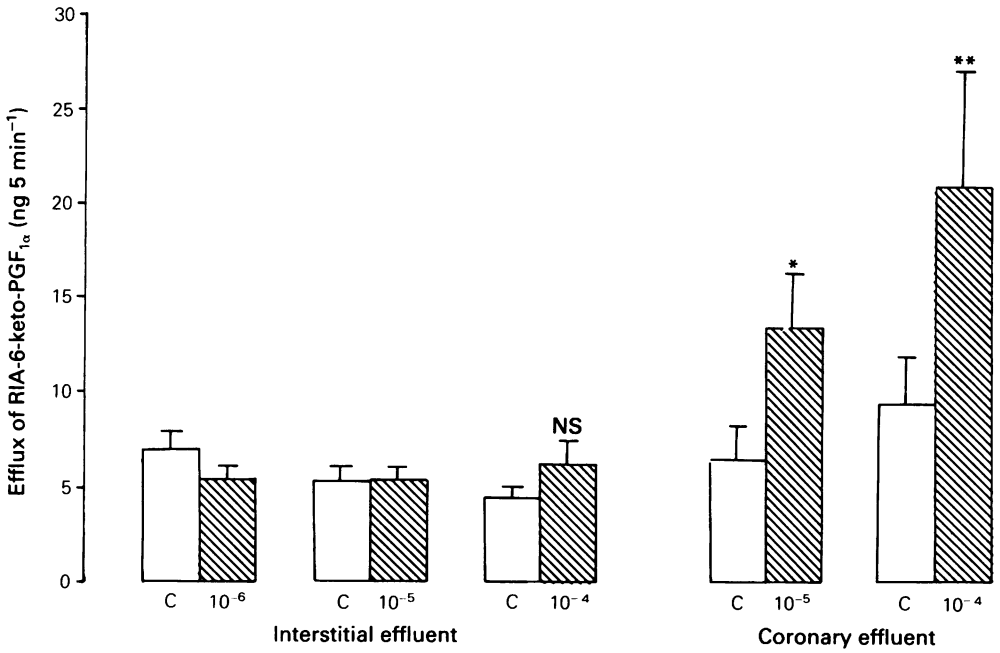

Figure 2 Perfused rabbit heart: the hearts were prepared to allow for separate collection of coronary and interstitial effluents. The columns indicate the cardiac formation of prostacyclin, as reflected by the coronary and interstitial efflux rates of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) during control periods (C, open columns) immediately preceding the periods of adenosine infusion, and during the infusions of adenosine (concentrations given below hatched columns). Data are expressed as mean of $n = 6$; s.e.means shown by vertical lines. * and ** indicate that the values differ significantly from the corresponding values before infusion of adenosine. NS: not significant.

$PGF_{1\alpha}$ in the coronary effluent, but did not affect the outflow in the interstitial fluid (Figure 2). When adenosine was administered at a concentration of 10^{-4}M the coronary efflux was augmented ($P < 0.01$) by about 120%. Also at this concentration adenosine failed to affect the outflow of 6-keto-$PGF_{1\alpha}$ in the interstitial fluid.

Efflux of labelled 6-keto-prostaglandin $F_{1\alpha}$ in hearts infused with [^{14}C]-Na-arachidonate

During infusion of ^{14}C-labelled Na-arachidonate, more than 85% of the radioactivity was retained in the organ. At the end of the washing period the basal efflux of radioactivity from the hearts was about 16,000 c.p.m. min^{-1}. When adenosine (12 μM) was infused the coronary flow was increased. The efflux of radioactivity was also raised, mainly in parallel to the increase in flow (Table 2). The amount of lipid-extractable activity, as indicated by the amount of the activity in the effluent adsorbed on the C-18 columns, was about 5% during basal perfusion. The amount of radioactivity co-chromatographing with the unlabelled standard of 6-keto-$PGF_{1\alpha}$ was about 6% of this lipid extractable activity.

The efflux of lipid extractable activity during infusion of adenosine was more than 15%, i.e. three times higher than the corresponding fraction collected during basal perfusion. The portion of the lipid extractable activity identified as 6-keto-$PGF_{1\alpha}$ in effluent collected during infusion of adenosine was not different from that during basal perfusion. This implies that the fractional amount of labelled 6-keto-$PGF_{1\alpha}$ in the coronary effluent during infusion of adenosine was elevated in parallel to the increase in efflux of lipid extractable activity (Table 2).

Discussion

The present experiments clearly demonstrate that adenosine in the perfusion medium can elicit efflux of 6-keto-$PGF_{1\alpha}$ from rabbit isolated heart. Since 6-keto-$PGF_{1\alpha}$ is a non-enzymatic hydrolysis product of PGI_2,

and since PGI_2 like other prostaglandins is not stored in the tissue (Piper & Vane, 1971), the data can be taken as evidence that adenosine stimulates the formation of prostacyclin in the rabbit heart. The concentrations required to elicit liberation of 6-keto-$PGF_{1\alpha}$ were 10^{-6}–10^{-4}M. In an earlier paper (Edlund *et al.*, 1983) we reported that in the same preparation, perfusion of the heart with a medium saturated with a gas mixture containing 8% O_2 instead of 95% O_2 was followed by a purine release into the interstitial effluent reaching a concentration of about 2×10^{-5}M. Since the interstitial effluent probably reflects the composition of the interstitial fluid it seems reasonable to conclude that a pathophysiological stimulus like severe hypoxia is sufficient to liberate adenosine in amounts which in turn are capable of eliciting formation of prostacyclin in the rabbit isolated heart.

Concerning the mechanism behind this stimulatory action, the current isotope experiments offer some information. It has been demonstrated earlier that labelled arachidonic acid, infused as in the present experiments, is incorporated into the phospholipids of the heart from which it can be released by physiological stimuli to serve as precursor in the formation of prostaglandins (Isakson *et al.*, 1976). Since the intracellular level of arachidonic acid in unstimulated cells is very low (Kunze & Vogt, 1971), the rate-limiting step in tissue prostaglandin formation is the mobilisation of substrate for cyclo-oxygenase, i.e. arachidonic acid liberation from the phospholipids in the cell membranes. This step is mediated physiologically by acyl hydrolases like phospholipase A_2 or possibly phospholipase C (cf. Blackwell & Flower, 1983). Based on these considerations it seems probable also that the currently observed stimulation of PGI_2 formation was based on acyl hydrolase activation. This assumption is supported by the observation that adenosine facilitated the efflux of labelled lipid extractable substances in the coronary effluent more markedly than the coronary efflux of radioactivity in general. Such an increase in the coronary efflux of lipid extractable substances indicates that specific liberation of lipid-bound radioactivity (i.e. labelled arachidonic acid) had occurred as a result of the administration of adenosine.

Prostacyclin is the main metabolite of arachidonic acid in the rabbit heart (DeDeckere *et al.*, 1977). Prostacyclin is mainly formed in vascular tissue (Moncada *et al.*, 1976; MacIntyre *et al.*, 1978; Marcus *et al.*, 1978), particularly in the endothelium. In the present experiments parallel collection of coronary and interstitial effluents revealed that adenosine facilitated efflux of 6-keto-$PGF_{1\alpha}$ into the venous effluent but not into the interstitial fluid. These data strongly support the concept of vascular formation of prostacyclin as the main source of 6-keto-$PGF_{1\alpha}$ in the present experiments, and hence, that adenosine stimulated prostacyclin synthesis in the endothelial cells.

It has been shown earlier that the cardiac actions of adenosine are potentiated by tissue acidosis (Merrill *et al.*, 1978). For this reason all the current flow measurements and prostacyclin efflux analyses were performed both at normal and low tissue pH. Although the data obtained confirmed the promoting action of tissue acidosis on the basal coronary flow, they provided no evidence of an augmentation of the coronary production of prostacyclin during increased tissue hydrogen concentrations. This observation does not support the theory that potentiation of the cardiac action of adenosine is connected to an increased formation of prostacyclin.

Taken together, the present experiments demonstrate that adenosine, in concentrations that can be obtained in response to physiological stimulation, facilitates coronary vascular formation of prostacyclin in the rabbit heart. It is beyond the scope of the present paper to discuss the physiological implications of such stimulation in detail, but it should be noted that a link between adenosine liberation from hypoxic or ischaemic myocardial cells and vascular formation of the most powerful vasodilator known, i.e. prostacyclin, can fulfil a purpose in adjusting coronary perfusion to the metabolic demand of the heart under certain circumstances.

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