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RELEASE OF TWO VASODILATORS, ADENOSINE AND PROSTACYCLIN, FROM ISOLATED RABBIT HEARTS DURING CONTROLLED HYPOXIA

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SUMMARY

1. The release of two locally formed vasodilators, adenosine and prostacyclin (PGI_2), from hearts subjected to different degrees of hypoxia was investigated. Isolated rabbit hearts were perfused according to Langendorff with Tyrode solution, saturated with gas mixtures containing 8–95 % O_2 and 5 % CO_2 in N_2. Coronary flow rate, O_2 extraction and uptake, and cardiac production of lactate, purines and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) were determined.

2. During perfusion of the hearts with a solution saturated with 95 % O_2, release of lactate, 6-keto-$PGF_{1\alpha}$ and purines was very low: lactate was liberated at a rate of about 5 μmol/100 g . min, purine release corresponded to 2 % of the total adenosine nucleotide content of the heart per hour and the release of 6-keto-$PGF_{1\alpha}$ was about 150 μmol/100 g . min.

3. During hypoxia there was a graded release of lactate and purines from the heart, as well as a liberation of 6-keto-$PGF_{1\alpha}$. Mild hypoxia (60 % O_2 in the gas mixture) elicited a 160 % increase in the formation of lactate and a 40 % increase in the release of purines. During severe hypoxia (8 % O_2 in the gas mixture) the release of lactate and purines increased by more than 2000 %. In contrast, the release of 6-keto-$PGF_{1\alpha}$ never increased more than 80 % at any degree of hypoxia, neither did it correlate to the severity of the hypoxia.

4. From these data we conclude that of the two vasodilating agents formed in the heart, adenosine and prostacyclin, the former is probably more important in the regulation of coronary flow.

INTRODUCTION

It is generally agreed that local metabolic factors rather than neurogenic influences are chiefly responsible for the adaptation of coronary flow to the metabolic demands of the myocardium. Already in 1961 Berne proposed that adenosine might be a link between the metabolic state of the heart and the tone in the coronary vessels. This 'adenosine hypothesis' states that if the coronary flow tends to decrease, the lowered

O_2 supply to the heart leads to anaerobic metabolism and a breakdown of energy-rich adenylic acids, with subsequent formation of adenosine. Adenosine is a potent vasodilator, and is released from the heart during hypoxia and ischaemia (Jacob & Berne, 1960; Imai, Riley & Berne, 1964).

However, hypoxia and ischaemia also stimulate the cardiac formation of prostaglandins (PG), (Kraemer & Folts, 1973; Wennmalm, 1975; Kent, Alexander, Pisano, Keiser & Cooper, 1973, Kraemer, Phernetton & Folts, 1976). Shortly after the discovery of the potent vasodilator and platelet anti-aggregatory PG prostacyclin (PGI_2) (Gryglewski, Bunting, Moncada, Flower & Vane, 1976), it was shown that this compound is produced by the animal heart following pharmacological stimulation (Isakson, Raz, Denny, Pure & Needleman, 1977) or hypoxia (De Deckere, Nugteren & Ten Hoor, 1977). Due to its formation in the coronary vessels and its biological properties, PGI_2 was also suggested to be involved, along with adenosine, in the (patho)physiological regulation of the coronary flow rate (De Deckere *et al.* 1977).

In order to elucidate the relative importance of these two proposed regulatory compounds we studied their parallel release during graded and controlled hypoxia in isolated rabbit hearts perfused according to Langendorff. The Langendorff heart preparation, while excellent for release studies, has a diminished basal coronary tone, which limits its suitability as model organ in studies aimed to analyse coronary flow. The main emphasis of the present investigation has therefore been given to the relation between the degree of hypoxia on the one hand and the magnitudes of release of adenosine and prostacyclin on the other.

METHODS

Preparation and perfusion of rabbit hearts

Rabbits of mixed strains and sexes, weighing 1·5–2·5 kg, were used for the study. The animals were killed by a blow on the head and exsanguinated by cutting one of the carotid arteries. The chest was opened and a catheter was quickly inserted in the aorta just above the coronary arteries, thus allowing rapid establishment of the coronary circulation with Tyrode solution. Another catheter was inserted in the pulmonary artery, and the pulmonary and caval veins were ligated. The heart was then removed to the perfusion apparatus, where coronary perfusion was continued. This modified Langendorff preparation (De Deckere & Ten Hoor, 1977) separates the coronary flow ($\dot{Q}_{rv}$) from the transmyocardial effluent ($\dot{Q}_i$). Thus $\dot{Q}_{rv}$ leaves the heart via the pulmonary artery, while $\dot{Q}_i$ reaches the surface of the heart through the interstitial space and via the lymphatics. The flow rate of $\dot{Q}_i$ is generally less than 5% of that of $\dot{Q}_{rv}$. $\dot{Q}_{rv}$ is collected from the catheter in the pulmonary artery, $\dot{Q}_i$ as it drips from the apex of the heart.

The Tyrode solution had the following composition (mM): NaCl, 136·9; KCl, 2·7; $CaCl_2$, 1·8; $MgCl_2$, 1·0; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4 and glucose 5·6. The pH of the solution was 7·4–7·5 and the temperature was kept at 38 °C. The perfusion pressure was 5·9 kPa (60 cmH_2O). The perfusion apparatus consisted of two columns supplied with Tyrode solution from different reservoirs and bubbled with gas from different containers. At the lower end the columns were connected via a stop-cock, which allowed instantaneous switching of perfusion from one of the columns (containing solution gassed with a mixture of 95% O_2 and 5% CO_2 = 'normoxic solution') to the other (containing solution gassed with 8–60% O_2 and 5% CO_2 in N_2 = 'hypoxic solution'). The solution used for perfusion during the preparation and stabilization periods was always normoxic. A cannula inserted between the stop-cock and the aortic catheter allowed sampling of inflow ('arterial') solution.

Procedure

After the stabilization period, which lasted 20–30 min, the experiments were started with normoxic perfusion for 8 min. Perfusion was subsequently switched to hypoxic medium, i.e. Tyrode solution equilibrated with gas mixtures containing either 60, 30, 20, 15 or 8 % O_2 and 5 % CO_2 in N_2. After 12 min of hypoxia, perfusion was switched back to normoxic solution for a final 10 min period. $\dot{Q}_{rv}$ and $\dot{Q}_i$ were collected at 1- and 2-min intervals, respectively, during these three periods and the weights of the collected effluents were used as a measure of flow rates without correction for density. 'Arterial' samples were also taken. All samples were collected on ice. The $\dot{Q}_{rv}$ samples were analysed for content of O_2 and lactate, while the $\dot{Q}_i$ samples, and in some experiments the $\dot{Q}_{rv}$ samples as well, were assayed for adenosine and its metabolites, and for 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2). Determinations of O_2 content were performed three times in the basal state, at 4, 6, and 8 min of hypoxia and again three times after hypoxia. Analyses of lactate (in $\dot{Q}_{rv}$), purines and 6-keto-$PGF_{1\alpha}$ (in $\dot{Q}_i$) were performed in all samples.

Twenty-four hearts were investigated, five at either of the hypoxia degrees 60, 30, 20, and 15 % (O_2 in the Tyrode solution) and four at 8 %. Only one degree of hypoxia was investigated in each heart.

Separately the effect of adding erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) to the Tyrode solution perfusing the heart was studied.

EHNA is an efficient inhibitor of adenosine deaminase (Schaeffer & Schwender, 1974) and was added to a final concentration of 3 μM in the Tyrode solution. Three experiments were performed. Following a normoxic equilibration period, hypoxia (20 % O_2 in the Tyrode solution) was introduced and maintained for 12 min, after which perfusion was switched back to normoxic solution. Samples for purine analysis were taken as above.

Analyses

The oxygen pressures in 'arterial' and 'venous' samples (i.e. solution from the cannulas above the aorta and from the pulmonary artery, respectively) were analysed in an IL 213 blood-gas analyser. The calculation of O_2 content assumes a solubility coefficient of 0·0224 (calculated from the results of Christoforides & Hedley-Whyte, 1969). The myocardial O_2 uptake has been calculated from the arterio-venous difference in O_2 content ($d(A-V)O_2$) and the coronary flow rate ($\dot{Q}_{rv}$).

Lactate was determined by a microfluorometric method as described by Jorfeldt & Juhlin-Dannfelt (1978).

Purines were analysed in unextracted samples using high performance liquid chromatography and absorbance detection (Fredholm & Sollevi, 1981; Fredholm, Hedqvist, Lindström & Wennmalm, 1982).

Analysis of 6-keto-$PGF_{1\alpha}$ was performed in unextracted samples in duplicate, using radio-immunoassay in 1:30 to 1:300 dilution (Patrono, Pugliese, Ciabattoni, Patrignani, Maseri, Chierchia, Peskar, Cinotti, Simonetti & Pierucci, 1982).

In each heart, the mean values of venous O_2 content and O_2 uptake (three determinations in each state), coronary flow (1–5 min of hypoxia expressed as a percentage of the basal flow) and lactate release (three basal determinations and eight during hypoxia, 5–12 min of hypoxia) were used for further calculations.

All results are presented as mean ± S.E. of the mean. When applicable, Student's t test has been used for calculating statistical differences.

RESULTS

O_2 uptake and release of lactate

The mean wet weight of the hearts was 6·7 ± 0·3 g. During perfusion of the hearts with normoxic Tyrode solution the beating frequency was 150–240/min. All hearts displayed powerful contractions, which were not subject to any visible deterioration during the normoxic perfusion period. In the basal state the coronary flow ($\dot{Q}_{rv}$) was 611 ± 17 ml/min . 100 g wet weight, and the $d(A-V)O_2$ was 6·8 ± 0·3 ml/l, resulting

in an O_2 uptake ($\dot{V}_{O_2}$) of 4·1 ± 0·2 ml/min . 100 g wet weight. The O_2 content in $\dot{Q}_{rv}$ was 12·8 ± 0·3 ml/l, corresponding to an O_2 pressure of 58 kPa (Fig. 1). A small net release of lactate, amounting to 5 μmol/min . 100 g wet weight, was found. The transmyocardial flow rate ($\dot{Q}_t$) was 19 ± 3 ml/min . 100 g wet weight.

Switching of perfusion to hypoxic medium induced marked changes in the mechanical activity of the heart, which were more pronounced the more severe the

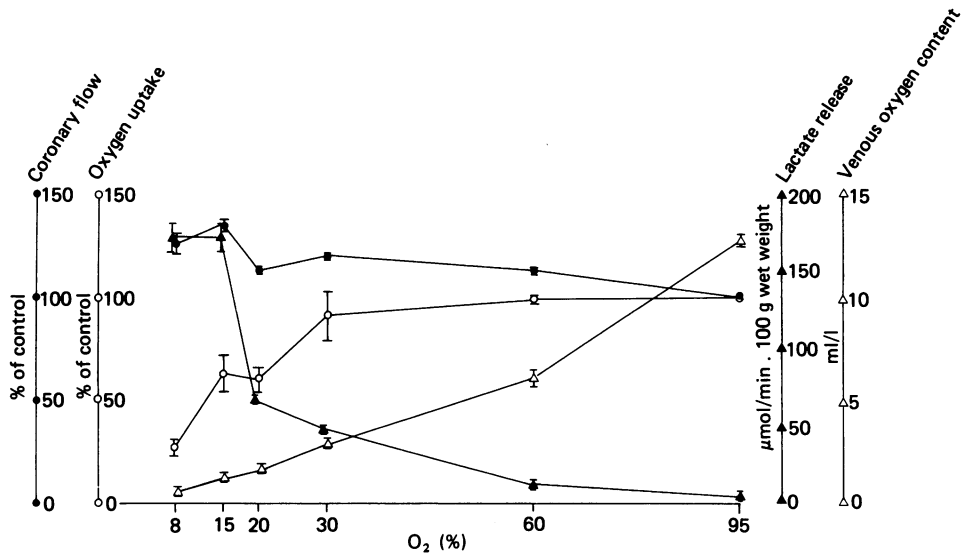

Fig. 1. Coronary flow (●—●), O_2 content in the coronary effluent (△—△), calculated O_2 uptake (○—○), and lactate release (▲—▲) in rabbit hearts perfused with Tyrode solution equilibrated with O_2 at different concentrations. Each point represents the mean value from twenty-four (normoxia) or five (hypoxia) experiments.

hypoxia. The heart rate fell and the contractions rapidly ceased. Simultaneously the heart dilated considerably, especially the atria and the auricles. When the O_2 in the Tyrode solution was lowered to 60 or 30 %, the O_2 content of the effluent decreased and the cardiac flow increased, resulting in an unchanged cardiac $\dot{V}_{O_2}$ compared to normoxic perfusion. Further reduction of the oxygen in the gas mixture, to 20 % or less, was, however, followed by a decreased myocardial O_2 consumption (Fig. 1). The $d(A-V)O_2$, which was unchanged at 60 % O_2, decreased below this figure in spite of very low venous levels of O_2 (corresponding to O_2 pressures below 3 kPa, Fig. 1). The decreased $d(A-V)O_2$ at 30 % O_2 or less was only partially compensated for by an increased coronary flow (Fig. 1). A significant increase in the release of lactate was obtained already at 60 % O_2, and this was greatly enhanced at more severe degrees of hypoxia (Fig. 1). At each level of hypoxia the release of lactate showed an initial peak followed by a decline to a plateau at which it was maintained throughout the hypoxic period (Fig. 2).

During the normoxic recovery period following the hypoxic perfusion, the mechanical activity was gradually restored. Complete recovery was, however, not achieved in all experiments, especially not after severe hypoxia. Within a few minutes the O_2

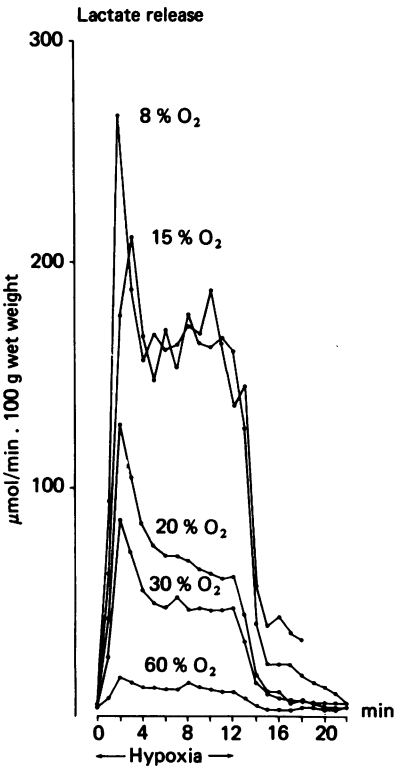

Fig. 2 Release of lactate in the coronary effluent from rabbit hearts in the basal state (0 min), during hypoxic perfusion with varying concentrations of O_2 (8–60 %) in the gas mixture equilibrating the perfusing medium (1–12 min), and during recovery with normoxic (95 % O_2) perfusion (13–22 min). Each point represents the mean from four or five hearts. Note that even with slight hypoxia (60 % O_2) there was a significant increase in lactate release. At all degrees of hypoxia there was an immediate rise, with a peak release within 3 min, then a decline to a steady-state level.

uptake and coronary flow had returned to the pre-hypoxic basal level, irrespective of the degree of the preceding hypoxia.

Purines

The basal release of purines in the transmyocardial effluent was low (Table 1). Adenosine was never recovered, and there were about equal concentrations of inosine and hypoxanthine. The release in the coronary effluent was significantly higher (Table 1); adenosine and hypoxanthine each constituted about 20 % of the total purine efflux and inosine the remaining part. The purine concentration in the total cardiac effluent was calculated to 0·4 μM (Table 1).

During hypoxic perfusion the release of purines increased progressively. Even a decrease to 60 % O_2 in the gas mixture elicited an increased release of purines in $\dot{Q}_i$, compared to the basal state (Fig. 3, Table 1). During more severe hypoxia there was a marked and progressive increase in both the concentrations and the release rates of the purines (Fig. 3, Table 1). When the release of purines was related to the O_2

TABLE 1. Concentrations and release rates of adenosine, inosine and hypoxanthine

	$\dot{Q}_i$						$\dot{Q}_{rv}$		Total cardiac effluent	
	Normoxia	Hypoxia					Normoxia	Hypoxia	Normoxia	Hypoxia
O_2 in perfusion medium (%)	95	60	30	20	15		95	8	95	8
Concentration of purines (μM)	2·2±0·2	3·3±0·4	4·5±0·9	8·8±1·3	12·5±4·2	19·3±4·9	0·4±0·1	6·4±1·3	0·4±0·1	6·6±1·1
Release of purines (nmol/min.100 g wet weight)	34±4	49±7	118±11	205±25	390±175	766±194	232±60	3770±398	286±72	4645±361
No. of expts.	24	5	5	5	5	4	4	4	4	4
Significance, $P <$	—	0·005	0·002	0·002	0·10	0·05	—	0·05	—	0·002

In each heart, basal values are the mean of 2–3 samples and hypoxic values are the mean of the samples drawn during 5–12 min of hypoxic perfusion. Values are given as mean±S.E. of the mean. Significance refers to the change in release rate during the different degrees of hypoxia compared to the corresponding release during normoxia in the same expt.

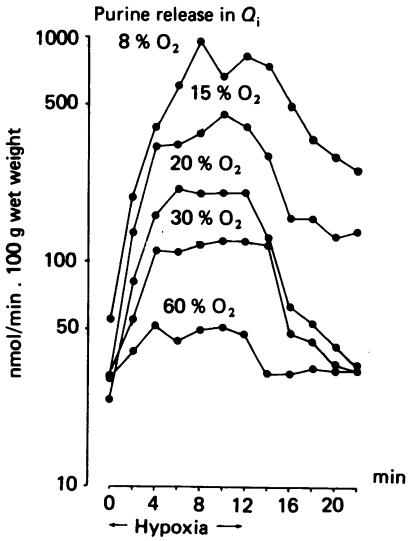

Fig. 3. Release of purines (adenosine + inosine + hypoxanthine) in the transmyocardial effluent from rabbit hearts during basal normoxia (0 min), varying degrees of hypoxia (2–12 min), and normoxic recovery (14–22 min). Each point represents the mean of four or five determinations. Note the progressively increasing purine release with more severe hypoxia.

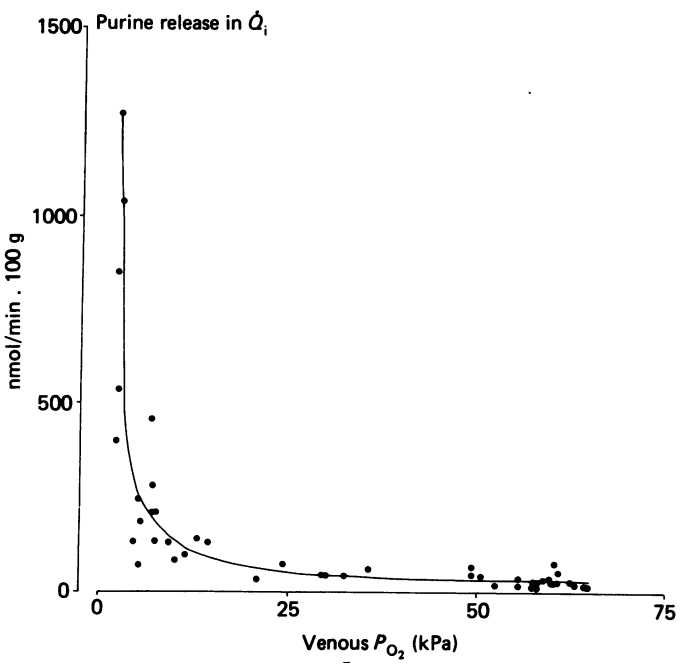

Fig. 4. Release of purines in the transmyocardial effluent ($\dot{Q}_i$) as a function of the O_2 pressure (P_{O_2}) in the coronary effluent ($\dot{Q}_{rv}$) during basal (normoxic) and hypoxic perfusion. Purine release is expressed as the mean value obtained from three determinations in the basal state and four determinations during 5–12 min of hypoxia. Venous P_{O_2} is the mean value obtained from three determinations in the basal as well as hypoxic state.

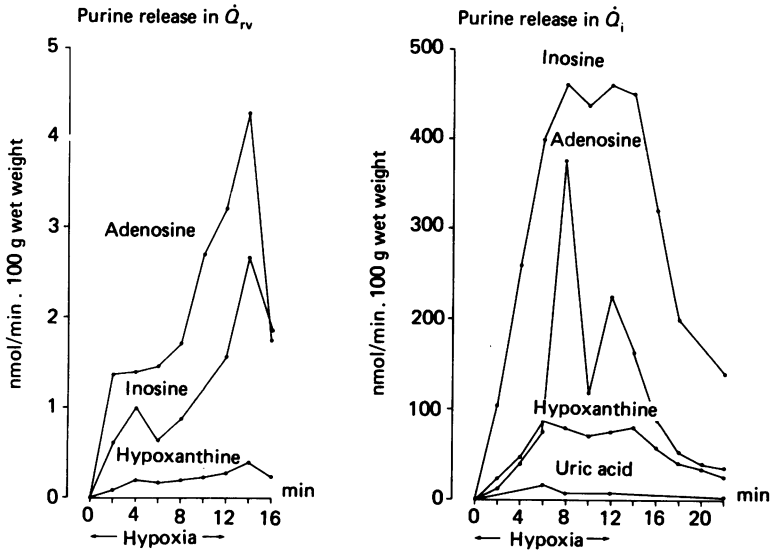

Fig. 5. Increase in release of adenosine and its metabolites above the basal (normoxic) level during severe hypoxia (8 % O_2 in the perfusing medium, 2–12 min) and normoxic recovery in the coronary ($\dot{Q}_{rv}$, 14–16 min) and transmyocardial ($\dot{Q}_i$, 14–22 min) effluents. Note that in $\dot{Q}_{rv}$ adenosine is the major purine, while inosine is in $\dot{Q}_i$. The release of uric acid is unaffected during hypoxia. Each point represents the mean of four determinations.

TABLE 2. Effect of EHNA (an inhibitor of adenosine deaminase), added to the perfusing medium, on the release of adenosine and its metabolites during basal normoxia and hypoxia (20 % O_2)

	Adenosine		Inosine		Hypoxanthine		Uric acid
Condition	Control	EHNA	Control	EHNA	Control	EHNA	EHNA
Basal	0	38±27	13±3	31±14	12±2	18±6	13±2
Hypoxia 20 % O_2	0	158±51	139±14	36±18	37±9	19±8	15±6

Five hearts were perfused with drug-free solution (control) and three hearts with solution containing 3 μM-EHNA. Values are given as nmol/min . 100 g wet weight, mean ± S.E. of the mean. From these data it is evident that the hypoxic increase in efflux of inosine and hypoxanthine was caused by an increased formation of adenosine. For further explanation, see text.

pressure in the $\dot{Q}_{rv}$, the release was found to be asymptotically increased at low O_2 pressures (Fig. 4). The maximal coronary flow during hypoxia (usually occurring with the first 3 min) was correlated to the total efflux of purines during the period ($r = 0{\cdot}77$). The total coronary flow during the hypoxia was, however, not correlated to this efflux ($r = 0{\cdot}07$).

The relationship between the purine release in $\dot{Q}_i$ and $\dot{Q}_{rv}$ was studied in four hearts during basal conditions and hypoxic perfusion (8 % O_2 in the gas mixture). The concentrations of the purines were 2–3 times higher in $\dot{Q}_i$ compared to $\dot{Q}_{rv}$, but the actual amounts released were about 5 times higher in $\dot{Q}_{rv}$. During hypoxia the dominant purine released in $\dot{Q}_{rv}$ was adenosine, but in $\dot{Q}_i$ it was inosine (Fig. 5). Hypoxanthine increased only slightly during hypoxia (Fig. 5). The basal release of

TABLE 3. Concentrations and release rates of 6-keto-$PGF_{1\alpha}$ in $\dot{Q}_l$ and $\dot{Q}_{rv}$ respectively

	$\dot{Q}_l$						$\dot{Q}_{rv}$		Total cardiac effluent	
	Normoxia	Hypoxia					Normoxia	Hypoxia	Normoxia	Hypoxia
O_2 in perfusing medium (%)	95	60	30	20	15	8	95	8	95	8
Concentration of 6-keto-$PGF_{1\alpha}$ (ng/ml)	2·5±0·3	3·1±0·9	2·4±0·7	2·1±0·8	1·6±0·4	1·5±0·3	0·02±0	0·04±0·01	0·07±0·02	0·14±0·02
Release of 6-keto-PGF_{α} (ng/min . 100 g wet weight)	34±2	42±6	52±5	61±17	38±7	50±7	23±7	36±11	47±10	90±8
No. of expts.	23	5	5	5	5	3	3	3	3	3
Significance, $P <$	—	n.s.	0·01	n.s.	n.s.	0·01	—	n.s.	—	n.s.

In each heart, basal values are the mean of three determinations and hypoxic values are the mean of 2–4 samples taken during hypoxic perfusion. Values are given as mean ± S.E. of the mean. Significance refers to the change in release rate during the different degrees of hypoxia compared to the corresponding release during normoxia in the same expts; n.s. indicates lack of statistical significance.

uric acid in $\dot{Q}_i$ (18 ± 9 nmol/min . 100 g wet weight) was low, and did not increase significantly during hypoxia (Fig. 5).

The effects of an adenosine deaminase inhibitor (EHNA) in the perfusing medium are shown in Table 2. Before EHNA was added, hypoxia (20 % O_2 in the gas mixture) elicited an increase in the release of purines in $\dot{Q}_i$, primarily inosine, and no adenosine was recovered. After addition of EHNA the total release of purines was of the same magnitude, but adenosine was now the major purine and the only one to increase during the period of hypoxia.

6-keto-PGF$_{1\alpha}$

The concentrations of 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of PGI_2, were some 100 times higher in $\dot{Q}_i$ compared to $\dot{Q}_{rv}$ and the major release (concentration × flow rate) was also via $\dot{Q}_i$ (Table 3). During hypoxic perfusion the total group displayed a slightly increased release of 6-keto-$PGF_{1\alpha}$ (44 %, $P < 0{\cdot}001$, $n = 24$), without correlation to the degree of hypoxia (Table 3). There was no correlation between the release of 6-keto-$PGF_{1\alpha}$ and the coronary flow.

DISCUSSION

The aim of the present investigation was to evaluate the relative production and release of the two vasodilating substances, adenosine and prostacyclin, formed in the heart during hypoxia. Adenosine is accepted as a regulator of the coronary flow. Since production of prostacyclin is stimulated by hypoxia, this compound has also been suggested to act in the regulation of the myocardial perfusion.

The preparation used in this study is well oxygenated and stable in the basal state, as previously reported (Edlund & Wennmalm, 1981). The basal data on coronary flow, $d(A-V)_{O_2}$, $\dot{V}_{O_2}$, and lactate production obtained here are well in accordance with those earlier results. The O_2 uptake was slightly higher in the present study; this may be explained by the fact that the rabbits used in this study were younger, with smaller hearts. The results during hypoxic perfusion are also in agreement with the previous study. Lowering the O_2 pressure in the gas mixture to 60 % resulted in a slight but significant increase in the production of lactate, indicating a certain degree of anaerobic metabolism.

During more severe hypoxia the decreased O_2 content in the perfusing medium was paralleled by an increase in coronary flow. This increase was, however, insufficient to compensate fully for the decreased O_2 content in the perfusing medium at 20 % O_2 or less. Consequently, $\dot{V}_{O_2}$ decreased and a large production of lactate appeared at 8–20 % O_2 in the Tyrode solution. The release of lactate displayed a biphasic pattern, with an immediate sharp rise in outflow, reaching a peak within 3 min of hypoxia, followed by a lower steady-state release.

Adenosine formed in the myocardium is to a considerable extent washed out via the blood vessels. The major part of the purines was washed out via the coronary effluent ($\dot{Q}_{rv}$); the amounts found in the interstitial flow collected on the surface of the heart ($\dot{Q}_i$) were smaller. In the isolated heart perfused with a salinic medium, the coronary flow is increased about 10-fold compared to the estimated *in vivo* flow. This leads to a considerable dilution of metabolites released in the coronary effluent and

may explain why the concentrations of purines were much higher in $\dot{Q}_i$. Once formed, adenosine is subjected to the intracellular actions of adenosine kinase (forming AMP), S-adenosyl-homocysteine (SAH) hydrolase (forming SAH) and adenosine deaminase (forming inosine) (Schrader, Schütz & Bardenheur, 1981; Schütz, Schrader & Gerlach, 1981). An increased formation of adenosine is detected extracellularly by an efflux of adenosine itself or of inosine (or its degradatives). Adenosine deaminase is present also in the cardiac effluent (Jacob & Berne, 1960). Such presence of adenosine deaminase may explain the small amounts of intact adenosine in $\dot{Q}_i$; the transmyocardial flow rate is rather slow, which would allow the deaminase to complete the inactivation of adenosine during the passage through the myocardium. The coronary flow is more rapid, and consequently adenosine was recovered in $\dot{Q}_{rv}$ to a greater extent.

Inosine may be derived not only from adenosine. AMP can be degraded along either or both of two routes. One goes via the 5′-nucleotidase to form adenosine, which is deaminated to inosine, the other via the action of the AMP deaminase to form inosinic acid (IMP) before dephosphorylation to inosine. In the heart, inosine is primarily formed via adenosine (Imai *et al.* 1964, Deuticke & Gerlach, 1966). To confirm this in the rabbit heart we studied the effect of adding an adenosine deaminase inhibitor (EHNA) to the perfusing medium (Table 2). Prior to EHNA, hypoxia elicited an increased release of inosine and hypoxanthine, but with EHNA only adenosine displayed an increase. This suggests that the hypoxic increase in the release of inosine (and to a smaller extent also hypoxanthine) in the absence of EHNA is based on an increased formation of adenosine instead of IMP. We are therefore inclined to consider the increased release during hypoxia as caused mainly by an augmented formation of adenosine.

During basal perfusion Schrader, Haddy & Gerlach (1977) found, in the effluent from isolated guinea-pig hearts, 4 nM-adenosine and a total purine concentration of 73 nM. The release rates were 5 and 48 nmol/min . 100 g, respectively. In another study the same group reported release rates of 9 and 104 nmol/min . 100 g, respectively (Schrader *et al.* 1981). Rubio, Wiedmeier & Berne (1974) reported a release of adenosine from guinea-pig hearts of 20 nmol/min . 100 g. In the present experiments we found 2–10 times higher values, possibly due to the differences in species and analytical techniques. Rubio & Berne (1969) measured the concentration of adenosine in the pericardial fluid in dogs and found 1·1 μM, probably representing the basal extracellular concentration in the myocardium. The transmyocardial effluent, $\dot{Q}_i$, should correspond fairly well to this and we measured twice as large a total purine content, but no adenosine. Miller, Belardinelli, Bacchus, Foley, Rubio & Berne (1979) studied the adenosine content in dog myocardium, and calculated the concentration in the interstitial fluid to be 2·4 μM, which relates well with our purine concentrations in $\dot{Q}_i$. Rubio & Berne (1969); Olsson (1970); Rubio *et al.* (1974) and Schrader *et al.* (1981), reported tissue concentrations of adenosine in different species in the same range.

It has been demonstrated in a number of studies that hypoxia and ischaemia elicit a breakdown of energy-rich adenylic acids, leading to an increased formation of adenosine and its metabolites (see e.g. Berne 1963; Gerlach, Deuticke & Dreisbach, 1963). Rubio *et al.* (1974) demonstrated a parallelism between coronary flow, tissue

adenosine levels and rate of release of adenosine at different levels of oxygen content in perfusing medium in isolated guinea-pig hearts. Our present data agree very well with their results, although we found an even more pronounced increase in the concentrations and release rates of the purines. Schrader *et al.* (1977, 1981) also reported hypoxia-induced release of purines (primarily inosine) from isolated guinea-pig hearts, of the same order of magnitude as in our experiments. The total myocardial content of adenine nucleotides (primarily ATP) and their derivatives has been reported to be (in μmol/100 g) 488 in the rabbit (Imai *et al.* 1964), 754 and 622 in the rat and the dog respectively (Rubio, Berne & Dobson, 1973), and 488 in the guinea-pig (Schrader & Gerlach, 1976). During 12 min of severe hypoxia (8% O_2) we found a loss of purines amounting to 56 μmol/100 g wet weight, which, according to the data referred above, should correspond to around 10% of the total content of adenine nucleotides of the heart. In contrast, during normoxic perfusion there is a loss of only 17 μmol/h, i.e. only 2%. This is in agreement with Bünger, Haddy & Gerlach (1975*a*), who studied the isolated guinea-pig heart and found essentially unchanged myocardial concentrations of high energy phosphates for 90 min during normoxic perfusion.

Recent studies (see Nees & Gerlach 1982) have shown that endothelial cells have particularly high levels of adenine nucleotides and that they release considerable amounts of adenosine when stimulated e.g. by hypoxia. It has therefore been suggested that a substantial proportion of the adenosine released into the coronary effluent may represent adenosine originating from endothelial cells rather than cardiac myocytes. Since this adenosine may never have been in contact with the smooth muscle cells of the coronary vessels, its biological role has been questioned. The $\dot{Q}_{rv}$ release of purines may, in fact, partly reflect purines originating from endothelial cells. By contrast $\dot{Q}_i$ is unlikely to be of predominantly endothelial origin. Since there was a principally similar pattern of release in $\dot{Q}_{rv}$ and $\dot{Q}_i$, our conclusion cannot be invalidated by an important endothelial cell contribution to the purine overflow into the venous coronary effluent.

The concentrations of purines in the interstitial fluid ($\dot{Q}_i$) of the present rabbit hearts are probably well within the range of physiological effects. Thus, Bünger, Haddy, Querengässer & Gerlach (1975*b*) studied isolated guinea-pig hearts and demonstrated a decrease in coronary vascular resistance with 10^{-7} M-adenosine in the perfusate and a maximal dilation around 5×10^{-6} M. Accordingly, Schrader *et al.* (1977), in the same preparation, noticed an increase in coronary flow even with 5×10^{9} M.

Prostacyclin is the major prostaglandin produced in the heart (Isakson *et al.* 1977, De Deckere *et al.* 1977) and due to its vasodilating properties it was suggested to take part in the regulation of coronary blood flow. Following anoxia De Deckere *et al.* (1977) detected an increased efflux of 6-keto-$PGF_{1\alpha}$ from the isolated rabbit heart. Our results likewise demonstrate an increased release of 6-keto-$PGF_{1\alpha}$ during hypoxia. This increase was, however, moderate and it derived from an elevation of the interstitial flow rate, the concentration of 6-keto-$PGF_{1\alpha}$ in the effluent not exceeding control. It is also worth noting that the increase in release rate did not correlate to the degree of hypoxia (Table 3). The basal concentration of 6-keto-$PGF_{1\alpha}$ in $\dot{Q}_i$ was rather high (2·5 ng/ml), certainly well above the ceiling for exerting

biological effects. In $\dot{Q}_{rv}$ the concentrations of 6-keto-$PGF_{1\alpha}$ were low, and the calculated value for the mixed cardiac effluent corresponds well with the results of Schrör, Köhler, Müller, Peskar & Rösen (1981) obtained in guinea-pig hearts.

Obviously there is a clear difference between the release patterns of the two vasodilating agents, adenosine and prostacyclin, produced in the heart. Both compounds are released in increased amounts during hypoxia; the increase in prostacyclin release is, however, very small, while adenosine production is enhanced many times over and is closely related to the degree of hypoxia.

As noted in the Introduction the possibilities to detect physiologically or pathophysiologically induced increases in coronary flow rate in the rabbit heart Langendorff preparation are limited, due to the low coronary tone prevailing already in the basal state. This may explain why the increase in coronary flow during hypoxia was rather low, and why there were no commonly occurring correlations between the release rates of purines or 6-keto-$PGF_{1\alpha}$ and the coronary flow. Rubio *et al.* (1974) and Schrader *et al.* (1977) studied isolated guinea-pig hearts perfused according to Langendorff and reported more pronounced elevations of the coronary flow during hypoxia (100–200 % increase) with good correlation to the efflux of purines. The basal coronary flow, expressed in ml/100 g tissue . min, in those studies on guinea-pig hearts was of the same order as that in the present rabbit hearts. The lack of more pronounced increases in coronary flow in our experiments may consequently reflect species differences.

In summary, isolated rabbit hearts perfused according to Langendorff and subjected to progressively increasing hypoxia display a parallel and gradual increase in the release of lactate (as a measure of anaerobic metabolism) and adenosine (and its degradation products). The production of PGI_2 is also stimulated by hypoxia, but only to a minor extent and not in parallel to the severity of the hypoxia. On the basis of these results we suggest that of the two vasodilating agents formed in the heart, adenosine and prostacyclin, the former is probably more important in the regulation of coronary flow.

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