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RELEASE OF ENDOTHELIAL MEDIATORS AND SYMPATHETIC TRANSMITTERS AT DIFFERENT CORONARY FLOW RATES IN RABBIT HEARTS

BY ÅKE WENNMALM*, GÜNTHER BENTHIN*, EWA KARWATOWSKA-PROKOPCZUK*, JAN LUNDBERG† and ANN-SOFI PETERSSON*

*From *the Department of Clinical Physiology, Gothenburg University, Gothenburg, and †the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden*

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SUMMARY

1. The release of three endothelial mediators, namely, endothelial-derived relaxing factor (EDRF), prostacyclin (PGI_2) and endothelin, and of two sympathetic neurotransmitters, noradrenaline and neuropeptide Y (NPY), from resting or sympathetically stimulated rabbit Langendorff hearts was investigated at normal or elevated coronary flow. The sympathetic nerves to the hearts were stimulated at 5 Hz for 30 s and the cardiac effluent was analysed for nitrite (metabolite of EDRF) with electron paramagnetic resonance spectrometry, for 6-keto-$PGF_{1\alpha}$ (metabolite of PGI_2) with gas chromatography/mass spectrometry, for endothelin- and NPY-like immunoreactivity with radioimmunoassay, and for noradrenaline and purines with liquid chromatography.

2. During perfusion of the hearts at normal flow ($35 \pm 1{\cdot}4$ ml min^{-1}) the effluent concentration of nitrite was $0{\cdot}15 \pm 0{\cdot}02$ μM, that of 6-keto-$PGF_{1\alpha}$ $0{\cdot}74 \pm 0{\cdot}08$ nM, and that of endothelin-like immunoreactivity $0{\cdot}18 \pm 0{\cdot}01$ pM. Nerve stimulation augmented the release of 6-keto-$PGF_{1\alpha}$ from 76 ± 8 to 99 ± 10 pmol $(3\ min)^{-1}$ ($P < 0{\cdot}05$), but did not affect the release of nitrite or endothelin-like immunoreactivity. Nerve stimulation also facilitated the outflow of noradrenaline and of NPY-like immunoreactivity by 52 ± 11 pmol $(3\ min)^{-1}$ and 19 ± 7 fmol $(3\ min)^{-1}$, respectively.

3. Elevation of the coronary flow to $79 \pm 3{\cdot}2$ ml min^{-1} did not affect the effluent concentrations of nitrite, 6-keto-$PGF_{1\alpha}$ and endothelin-like immunoreactivity, implying that their outflows were augmented. Sympathetic stimulation at elevated coronary flow did not further augment the outflow of endothelial mediators or of NPY-like immunoreactivity, but increased the outflow of noradrenaline by 62 ± 12%, in comparison to stimulation at normal flow. Perfusion of the heart with the noradrenaline uptake blocker desipramine (5 μM) completely abolished the promoting effecting of elevated coronary flow on noradrenaline outflow during sympathetic stimulation.

4. These data indicate that an increase in coronary flow in perfused rabbit hearts is paralleled by a corresponding facilitation of the formation of the endothelial mediators, EDRF, prostacyclin and endothelin. Such an elevation of mediator

formation does not affect nerve stimulation-induced release of sympathetic transmitters in the heart.

INTRODUCTION

Recent data indicate that the vascular endothelium, via formation of mediators affecting the tone of the underlying vascular smooth muscle cells, may play an active role in the regulation of blood flow. The endothelium has been shown to produce both smooth muscle relaxing and constricting agents. Those hitherto identified are the vasodilators endothelial-derived relaxing factor (EDRF) and prostacyclin (PGI_2), and the vasoconstrictor endothelin. PGI_2 is the most thoroughly investigated of these mediators. It is a potent, unstable smooth muscle relaxing agent, and in addition a powerful platelet anti-aggregatory compound (Gryglewski, Bunting, Moncada, Flower & Vane, 1976). PGI_2 is the major arachidonate derivative formed in the heart (De Deckere, Nugteren & Ten Hoor, 1977; Isakson, Raz, Denny, Pure & Needleman, 1977; Wennmalm, 1979*a*).

Endothelial-derived relaxing factor was originally described as an obligatory component in the relaxation of arterial smooth muscle by acetylcholine (Furchgott & Zawadzki, 1980). It was subsequently found to mediate the smooth muscle relaxation induced by several known vasodilators (see Vanhoutte, 1989). The short half-life of EDRF was properly explained when it was identified as nitric oxide, a highly unstable gas which is oxidized to nitrite and nitrate in physiological solutions (Palmer, Ferrige & Moncada, 1987). Albeit acting through different intracellular messenger systems, EDRF and prostacyclin share the properties to be vasodilators and platelet anti-aggregatory agents (see Moncada, Palmer & Higgs, 1987).

Endothelin, originally described as an incubation product of porcine aortic endothelial cells (Yanagisawa, Kurihara, Kimura, Tomobe, Kobayashi, Mitsui, Yazaki, Goto & Masaki, 1988), is actually a family of closely related 21-residue peptides. Endothelins elicit long-lasting hypertensive actions in most tissues and species investigated. Circulating levels of endothelin have been detected in humans under various conditions (see Karwatowska-Prokopczuk & Wennmalm, 1990*a*).

We recently reported that universal elevation of perfusion pressure at constant flow in the rabbit coronary circulation augments cardiac formation of PGI_2 substantially (Karwatowska-Prokopczuk, Ciabattoni & Wennmalm, 1989), possibly as a consequence of pressure-induced stretching of the coronary endothelial cells. In the present study we addressed the effect of an increase in coronary flow on the release of endothelial mediators, by analysing the formation of EDRF, prostacyclin, and endothelin in the cardiac effluent during perfusion at two different flow rates. Since some of these mediators may modulate the release of sympathetic transmitters, we also determined the outflow of noradrenaline and neuropeptide Y (NPY) evoked by stimulation of the sympathetic nerves to the heart under these conditions.

METHODS

Preparation and perfusion of rabbit hearts

Rabbits of mixed strains, 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 the left carotid artery. 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. The heart with intact right and left postganglionic sympathetic nerve supply was dissected out according to the method of Hucovic & Muscholl (1962) and further developed by Löffelholz & Muscholl (1969). The heart was then removed and transferred to the perfusion apparatus where coronary perfusion at constant pressure was continued. The sympathetic nerves were passed through ring platinum electrodes and the right and left nerves were stimulated simultaneously with pulses of supramaximal intensity (10–15 V) and a duration of 1 ms delivered by a Grass Model S88 stimulator. The cardiac effluent was collected dripping from the apex of the heart. Heart rate was recorded visually. Contractility was recorded via a lever attached with one end to the apex of the heart and the other to an isotonic transducer, connected to a Grass Model 7D recorder.

The Tyrode solution had the following composition (mM): NaCl, 137; KCl, 2·7; $CaCl_2$, 1·8; $MgCl_2$, 1·0; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4; glucose, 5·6; and ethylenediaminetetraacetic acid (EDTA), 0·1. The pH of the solution was 7·3–7·5 after equilibration with 3% CO_2 in O_2, and the temperature was kept at 38 °C.

Procedure

After the stabilization period, which lasted for 20–30 min, the experiments were started with perfusion at 'normal' coronary flow (constant pressure perfusion at 70 cmH_2O). Perfusate was collected during 3 min; this collection period is referred to as 'rest'. Immediately following this the sympathetic nerves were stimulated at 5 Hz for 30 s. Perfusate was collected from the beginning of the stimulation for 3 min; this collection period is called 'nerve stim'. After nerve stimulation, perfusion was maintained at normal flow for another 4 min, after which it was increased by elevation of the perfusion pressure to 140 cmH_2O. Following 5 min of perfusion at this elevated flow a 3 min effluent collection period (rest), followed by sympathetic stimulation with simultaneous effluent collection (nerve stim), were performed as during perfusion at normal flow.

Cardiac effluent was collected on ice. After volume determination the effluent was divided into portions for analysis of 6-keto-$PGF_{1\alpha}$, nitrite, endothelin and NPY, purines, and noradrenaline. The samples were immediately frozen and kept at −20 °C until the analyses, which were performed within 1 month.

Analyses

Analysis of nitrite (NO; oxidation product of EDRF was performed according to Wennmalm, Lanne & Petersson (1990). In summary, samples were degassed at about 120 mmHg for at least 1 h, equilibrated with He at atmospheric pressure, and added to a haemoglobin (Hb)–agarose column. The column was made up with 400 μl of a commercial gel of bovine Hb–agarose (Sigma), diluted with 1 ml of water, degassed at subatmospheric pressure and reduced with 10 mg of sodium dithionite in 1 ml of 0·1 M-HEPES buffer, pH 7·0. Dithionite (2 mg in 1 ml of HEPES buffer) was then added to the column, and the dithionite–sample mixture was left for at least 15 min, in order to ensure quantitative conversion of NO_2^- to NO. After this the mixture was allowed to pass through the column at a low flow rate. When the mixture had passed through the column it was frozen in liquid nitrogen. Standard curves were prepared by addition of known amounts of $NaNO_2$ (1–100 nmol) to samples of Tyrode solution before they were degassed. The NO content of the columns was determined using electron paramagnetic resonance spectrometry (EPR). The EPR spectra were recorded at a temperature of 77 K on a Varian E-3 EPR spectrometer at a microwave frequency of 9·17 GHz and a microwave power of 8 mW. Spectra were scanned from about 3000 to 3500 gauss with a modulation amplitude of 8 gauss and a rate of 25 gauss s^{-1}. When the standard of sodium nitrite was exchanged for sodium nitrate ($NaNO_3$) no EPR spectrum was observed, indicating that nitrate was not converted to NO by dithionite.

The prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$, was analysed with a stable isotope dilution technique using gas chromatography/mass spectrometry as follows. After addition of a tetradeuterated internal standard of 6-keto-$PGF_{1\alpha}$ (1 ng ml^{-1}) a 2·5 ml portion of the sample was treated for 40 min with 30 mg methoxyamine-hydrochloride dissolved in 0·75 ml acetate buffer (1·5 M, pH 4·8). After acidification the reaction products were adsorbed on a disposable C18 cartridge (Sep-Pak, Waters), washed, and eluted with 2 ml of ethyl acetate. After evaporation the residue was dissolved in 1 ml of 15% isopropanolol in ethyl acetate (v/v) and applied to a

disposable silica cartridge (Sep-Pak, Waters). After washing, the cartridge was eluted with 2 ml 40% isopropanolol in ethyl acetate (v/v). After evaporation to dryness the sample was converted to its pentafluorobenzyl ester, dried, and dissolved in 2 ml 45% ethyl acetate in hexane (v/v). It was subsequently adsorbed to a disposable dihydroxy cartridge (2-OH, Analytichem), washed, and eluted with 2 ml of 60% ethyl acetate in hexane (v/v). After evaporation, the residue was converted to its trimethyl-silyl ether. Upon analysis the sample was dried and dissolved in 10 μl hexane. A 5 μl portion was injected in a Varian 3400 gas chromatograph equipped with a 20 m medium polarity capillary column and operated at 275 °C. The gas chromatograph was connected to a Finnigan Incos 50 mass spectrometer operated in the negative ion/chemical ionization mode with methane as reactant gas. Selective ion monitoring was performed at mass number = 614 for the sample content of 6-keto-$PGF_{1\alpha}$ and at mass number = 618 for the tetradeuterated internal standard.

The content of endothelin-like immunoreactivity was analysed using antiserum RAS 6901 (Peninsula, Belmont, CA, USA) raised against endothelin-1 in rabbits. Endothelin-1 labelled with ^{125}I (Amersham, Bucks, UK) was used as tracer. The detection limit of the assay is 0·40 fmol per tube and the intra-assay variation for a 40 pM standard is 5%. This antiserum has the following cross-reactivity with peptides of the endothelin family: endothelin-1, 100%, endothelin-2, 47%, endothelin-3, 30%, and big endothelin-1, 31%. For further details see Pernow, Hemsén & Lundberg (1989). NPY-like immunoreactivity was detected using the N1 antiserum which does not cross-react (< 0·1%) with peptide YY or bovine pancreatic polypeptide (see Theodorsson-Norheim, Hemsén & Lundberg, 1985, for details and validation).

Purines were analysed in unextracted samples as adenosine, inosine and hypoxanthine using liquid chromatography with adsorbance 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.

Samples for analysis of noradrenaline were immediately acidified with acetic acid (final concentration 0·05 M). After thawing they were analysed using liquid chromatography with amperometric detection according to Hjemdahl, Daleskog & Kahan (1979). The recovery of dihydroxybenzylamine added to the perfusate and carried through the entire procedure of purification and determination was about 60%.

All results are presented as mean ± S.E.M. When applicable, Student's t test has been used for calculation of statistical differences. A $P < 0.05$ has been considered significant.

RESULTS

Normal coronary flow

The spontaneous heart rate was 122 ± 6 beats min^{-1} and the normal coronary flow was 35 ± 1·4 ml min^{-1}. All hearts displayed powerful contractions, which were not subject to any visible deterioration during perfusion at normal coronary flow. The effluent concentration of purines (sum of adenosine, inosine and hypoxanthine) was 0·15 ± 0·02 μM.

The effluent concentration of nitrite at rest was 0·15 ± 0·02 μM, and its outflow during the initial perfusate collection period was 12 ± 1·8 nmol $(3\ min)^{-1}$. The effluent concentration of 6-keto-$PGF_{1\alpha}$ was 0·74 ± 0·08 nM, and its outflow during perfusate collection at rest was 76 ± 8 pmol $(3\ min)^{-1}$. The corresponding figures for endothelin-like immunoreactivity were 0·18 ± 0·01 pM, and 19 ± 1·5 fmol $(3\ min)^{-1}$. The effluent concentration of endothelial mediators, and their outflow during the effluent collection periods are shown in Fig. 1.

Stimulation of the sympathetic nerves to the heart elevated the heart rate to 177 ± 12 beats min^{-1}, and increased the contractile force by 44 ± 5%. Purine outflow was not affected (Table 1). Nerve stimulation also induced an outflow of noradrenaline into the effluent, amounting to 52 ± 11 pmol $(3\ min)^{-1}$ (Fig. 2). In addition

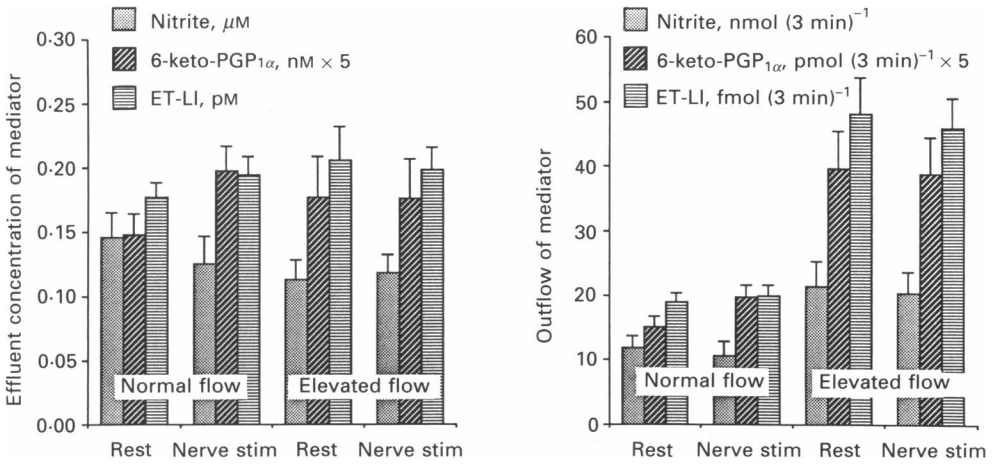

Fig. 1. Coronary effluent content of the endothelial mediators nitrite (oxidation product of nitric oxide (EDRF)), 6-keto-$PGF_{1\alpha}$ (dehydration product of prostacyclin), and endothelin (assessed as endothelin-like immunoreactivity, ET-LI). The left panel displays the effluent concentrations of these mediators, and the right panel presents the outflow of mediators in 3 min perfusate collection periods. The hearts ($n = 13$) were perfused at normal and elevated coronary flow ($35 \pm 1{\cdot}4$ and $79 \pm 3{\cdot}2$ ml min^{-1}, respectively). In either condition effluent from the hearts was collected in the basal state (Rest) and during sympathetic nerve stimulation (Nerve stim, 5 Hz, 30 s). Columns and bars represent mean ± S.E.M.

TABLE 1. Heart rate, inotropic response and outflow of purines in Langendorff perfused rabbit hearts ($n = 13$) subjected to sympathetic nerve stimulation (5 Hz, 30 s) at two different coronary flow rates. Data presented as mean ± S.E.M.

	Normal coronary flow ($35 \pm 1{\cdot}4$ ml min^{-1})		Elevated coronary flow ($79 \pm 3{\cdot}2$ ml min^{-1})	
	Rest	Nerve stim	Rest	Nerve stim
Heart rate (beats min^{-1})	122 ± 6	177 ± 12	138 ± 6	180 ± 7
Inotropic response (% over basal)	—	44 ± 5	—	55 ± 12
Effluent concentration of purines (μM)	$0{\cdot}15 \pm 0{\cdot}02$	$0{\cdot}15 \pm 0{\cdot}01$	$0{\cdot}14 \pm 0{\cdot}02$	$0{\cdot}18 \pm 0{\cdot}04$
Outflow of purines (nmol $(3\ min)^{-1}$)	$16 \pm 2{\cdot}5$	$16 \pm 1{\cdot}6$	$32 \pm 3{\cdot}2$	$39 \pm 7{\cdot}5$

the outflow of NPY-like immunoreactivity was enhanced, by 19 ± 7 fmol $(3\ min)^{-1}$ ($P < 0{\cdot}05$, Table 2).

During nerve stimulation the effluent concentration of 6-keto-$PGF_{1\alpha}$ was augmented moderately, to $0{\cdot}99 \pm 0{\cdot}10$ nM ($P < 0{\cdot}01$), as was its outflow during the perfusate collection period ($P < 0{\cdot}05$, Fig. 1). The effluent concentrations of nitrite or endothelin-like immunoreactivity, or the outflow rates of these mediators, were not affected by sympathetic nerve stimulation (Fig. 1).

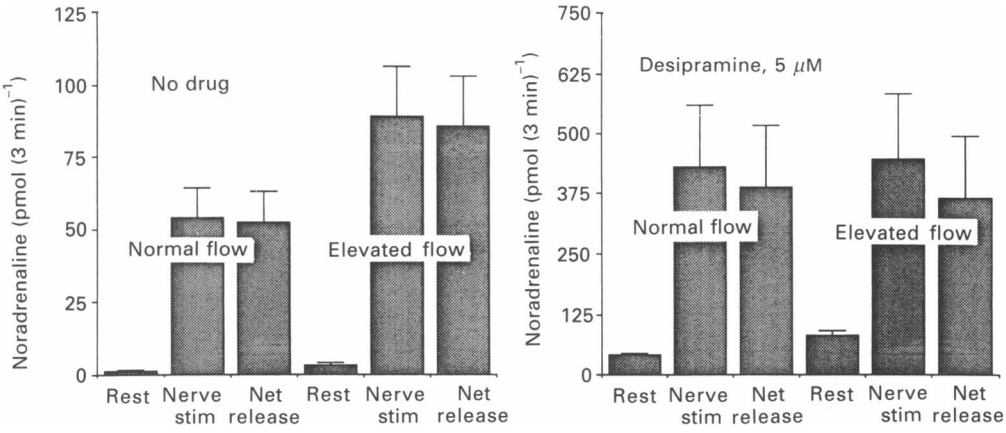

Fig. 2. Coronary effluent content of noradrenaline in rabbit hearts perfused without drug (left panel, $n = 13$), and with the noradrenaline uptake inhibitor desipramine (5 μM, right panel, $n = 7$). The hearts were perfused at normal and elevated coronary flow ($35 \pm 1{\cdot}4$ and $79 \pm 3{\cdot}2$ ml min^{-1}, respectively). In either condition effluent from the hearts was collected for 3 min in the basal state (Rest) and during sympathetic nerve stimulation (Nerve stim, 5 Hz, 30 s). Columns and bars represent mean ± S.E.M.

TABLE 2. Net outflow (outflow during stimulation − outflow at rest) of noradrenaline and neuropeptide Y from Langendorff perfused rabbit hearts ($n = 13$) in response to sympathetic nerve stimulation (5 Hz, 30 s) during perfusion at normal ($35 \pm 1{\cdot}4$ ml min^{-1}) and elevated ($79 \pm 3{\cdot}2$ ml min^{-1}) coronary flow. Effluent was collected during 3 min. Data presented as mean ± S.E.M.

	Normal coronary flow	Elevated coronary flow	P value
Noradrenaline (pmol (3 min)$^{-1}$)	52 ± 11	86 ± 17	$< 0{\cdot}001$
Neuropeptide Y (fmol (3 min)$^{-1}$)	19 ± 7	48 ± 19	$> 0{\cdot}05$

Elevated coronary flow

When the coronary flow was increased to $79 \pm 3{\cdot}2$ ml min^{-1} the spontaneous beating rate of the hearts was not affected. There was, however, a marked decrease in the contractile amplitudes, by 30–80%. The effluent concentration of purines was not affected (Table 1).

The effluent concentrations of endothelial mediators was not affected by the elevated coronary flow. Nitrite concentration displayed a slight tendency to decrease, and 6-keto-PGF$_{1\alpha}$ and endothelin-like immunoreactivity to increase; none of these numerical changes attained statistical significance (Fig. 1). Since the effluent concentrations were not diminished, the outflow rates of mediators were enhanced in relation to the increase in coronary flow. Thus, nitrite outflow increased from $12 \pm 1{\cdot}8$ to $22 \pm 3{\cdot}9$ nmol (3 min)$^{-1}$ ($P < 0{\cdot}05$), 6-keto-PGF$_{1\alpha}$ from 76 ± 8 to 198 ± 30 pmol (3 min)$^{-1}$ ($P < 0{\cdot}001$), and endothelin-like immunoreactivity from 19 ± 1 to 48 ± 6 fmol (3 min)$^{-1}$ ($P < 0{\cdot}001$).

Sympathetic nerve stimulation during elevated coronary flow induced chronotropic and inotropic responses that did not differ from the corresponding effects at normal flow (Table 1). The effluent concentration of purines was not affected

either. The net outflow of noradrenaline in the cardiac effluent was 86 ± 17 pmol $(3 \text{ min})^{-1}$, which is significantly ($P < 0.001$) more than the net outflow during stimulation at normal pressure (52 ± 11 pmol $(3 \text{ min})^{-1}$). Also the outflow of NPY was augmented by 48 ± 19 fmol $(3 \text{ min})^{-1}$ during stimulation at elevated coronary flow ($P < 0.05$, Table 2). There was no effect of nerve stimulation at elevated flow on the endothelial mediators; neither effluent concentration nor outflow per period of nitrite, 6-keto-$PGF_{1\alpha}$ or endothelin-like immunoreactivity were changed (Fig. 1).

Effect of inhibition of noradrenaline uptake with desipramine

Hearts perfused during the entire experiment with desipramine (5 μM; Ciba-Geigy) displayed a tendency to be arrhythmic. Apart from this, the hearts in this series did not differ from those perfused without drug with respect to coronary flow, beating frequency or contractility. The chronotropic and inotropic responses to sympathetic nerve stimulation did not differ from those in hearts perfused without drug with respect to amplitude. The duration of these responses was, however, substantially facilitated (not shown).

The basal outflow of noradrenaline was enhanced in comparison to the drug-free hearts (Fig. 2). The most marked effect of the drug was, however, on the outflow of noradrenaline in response to sympathetic stimulation. The net outflow of noradrenaline at normal coronary flow was 389 ± 127 pmol $(3 \text{ min})^{-1}$, which is more than 7 times the corresponding outflow in the absence of drug. During perfusion at elevated coronary flow there was a higher resting efflux of noradrenaline, in comparison to perfusion at normal flow. Nerve stimulation at elevated flow elicited a net outflow of noradrenaline amounting to 363 ± 129 pmol $(3 \text{ min})^{-1}$. The outflows of noradrenaline during normal and elevated coronary flow, respectively, in the presence of desipramine (5 μM) did not differ significantly.

DISCUSSION

In the present study the cardiac formation of three endothelial mediators, EDRF, prostacyclin and endothelin, was followed by analysis of nitrite, 6-keto-$PGF_{1\alpha}$, and endothelin-like immunoreactivity in the effluent from rabbit hearts perfused at normal and elevated coronary flow. Elevation of coronary flow did not change the mediator concentrations in the cardiac effluent, suggesting that their formation rates were increased. The present experiments also demonstrated that the induced changes in mediator formation did not affect stimulation-induced release of sympathetic transmitters from the adrenergic nerves in the heart.

The mechanical performance of the heart, and the cardiac effluent concentration of purines, were used as coarse indices of the metabolic state of the myocardium in the present experiments. We have previously demonstrated (Edlund, Fredholm, Patrignani, Patrono, Wennmalm & Wennmalm, 1983) that the purine outflow in this preparation during well-oxygenated conditions is about 280 nmol min^{-1} $(100 \text{ g wet weight})^{-1}$. The highest outflow of purines in the present experiments was about 40 nmol $(3 \text{ min})^{-1}$ (during elevated pressure), corresponding to an outflow of about 170 nmol min^{-1} $(100 \text{ g wet weight})^{-1}$. This low outflow of purines, and the well-

maintained chronotropic and inotropic responses to sympathetic stimulation, allow us to conclude that the present hearts were not hypoxic, and hence, that our results were not influenced by a unfavourable metabolic situation in the myocardium.

There are few data available on the release of endothelial mediators from perfused organs. Amezcua, Palmer, De Souza & Moncada (1989) reported that acetylcholine (1 μM) given during 1 min released about 500 pmol of nitric oxide in Langendorff perfused rabbit hearts. They did, however, not state the basal efflux of NO in their preparation. In isolated guinea-pig heart the basal release of NO was reported to average 164 pmol min^{-1}, to be substantially increased by infusion of bradykinin (Kelm & Schrader, 1988). The latter authors also observed that perfusion of the hearts with the NO scavenger oxyhaemoglobin reduced the cyclic GMP release into the coronary circulation.

In a previous study we reported an outflow of 6-keto-$PGF_{1\alpha}$ from rabbit hearts, using gas chromatography/mass spectrometry as in the current experiments, averaging 30 ng $(3\ min)^{-1}$ (Karwatowska-Prokopczuk & Wennmalm, 1990*b*). In the present study the outflow of 6-keto-$PGF_{1\alpha}$ amounted to about 27 ± 3 ng $(3\ min)^{-1}$ (76 ± 8 pmol $(3\ min)^{-1}$), i.e. very close to that observed earlier.

Also with respect to endothelin few if any data on efflux rates are available. In normal subjects the plasma level has been reported to be around 3 pg ml^{-1} (Koyama, Nischzawa, Moril, Tabata, Inoue & Yamaji, 1989) or up to 0·69 pg ml^{-1} (Cernacek & Stewart, 1989). Data on efflux from isolated organs have not been reported.

The levels of nitrite and endothelin-like immunoreactivity currently reported consequently appear to be the first on basal outflow in this preparation. The parallel analyses of nitrite, 6-keto-$PGF_{1\alpha}$, and endothelin-like immunoreactivity call for a comment on the relation between their concentrations in the cardiac effluent. On a molar basis, nitrite was most abundant, appearing in the effluent in a concentration of about 0·15 μM. NO, the active parent compound to nitrite, is the most unstable of the mediators analysed here, having a $t_{\frac{1}{2}}$ of 6–50 s (Griffith, Edwards, Lewis, Newby & Henderson, 1984; Förstermann, Trogisch & Busse, 1985). The prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$, was released to produce an effluent concentration of about 0·75 nM; its concentration was consequently about 200 times lower than that of nitrite. Prostacyclin is considerably more stable than NO, but disappears in biological solutions within 10 min (Gryglewski *et al.* 1976). Endothelin-like immunoreactivity appeared in a concentration of about 0·15 pM in the cardiac effluent, i.e. six orders of magnitude lower than the concentration of nitrite, and 5000 times lower than that of 6-keto-$PGF_{1\alpha}$. Endothelin is quite stable in biological solutions (Pernow *et al.* 1989). Hence, the effluent concentrations of these mediators was inversely related both to their $t_{\frac{1}{2}}$ and to their respective molecular weights.

The relative potencies of these mediators is also of interest in connection with their mutual effluent concentrations. The half-maximal effective concentration (EC_{50}) of endothelin was reported to be 0·4 nM in isolated specimens of vascular tissue (Yanagisawa *et al.* 1988). In the rabbit heart we obtained evidence of a somewhat higher EC_{50}, being in the 1–5 nM range (Karwatowska-Prokopczuk & Wennmalm, 1990*b*). The concentration giving 50% maximal inhibition (IC_{50}) of NO and prostacyclin in platelets aggregating in response to various agents have been reported to be 0·3–1·1 μM and 1·5–12 nM (Radomski, Palmer & Moncada, 1987).

Making comparisons between these EC_{50} and IC_{50} levels, obtained in various systems, with the mediator release obtained in the present experiments may be misleading, and the results must be interpreted with great caution. Keeping this in mind, it is yet interesting that the current release of endothelin-like immunoreactivity was 3–4 orders of magnitude lower than the observed EC_{50}, that the release of prostacyclin was 1–10 times lower than the observed IC_{50}, and that the release of nitrite was only 2–6 times lower than the observed IC_{50}. According to these data, nitrite and prostacyclin would be released in the basal state in concentrations sufficient to elicit borderline biological activity, while endothelin would be far from this. Previous data from our laboratory, as well as from others, support the concept that endogenously formed NO and prostacyclin in fact have an impact on coronary flow in the Langendorff perfused heart (Wennmalm, 1979*b*; Amezcua *et al.* 1989; Wennmalm, Karwatowska-Prokopczuk & Wennmalm, 1989).

Elevation of the coronary flow did not change the mediator concentration in the cardiac effluent, indicating that the release of endothelial mediators from the vascular wall was enhanced sufficiently to meet the increase in flow. We have earlier demonstrated that adenosine-induced vasodilation in the rabbit heart augments the efflux of 6-keto-$PGF_{1\alpha}$, and that both the flow effect and the stimulated efflux of 6-keto-$PGF_{1\alpha}$ are obstructed by purinoceptor antagonism (Karwatowska-Prokopczuk, Ciabattoni & Wennmalm, 1988). The present data are in harmony with the concept that endothelial mediator efflux is related to the flow rate, and extends the observation to EDRF and endothelin as well. The physiological significance of such a flow-related formation of mediators is not obvious; it may be speculated that the release of NO and prostacyclin is associated with the anti-platelet effect characteristic of healthy vascular endothelium.

Sympathetic nerve stimulation elicited a 30% increase in the release of 6-keto-$PGF_{1\alpha}$, in accordance with previous observations in this preparation (Wennmalm, FitzGerald & Wennmalm, 1987). Furthermore, sympathetic nerve stimulation resulted in an outflow of noradrenaline and NPY-like immunoreactivity into the cardiac effluent. This outflow of noradrenaline and NPY-like immunoreactivity was enhanced when the stimulation was performed in association with elevated coronary flow. In order to clarify the basis for the increased release of noradrenaline an additional series of experiments was performed. It is generally accepted that a major portion of the amine released by nerve stimulation is normally recaptured into the nerves, and that reuptake plays an important role in the termination of action of the noradrenaline released (Rosell, Kopin & Axelrod, 1963). In the present experiments the elevation in coronary flow might have impaired such reuptake by facilitating wash-out of transmitter more efficiently. An alternative mechanism behind the increased outflow of noradrenaline might have been that the release of noradrenaline per nerve impulse was increased, possibly by some mechanism triggered by the increase in flow. To reveal whether the increased efflux of noradrenaline during stimulation at elevated flow was based on an increased release of transmitter, or alternatively, was the result of an impaired reuptake, some hearts were perfused with the noradrenaline uptake inhibitor desipramine (5 μM) added to the solution perfusing the heart. Desipramine completely abolished the difference between the outflows of noradrenaline which was seen in the drug-free experiments at normal and

elevated coronary flow, respectively. This is consistent with the view that the increased flow enhanced noradrenaline outflow in the effluent by promoting wash-out of transmitter. It also rules out the alternative, i.e. that the increased outflow of noradrenaline would be based on an increased release per nerve impulse.

In conclusion, the endothelial mediators EDRF, prostacyclin, and endothelin are continuously released from the coronary endothelium of the perfused rabbit heart; EDRF and prostacyclin in concentrations that may attain biological activity. Elevation of coronary flow is met by an increase in mediator release, which maintains their concentration in the effluent at the pre-set level. The release of mediators does not affect stimulation-evoked liberation of sympathetic transmitter under the conditions investigated. Further studies are required to reveal the interplay between mediator release, flow rate, and vascular tone in the coronary circulation.

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