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ACTIONS OF PROSTAGLANDIN E_2 ON MYOCARDIAL MECHANICS, CORONARY VASCULAR RESISTANCE AND OXYGEN CONSUMPTION IN THE GUINEA-PIG ISOLATED HEART PREPARATION

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1 In isolated, electrically driven (3 Hz) hearts of guinea-pigs the action of prostaglandin E_2 on left ventricular pressure (LVP), oxygen consumption ($\dot{Q}O_2$) and coronary vascular resistance (CVR) was studied by establishing cumulative concentration-response curves. The hearts were perfused at a constant flow (10 ml/min) with Tyrode solution (Ca^{++} 1.8 mM) at 32° C.

2 Under control conditions prostaglandin E_2 ($2.86 \times 10^{-11} - 1.43 \times 10^{-7}$ M) decreased LVP, $\dot{Q}O_2$ and CVR in a concentration-dependent manner by maximally 27, 18 and 38%, respectively ($P < 0.05$).

3 After reserpine pretreatment there were lower initial values for all parameters measured. The effect of prostaglandin E_2 on LVP and $\dot{Q}O_2$ was abolished, but CVR was further diminished, depending on the concentration.

4 The results seem to support the hypothesis of an interaction of prostaglandin E_2 with endogenous catecholamines as far as the effects on LVP and $\dot{Q}O_2$ are concerned. In contrast, prostaglandin E_2 seems to have a direct action on CVR, which is independent of the presence of catecholamines.

Introduction

Investigations into the action of prostaglandins on myocardial muscle preparations have been the subject of numerous studies (for references see Nakano, 1973). However, the results were divergent and generally acceptable explanations for these differences (which obviously seem to be more than simple species differences) are lacking. One possible cause may be the different methods of investigation. Thus, it is difficult to draw definite conclusions about changes in myocardial contractile force by prostaglandins from experiments with spontaneously beating preparations, since it is well known that the prostaglandins of the E series in particular also possess marked chronotropic actions (for references see Hedquist, 1973). Furthermore, especially for the E prostaglandins, their possible interaction with adrenergic neurotransmission must be taken into account; this in itself should induce alterations in myocardial contractile force and heart rate (see Hedquist, 1973).

Finally, there are striking differences between the atrial and ventricular myocardium, in their morphology (Forssmann & Girardier, 1966; Fawcett & McNutt, 1969; McNutt & Fawcett, 1969) as well as in their responsiveness to stimulation by exogenous drugs (Webler, 1974). These seem to have been overlooked at least by those authors, who tend to extend their results, obtained in isolated atrial preparations to the heart in general.

It was the aim of this study to investigate the actions of prostaglandin E_2 on factors, which are assumed to be of importance for myocardial performance, such as left ventricular pressure development, myocardial oxygen consumption and coronary vascular resistance in well defined isolated ventricle preparations in relation to their basic adrenergic activity.

Methods

Design of the experiment

In guinea-pigs (300-400 g body weight) of either sex, thoracotomy was performed under light ether anaesthesia after pretreatment with heparin (10 mg/kg i.p.). The aorta was cannulated as soon as possible and the heart perfused according to the

Langendorff technique with Tyrode solution at room temperature during the time of preparation, including cannulation of the pulmonary artery and ligation of all other blood vessels near the heart. A fluid-filled rubber-balloon-catheter was inserted into the left ventricle via the mitral ostium and connected with a Statham P 23 Db pressure transducer for measuring left ventricular actively developed pressure (LVP) and its derivatives: contraction velocity ($LVdP/dt_{max}$) and relaxation velocity ($LVdP/dt_{min}$). Characteristics of the system for pressure measurement were: resonant frequency 100 Hz, quenching factor about 0.5, time constant 1 ms, maximal frequency response: 24 Hz. Time to peak tension (TPT), which is not influenced by changes in pressure development, was estimated graphically from recordings at high paper speed (100 mm/second). Myocardial oxygen consumption (Q_{O_2}) was monitored by polarimetry (platinum-electrodes, Clark principle, sensitivity: 25-33 nA/mmHgO_2, provision for adjustment: 90% of final value within <1 s) of the pulmonary artery outflow as described elsewhere (Klaus & Krebs, 1969). In order to prevent limitation in oxygen supply to the heart in each experiment oxygen tension (P_{O_2}) was about 650 mmHg in the inflowing solution and higher than 200 mmHg in the outflow.

During the experiments the heart was perfused at constant flow (10 ml/min) with Tyrode solution of the following composition (mM); Na^+ 149, K^+ 5.4, Ca^{2+} 1.8, Mg^{2+} 1.05, Cl^- 145, $H_2PO_4^-$ 0.42, HCO_3^- 11.9, THAM 10.0, disodium edetate (EDTA) 0.05, glucose 11.0, pH 7.4, equilibrated with 95% O_2 and 5% CO_2 at 32° C and electrically driven at a constant rate of 180 beats/min (Grass stimulator S5, impulse duration 4 ms, 40V).

Coronary perfusion pressure was measured by Statham P 23 Bb pressure-transducer in the coronary inflow tract and was considered to be a direct expression of coronary vascular resistance (CVR), because the perfusion flow was held constant.

After an equilibration period of about 20 min, prostaglandin E_2 was added in a cumulative way. Experiments did not last longer than 120 minutes. In some animals reserpine (Serpasil) 7 mg/kg was given intraperitoneally once 16-24 h before the experiment.

Preparation of prostaglandin E_2 solutions

Stock solutions of prostaglandin E_2 were prepared as follows: 5 mg was dissolved in 0.5 ml 95% ethanol, 4.5 ml Na_2CO_3 (20 mg/100 ml) was added, the final pH checked (6-7.5), and the solution was stored frozen at −25° C not longer than 1 month. Immediately before starting an experiment dilutions were made from the stock solutions to give a concentration of 5 μg/ml prostaglandin E_2, aliquots of which were added to the perfusion fluid.

Statistical analysis

Statistical evaluation was performed using Student's *t*-test for comparing the means of the variables, which were tested with and without maximum concentrations of prostaglandin E_2. The coefficient of regression (b_{yx}) as obtained by analysis of covariance was also tested for significance. The means and their standard error ($\bar{x} \pm$ s.e. mean) are quoted in the text. *n* is the number of observations. The level of significance for α in each case was 0.05.

Results

Time of constant performance, coronary vascular resistance and O_2 consumption

In a first series of experiments LVP (mmHg), left ventricular contraction (LVP/dt_{max}) and relaxation ($LVdP/dt_{min}$) velocities (mmHg/s), time to peak tension (TPT) (ms), mean CVR (mmHg/ml) and Q_{O_2} (μl O_2/g ventricular dry weight per min) were measured under control conditions over a total time of 120 min after stabilization of the preparations from reserpine-treated and non-treated animals. Apart from a small and insignificant decrease of about 4.5% in Q_{O_2} in non-treated animals essentially no changes in any of the parameters were observed ($n = 6$). Thus, at least for a period of 120 min, our experimental conditions may be assumed to maintain a stable performance of the heart preparations.

Influence of reserpine-treatment

In the experiments on the possible role of catecholamines in the action of prostaglandin E_2, hearts from reserpine-treated animals were used. The actual degree of catecholamine depletion was checked by the addition of tyramine (5 μg/ml) to the perfusion fluid, in another series of experiments. Tyramine produced only small increases in myocardial performance without alterations in CVR and Q_{O_2}, indicating a sufficient depletion of catecholamines by reserpine.

As summarized in Table 1, there was a marked diminution by reserpine treatment of myocardial contractile force and CVR. On the other hand, only an insignificant decrease in Q_{O_2} was observed.

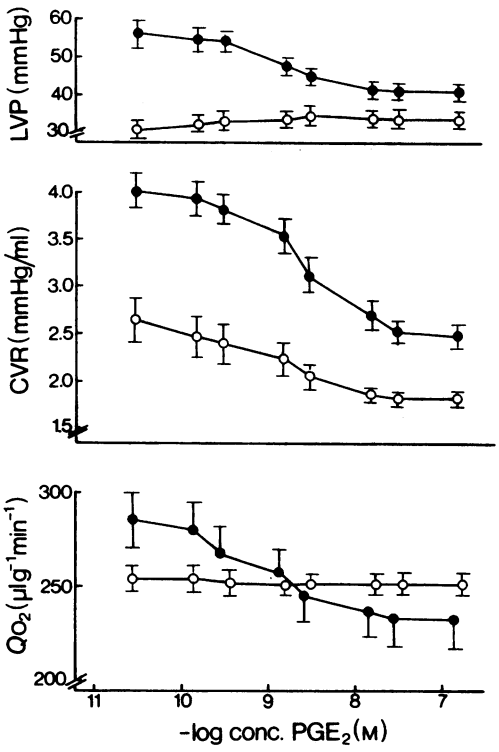

Figure 1 Changes in left ventricular pressure (LVP) (mmHg), coronary vascular resistance (CVR) (mmHg/ml) and oxygen consumption QO_2 (µl/g ventricular dry weight per min.) in response to − log molar concentration of prostaglandin E_2 (PGE_2) in control ((•) $n = 8$) and reserpine pretreated ((○) ,$n = 8$) animals. Vertical lines show s.e. mean.

Effects of prostaglandin E_2 in control animals

Prostaglandin E_2 in concentrations ranging from 2.86×10^{-11} to 1.43×10^{-7}M was studied. At 2.86×10^{-11}M prostaglandin E_2 did not alter myocardial performance, CVR or QO_2. However, if the concentration of prostaglandin E_2 was raised, there occurred a concentration-dependent decrease in cardiac performance, coronary vascular resistance and oxygen consumption. Changes in LVP, CVR, and QO_2 are shown in Figure 1 (•), changes in $LVdP/dt_{max}$, $LVdP/dt_{min}$ and TPT in Table 2 (left side). All changes, except the decrease in QO_2 became statistically significant at 2.86×10^{-9}M prostaglandin E_2, if compared with the respective control values. However, for QO_2 a significant regression-line with $b_{yx} = -21.63 \pm 3.11$ was obtained.

Effects of prostaglandin E_2 in reserpine-treated animals

In reserpine-treated guinea-pigs the effects of the same range of prostaglandin E_2 concentrations, i.e. 2.86×10^{-11} to 1.43×10^{-7}M, were studied. In contrast to control animals, in reserpine-treated guinea-pigs which had lower control levels of LVP, CVR and QO_2, there were no further changes in LVP and QO_2, when additive concentrations of prostaglandin E_2 were applied (Figure 1 (○)). The same was true for $LVdP/dt_{max}$, $LVdP/dt_{min}$ and TPT (Table 2, right side).

In contrast, a concentration-dependent decrease in CVR was also seen after the animals had been reserpine-treated: the difference became significant at 2.86×10^{-9}M prostaglandin E_2, i.e. at the same concentration as in the non-treated group. However, the coefficient of regression was only -0.38 ± 0.04 as compared with -0.72 ± 0.04 ($P < 0.05$) in the non-reserpine-treated group. Thus, the curves are not parallel. Changes in LVP and CVP are demonstrated in representative experiments with a control heart and a heart from a reserpine-treated guinea-pig in Figure 2.

Discussion

The above results provide clear evidence for a concentration-dependent decrease in myocardial performance, coronary vascular resistance and myocardial oxygen consumption in constant-flow perfused, electrically stimulated isolated heart of

Table 1 Left ventricular pressure (LVP), left ventricular contraction velocity ($LVdP/dt_{max}$) and relaxation velocity ($LVdP/dt_{min}$), time to peak tension (TPT), mean coronary vascular resistance (mCVR) and oxygen consumption (QO_2) of control and reserpine-treated guinea-pigs

	n	*LVP (mmHg)*	*$LVdP/dt_{max}$ (mmHg/s)*	*$LVdP/dt_{min}$ (mmHg/s)*	*TPT (ms)*	*mCVR (mmHg/ml)*	*QO_2 (µlg^{-1}min^{-1})*
Control	8	55.8 ± 3.6	742 ± 53	501 ± 41	117.5 ± 2.5	4.00 ± 0.18	285 ± 15
Reserpine	8	30.4 ± 2.1	374 ± 28	249 ± 18	136.9 ± 1.6	2.69 ± 0.25	254 ± 13
Significance		+	+	+	+	+	NS

QO_2 = µl O_2 per g ventricular dryweight and per minute.

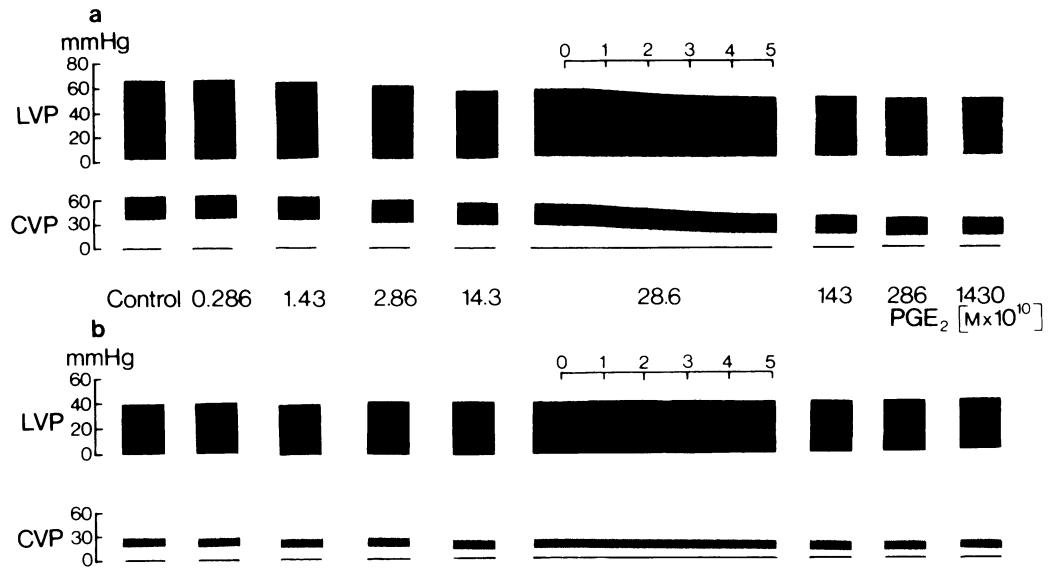

Figure 2 Changes in left ventricular pressure (LVP) and coronary vascular pressure (CVP) produced by increasing concentrations of prostaglandin E_2 (PGE_2) in representative control (a) and reserpine-pretreated animals (b). At 2.86×10^{-9}M, a continuous record lasting 5 min is shown; at the other concentrations of prostaglandin E_2 only the final steady effect is shown. Note the differences in initial values of LVP and CVP between (a) and (b), the decrease in LVP for (a) but not (b) and the diminution of CVP for both groups, although more pronounced for (a), with the same final value.

Table 2 Changes in left ventricular contraction velocity ($LVdP/dt_{max}$), relaxation velocity ($LVdP/dt_{min}$) and time to peak tension (TPT) by cumulative addition of prostaglandin E_2 (PGE_2) in reserpine-treated and non-treated animals

	No reserpine (n = 8)			*Reserpine* (n = 8)		
PGE_2 (M)	*$LVdP/dt_{max}$ (mmHg/s)*	*$LVdP/dt_{min}$ (mmHg/s)*	*TPT (ms)*	*$LVdP/dt_{max}$ (mmHg/s)*	*$LVdP/dt_{min}$ (mmHg/s)*	*TPT (ms)*
2.86×10^{-11}	742 ± 53	509 ± 41	118 ± 2	381 ± 27	262 ± 19	137 ± 2
1.43×10^{-10}	727 ± 49	489 ± 33	120 ± 2	401 ± 25	268 ± 20	134 ± 2
2.86×10^{-10}	684 ± 38	468 ± 23	120 ± 2	404 ± 23	266 ± 19	134 ± 2
1.43×10^{-9}	618 ± 39	424 ± 23	125 ± 3	429 ± 25	274 ± 18	133 ± 2
2.86×10^{-9}	571 ± 36*	401 ± 21*	126 ± 3*	432 ± 25	282 ± 21	132 ± 2
1.43×10^{-8}	528 ± 37*	368 ± 20*	129 ± 3*	430 ± 24	279 ± 18	132 ± 2
2.86×10^{-8}	525 ± 39*	366 ± 19*	129 ± 3*	437 ± 25	282 ± 18	132 ± 2
1.43×10^{-7}	521 ± 37*	364 ± 17*	129 ± 3*	437 ± 25	284 ± 19	132 ± 2

Values are mean ± s.e. mean

* Significant in comparison with value in presence of 2.86×10^{-11}M prostaglandin E_2

the guinea-pig by prostaglandin E_2. This is in contrast to many investigations on the actions of E-prostaglandins, carried out with isolated atria or spontaneously beating heart preparations, where either no changes or increases in contractile force were described (for references see Nakano, 1973).

On the other hand, our results agree well with an investigation reported by George, Paddock & Kadowitz (1972), who described a marked decrease in contractile force of isolated, electrically driven hearts of rats by prostaglandin E_1 in a concentration of 1×10^{-8}M. However, the transient preceding increase in contractile force as well as a tendency for contractile force to return towards control values, while the perfusion was continued (George *et al.*, 1972), could not be detected under our conditions. As shown in this paper, the effect of prostaglandin E_2 was essentially stable, which was confirmed in a more detailed study, that gave no evidence for tachyphylaxis to prostaglandin E_2 in either guinea-pig ventricles or atria (Krebs & Schrör, 1975).

A probable explanation of the decrease in contractile force could be seen in the fact that the electrical stimulation of cardiac sympathetic fibres, which are involved in the maintenance of constant myocardial performance in non-treated animals, is inhibited by prostaglandin E_2. This is in agreement with previous reports, in which inhibition by prostaglandin E_1 and E_2 of adrenergic transmitter release in the sympathetically innervated rabbit heart (Hedquist & Wennmalm, 1971) and decrease by prostaglandin E_1 of the release of noradrenaline in the rat heart *in situ* with an intact nervous system (Papanicolaou, Meyer & Milliez, 1971) were found. This hypothesis is further supported by the lack of any effects on myocardial performance, if the animals are reserpine-treated, i.e. depleted of their endogenous catecholamine content.

On the other hand, there is good evidence in favour of a direct dilatory action of prostaglandin E_2 on coronary vessels, because this effect persisted also in catecholamine-depleted hearts. However, the participation of some residual catecholamines, combined with a greater responsiveness of coronary vessels than of ventricular myocardium to catecholamine action offers another possible explanation.

Hence, if effects of prostaglandins in isolated hearts are studied, the endogenous transmitter content has to be considered in every case. This seems to have a greater influence in ventricular than in left atrial preparations of the same animal. In contrast to ventricular muscle, the contractile force of atrial muscle was increased as a result of a direct action of prostaglandin E_2. This effect on atrial muscle could even be enhanced by prior reserpine-treatment (Krebs & Schrör, 1975). The result, that the isoprenaline concentration-response curve for increase in actively developed left ventricular pressure is shifted to the right and downwards in control but not in reserpine-treated animals (Ca^{++} 1.8 mM) (Schrör, Becker, Berg & Krebs, 1975), provides additional evidence for the view that many of the differences reported, e.g. so-called 'inotropic' actions, seem to be understandable on the basis of the different models and methods of investigations used.

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