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The Action of the Dihydro Derivatives of Prostacyclin – (6R)-PGI_1 and (6S)-PGI_1 on the Heart and the Coronary Vasculature

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Summary. The action of the dihydro prostacyclins, (6R)-PGI_1 and (6S)-PGI_1, was studied on the isolated guinea pig heart and bovine coronary artery strips. PGE_2 and PGI_2 were used as standards.

In the isolated guinea pig heart (6S)-PGI_1 decreased the coronary perfusion pressure (CPP), myocardial force of contraction (MFC) and oxygen consumption (QO_2). (6R)-PGI_1 did not produce a significant change in these parameters. The ED_{50} (50% of maximum coronary dilation) was approximately 20 times higher for (6S)-PGI_1 than for PGI_2 or PGE_2.

Treatment of the hearts with reserpine + tyramine abolished the (6S)-PGI_1-induced decrease in MFC but not the decrease in the CPP. The same pattern of responses was seen with PGE_2.

Bovine coronary artery strips were contracted by both (6S)-PGI_1 and (6R)-PGI_1, the ED_{50} (50% of maximum increase in tension) being 5 and 10 times higher than that for PGE_2. The (6S)-PGI_1-induced contraction was preceeded by a small relaxation, which, however, was much less than that seen after PGI_2.

It is concluded that the hydration of the 5,6 double bound in the PGI_2 molecule results in an almost complete loss of PGI_2-like activity and generates PGE-like activity. The same biological activity of both dihydro prostacyclins in the isolated coronary artery strip but not in the intact coronary vascular bed leads to suggest that the sites of action in these systems are different.

Key words: Dihydro-PGI_2 – Prostacyclin (PGI_2) – Bovine coronary artery – Guinea pig heart – Myocardial mechanics – Coronary vascular tone.

Introduction

Prostacyclin (PGI_2) is a recently discovered product of arachidonic acid transformation via the cyclooxygenase pathway (Moncada et al., 1976). Release of PGI_2 has been demonstrated for isolated perfused hearts of several animal species (Schrör et al., 1977, 1978; de Deckere et al., 1977) and a potent coronary dilating activity was shown (Schrör and Moncada, 1978, Link et al., 1978). PGI_2 is an unstable compound, the half-life in aqueous solution being about 5 min (Johnson et al., 1976). Because of this and the possible biological significance of PGI_2 in prevention of platelet aggregation and modulation of local perfusion in vivo (Moncada and Vane, 1977; Armstrong et al., 1978), more stable analogs of PGI_2 have been synthesized, among them dihydro-derivatives, which also show inhibition of platelet aggregation (Togna et al., 1977) and have a protective activity against indomethacin-induced gastric erosions (Whittle et al., 1978).

Here, it is reported about the action of the dihydro-derivatives of prostacyclin, (6R)-PGI_1 and (6S)-PGI_1 (Fig. 1) on the coronary artery in vitro and the intact coronary vascular bed. Moreover, the action of both substances on the myocardial force of contraction and oxygen consumption was studied. PGE_2 and PGI_2 were used as standards.

Methods and Materials

Guinea Pig Hearts. Guinea pigs of either sex (body weight 300–400 g) were treated with heparin (10 mg/kg i.p.) and killed by a blow on the head. The heart was removed and placed into the perfusion apparatus after the aorta and pulmonary artery had been cannulated and the pulmonary and caval veins were ligated.

Perfusion was performed at constant volume (10 ml/min) via the aorta with oxygenated (5% CO_2 in O_2) Tyrode's solution at 35° C. The hearts were electrically driven at a constant rate of 180 beats/min (Grass stimulator S9, 40 V, 4 ms). Mean coronary perfusion pressure (CPP) was measured in a branch of the aortic inflow tract. A fluid-filled rubber balloon catheter was inserted into the left ventricle via the mitral ostium to measure the peak left ventricular actively developed pressure (LVP). The preload was adjusted to 0 to +2 mm Hg at the beginning and did not change during the experiment. Myocardial oxygen consumption (QO_2) was monitored polaro-

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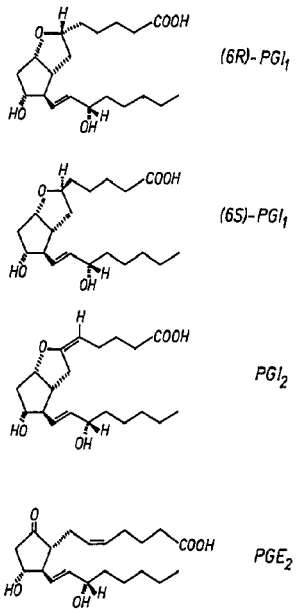

Fig. 1. The chemical structure of the 5,6-dihydro prostacyclins *(6S)-PGI*$_1$ and *(6R)-PGI*$_1$ as compared to *PGE*$_2$ and *PGI*$_2$

graphically in the pulmonary artery outflow with platinum electrodes as described elsewhere (Klaus and Krebs, 1968) and is referred to ventricular dry weight (v.d.w.).

Bovine Coronary Arteries. Bovine hearts were obtained immediately after slaughter, immersed in oxygenated Krebs-bicarbonate solution and transported to the laboratory (total time 20–30 min). The left descending coronary artery was dissected, cleaned of visible fat and cut helically into strips of about 30 × 2 mm. The strips were suspended under 2 g tension in a 10 ml muscle chamber at 37° C in Krebs-bicarbonate solution, equilibrated with 5 % CO_2 in O_2. Responses to the substances were recorded isometrically using strain-gauge transducers (TF 3, Fleck, Mainz).

The strips relaxed during the first 2 h of observation. The tone became increased in most of the preparations during the next 2 h and then remained stable for another 2–3 h. Measurements were made after this equilibration period of about 4 h had elapsed. Some preparations (10–20 %) did show spontaneous alterations in tone and were not used.

Substances and Solutions. Reserpine (Serpasil®, CIBA, Basel), PGE_2, (6R)-PGI_1, (6S)-PGI_1, PGI_2-Na (Schering, Berlin), tyramine-hydrochloride (Merck, Darmstadt) were available for our investigations.

Stock solutions (1 mg/ml) of PGE_2, (6R)-PGI_1 and (6S)-PGI_1 were prepared in phosphate buffer (50 mM) pH 7.2, and diluted with the perfusion medium to the concentration required, immediately prior to the experiment. PGI_2-Na was prepared as a stock (1 mg/ml) in 0.1 M NaOH and diluted with this solvent 10 and a 100-fold. Aliquots were added to the bath fluid (coronary artery strips) or infused with an infusion pump (Braun, Melsungen) at constant speed of 0.1 ml/min (guinea pig heart). The final pH was 7.4. All concentrations are referred to the final concentration of the free acid in the perfusion or bath fluids, respectively.

Experimental Protocol. After stable coronary vascular tone had been observed, PGE_2, (6R)-PGI_1 and (6S)-PGI_1 were added in a cumulative way of application. At each step an equilibration period of 10 min (duration of infusion or presence of the substances in the bath fluid) was allowed. Measurements were performed at the end of the equilibration period. Cumulative dose-response relationship was not studied with PGI_2 in the coronary artery strips because of the short half-life of the compound, which in our hands was about 8 min (Krebs-bicarbonate solution, 37° C, pH 7.4). After the maximum reaction had been obtained, substances were washed out for 20 (guinea pig heart) or 30 min (coronary artery strips) and afterwards the highest concentration of the drug was administered again.

Some of the guinea pigs were treated with reserpine (5 mg/kg i.p.) once 18–24 h prior to surgery. These hearts additionally received 3 $\times 10^{-6}$ M tyramine before the dose-response relationship was obtained.

Statistics. Statistical analysis was performed using the *t*-test. The mean and standard error ($\bar{x} \pm$ S.E.M.) are quoted in the text. The level of significance was 0.05. *n* is the number of observations.

In the study with the guinea pig heart, there was a time-dependent decrease in the LVP as seen from untreated control hearts. Statistical analysis of the action of the substances on myocardial force of contraction was, therefore, done by comparing the data obtained in presence of maximum concentration of the drug with those after the wash-out period.

Results

Guinea Pig Heart

Perfusion of untreated guinea pig hearts for 90 min with Tyrode solution after the end of the equilibration period (30–40 min after finishing the surgery) was followed by a time-dependent decrease in the LVP from 81 ± 5 to 65 ± 4 mm Hg ($P < 0.05$, $n = 12$). The CPP remained unchanged, 68 ± 4 mm Hg at the beginning and 72 ± 5 mm Hg after 90 min perfusion ($P < 0.05$, $n = 12$).

Cumulative application of PGE_2 and PGI_2 ($3 \times 10^{-9} - 3 \times 10^{-7}$ M) leads to a dose-dependent decrease in the CPP, which was completely reversible after a 20 min wash-out period. The maximum decrease amounted to 42 and 34 % of the initial value ($P < 0.01$). PGE_2 did also produce significant decrease in the LVP and $\dot{Q}O_2$, whereas PGI_2 did not (Table 1).

Cumulative application of (6S)-PGI_1 ($3 \times 10^{-8} - 1 \times 10^{-5}$ M) did also diminish the CPP for the same extent as seen with PGE_2 and PGI_2, 30 % of the initial value ($P < 0.01$). There was also a decrease in LVP and $\dot{Q}O_2$ ($P < 0.05$). All these effects were reversible after a 20 min wash-out period. In contrast to this, (6R)-PGI_1 ($3 \times 10^{-8} - 1 \times 10^{-5}$ M) did not produce any significant change in LVP, CPP and $\dot{Q}O_2$ (Table 1).

By comparing the relative potencies of PGE_2, PGI_2 and (6S)-PGI_1 in decreasing the CPP, an approximately 20 times lesser potency of (6S)-PGI_1 as compared to either PGE_2 or PGI_2 was found (Fig. 2).

Because this pattern of responses might indicate an inhibition of stimulus-induced noradrenaline release by (6S)-PGI_1, some additional experiments were performed with reserpinized animals.

Pretreatment of the hearts with reserpine + tyramine produced a decrease in LVP, $\dot{Q}O_2$ and CPP, due

Table 1. Effects of PGE_2, PGI_2, (6R)-PGI_1 and (6S)-PGI_1 on coronary perfusion pressure (CPP), left ventricular actively developed pressure (LVP) and myocardial oxygen consumption (QO_2) in the guinea pig isolated heart

Group	n	CPP (mm Hg)			LVP (mm Hg)			QO_2 (µl/g v.d.w./min)		
		CON_1	DRUG	CON_2	CON_1	DRUG	CON_2	CON_1	DRUG	CON_2
PGE_2	4–6	59±7	34±5*	63±8	87±9	53±5*	72±7	299±64	229±22*	285±46
PGI_2	6–9	56±5	37±4*	58±5	66±4	49±4	48±2	235±33	185±22	204±35
(6S)-PGI_1	8	61±4	43±4*	55±6	94±8	67±5*	80±6	359±27	261±16*	339±26
(6R)-PGI_1	6–8	51±6	64±6	61±6	82±8	59±3	66±4	326±27	349±38	313±38

CON_1: initial value before application of substances, CON_2: values after a 20 min wash-out period of the substances. DRUG: values at the highest concentration of the substances used: 3×10^{-7} M (PGE_2, PGI_2) or 1×10^{-5} M [(6R)-PGI_1, (6S)-PGI_1]. The data were taken from a cumulative dose-response curve. Statistical comparison was made between DRUG and CON_2 by the t-test for paired observations.
* $P < 0.01$

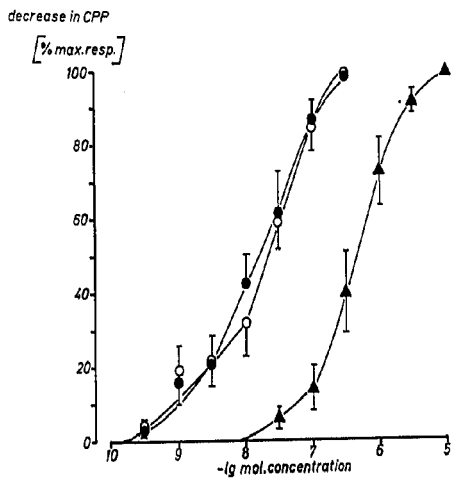

Fig.2. Cumulative dose-response curve for the decrease in the coronary perfusion pressure (*CPP*) in the guinea pig heart as induced by (6S)-PGI_1 (▲), PGE_2 (●) and PGI_2 (○). The effect is plotted in percent of maximum coronary dilation in each animal. Each point represents the mean and standard error ($\bar{x}$ ± S.E.M.) of 6–8 observations

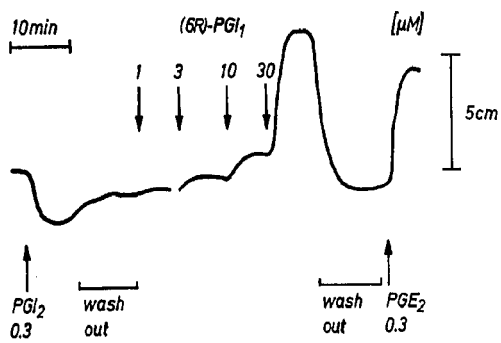

Fig.3. Action of *(6R)-PGI_1* on the isolated bovine coronary artery. An isolated coronary artery strip is relaxed by *PGI_2* and contracted by *PGE_2*. Cumulative application of *(6R)-PGI_1* leads to a dose-dependent contraction. Doses of *(6R)-PGI_1* below 1 µM were ineffective (not shown). All the doses, given, represent the final concentration in the bath fluid

to depletion of cardiac noradrenaline stores as described earlier for this model (Krebs and Schrör, 1975). (6S)-PGI_1 still diminished the CPP from 44 ± 3 to 35 ± 3 mm Hg ($P < 0.05$, $n = 6$), by 21 % of the initial value. PGE_2 decreased the CPP from 52 ± 5 to 40 ± 4 mm Hg ($P < 0.05$, $n = 6$), by 23 %. These effects were dose-dependent and completely reversibel after a 20 min wash-out period (not shown). In contrast, the drug-induced decrease in LVP was abolished: 35 ± 2 mm Hg in presence of 3×10^{-7} M PGE_2 and 32±4 mm Hg after wash-out ($P > 0.05$, $n = 6$); 38±4 mm Hg in presence of 1×10^{-5} M (6S)-PGI_1 and 40±5 mm Hg after wash-out ($P > 0.05$, $n = 6$). The wash-out time of 20 min was determined to be optimal in respective pilot experiments. Longer wash-out periods of e.g. 30 min did not change the MFC, while the coronary dilating effect was fully reversible already after 15–20 min.

Coronary Artery Strips

In isolated coronary artery strips PGI_2 did produce dose-dependent relaxation (threshold 1×10^{-8} M), PGE_2 dose-dependent contraction (threshold 3×10^{-8} M) (Figs. 3 and 4).

Cumulative application of (6R)-PGI_1 was followed by a dose-dependent contraction of the coronary artery strip ($1 \times 10^{-6} - 1 \times 10^{-4}$ M). Concentrations below 1×10^{-6} M were ineffective ($n = 8$) (Fig. 3). The maximum increase in tension amounted to 4.26 ± 0.15 g at 1×10^{-4} M ($n = 4$).

Cumulative application of (6S)-PGI_1 also lead to a contraction of the coronary artery strips at apparently the same concentration range as seen with the (6R)-derivative. However, in contrast to this, the (6S)-PGI_1-induced contraction was preceeded by a small relaxa-

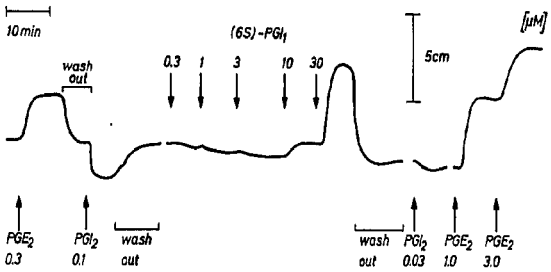

Fig. 4. Action of *(6S)-PGI*$_1$ on the isolated bovine coronary artery. An isolated coronary artery strip is contracted by *PGE*$_2$ but relaxed by *PGI*$_2$. Cumulative application of *(6S)-PGI*$_1$ is followed by a small relaxation at concentrations between 0.3–3 μM. At higher concentrations there is a contraction. The response to 30 μM *(6S)-PGI*$_1$ can be matched with about 2 μM PGE_2, indicating a 15-fold lesser activity of *(6S)-PGI*$_1$ in this experiment. All the doses, given, represent the final concentration in the bath fluid

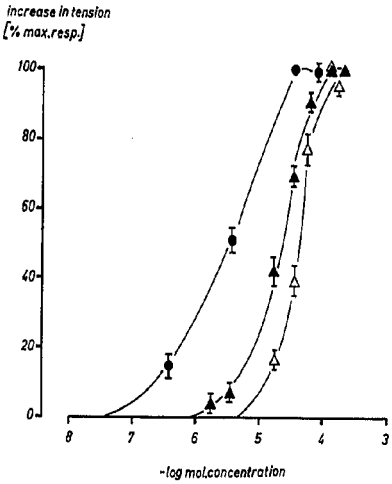

Fig. 5. Cumulative dose-response curve for the increase in muscular tension in the isolated bovine coronary artery as induced by (6S)-PGI_1 (▲), (6R)-PGI_1 (△) and PGE_2 (●). The effect is plotted in percent of maximum increase in tension in each artery strip. Each point represents the mean and standard error ($\bar{x} \pm$ S.E.M.) of 4 observations

tion at concentrations between $3 \times 10^{-7} - 3 \times 10^{-6}$ M, which was seen in 7 of 8 experiments. This relaxation was much less than that, obtained by PGI_2 (Fig. 4) and was followed by the same pronounced contraction as with (6R)-PGI_1. The maximum response was obtained at 1×10^{-4} M and amounted to 4.16 ± 0.47 g ($n = 4$).

Additionally, the increase in tension was estimated in percent of the individual maximum response of each strip and compared to that of PGE_2. The ED_{50} for (6S)-PGI_1 and (6R)-PGI_1 was 2×10^{-5} M and 4×10^{-5} M, respectively, that for PGE_2 4×10^{-6} M. This indicates a 5–10 times lesser potency of the dihydro prostacyclins (Fig. 5).

Discussion

The results show that (6S)-PGI_1 slightly mimics the naturally occurring PGI_2 with reference to its coronary dilating capacity, whereas (6R)-PGI_1 does not. Moreover, both stable analogs of PGI_2 produce a pronounced contraction of the isolated coronary artery strip, which is rather PGE-like and might also be involved in the coronary dilating activity of (6S)-PGI_1 in the guinea pig heart.

Both (6R)- and (6S)-PGI_1 have been reported to inhibit platelet aggregation, (6S)-PGI_1 being 10 times more potent than the (6R)-derivative but 10–100 times less potent than PGI_2 (Togna et al., 1977). We have found a 20 times smaller potency for (6S)-PGI_1 in comparison to PGI_2 with respect to its coronary dilating activity in the guinea pig heart. However, as shown previously (Schrör and Moncada, 1978) and confirmed in this study, both PGE_2 and PGI_2 are equipotent coronary dilating agents in this system. Thus, it is questioned, whether this activity produced by (6S)-PGI_1 is more PGI_2- or PGE_2-like.

From our data we suggest that the predominant activity of (6S)-PGI_1 is that of an E-type prostaglandin. Evidences, presented in support of this are: (1) decrease in myocardial force of contraction and QO_2 by (6S)-PGI_1 similar to that seen after PGE_2 in this model (Krebs and Schrör, 1975) or PGE_1 (Schrör, unpublished) but unlike PGI_2, which has only little if any myocardial depressant activity and does not change the myocardial oxygen consumption (Schrör and Moncada, 1978). (2) Maintenance of the coronary dilating activity of both (6S)-PGI_1 and PGE_2 in reserpine + tyramine treated guinea pigs, while the decreases in LVP and QO_2 are abolished. This indicates an inhibition of stimulus-induced noradrenaline release, which is well known for E-type prostaglandins (Hedqvist and Wennmalm, 1971) but was not shown for PGI_2 (Wennmalm, 1978). (3) A predominant contractile activity of (6S)-PGI_1 in the isolated coronary artery strip. This is the same response as seen with PGE_2, whereas PGI_2 does profoundly relax that tissue (Dusting et al., 1977). (4) An about one order of magnitude lesser activity of (6S)-PGI_1 in comparison to PGE_2 in both assay systems.

The small relaxation found with (6S)-PGI_1 on the coronary artery strip might be explained by residual

PGI_2-like activity, for series 1 prostaglandins have been shown to be much less coronary active agents than the corresponding series 2 compounds (Schrör and Merchant, unpublished). Alternatively, (6S)-PGI_1 may act similar to PGE_1, which has been reported previously to produce a small relaxation at low doses on the bovine coronary artery, which is followed by a contraction at high doses (Fricke and Schrör, 1978). Moreover, PGE_1 is known to inhibit platelet aggregation (Smith et al., 1974) as does (6S)-PGI_1 (Togna et al., 1977).

The biological activity of both dihydroprostacyclins was the same in the isolated coronary artery strip but not in the intact coronary vascular bed. This offers the interesting possibility that the sites of action in these systems are different.

Whether these speculations are justified, needs to be established. With respect to the cardiac and coronary actions, our data do not support the idea of a possible replacement of the physiological PGI_2 by its dihydro derivatives.

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