The Action of the Dihydro Derivatives of Prostacyclin —
(6R)-PGI₁ and (6S)-PGI₁ on the Heart and the Coronary Vasculature

K. Schrör
Pharmakologisches Institut, Universität Köln, Gießeler Strasse 24, D-5000 Köln 41, Federal Republic of Germany

Summary. The action of the dihydro prostacyclins, (6R)-PGI₁ and (6S)-PGI₁, was studied on the isolated guinea pig heart and bovine coronary artery strips. PGE₂ and PGI₂ were used as standards.

In the isolated guinea pig heart (6S)-PGI₁ decreased the coronary perfusion pressure (CPP), myocardial force of contraction (MFC) and oxygen consumption (QO₂). (6R)-PGI₁ did not produce a significant change in these parameters. The ED₅₀ (50% of maximum coronary dilation) was approximately 20 times higher for (6S)-PGI₁ than for PGI₂ or PGE₂.

Treatment of the hearts with reserpine + tyramine abolished the (6S)-PGI₁-induced decrease in MFC but not the decrease in the CPP. The same pattern of responses was seen with PGE₂.

Bovine coronary artery strips were contracted by both (6S)-PGI₁ and (6R)-PGI₁, the ED₅₀ (50% of maximum increase in tension) being 5 and 10 times higher than that for PGE₂. The (6S)-PGI₁-induced contraction was preceded by a small relaxation, which, however, was much less than that seen after PGI₂.

It is concluded that the hydration of the 5,6 double bound in the PGI₂ molecule results in an almost complete loss of PGI₂-like activity and generates PGE₂-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-PGI₁ — Prostacyclin (PGI₂) — Bovine coronary artery — Guinea pig heart — Myocardial mechanics — Coronary vascular tone.

Introduction

Prostacyclin (PGI₂) is a recently discovered product of arachidonic acid transformation via the cyclooxy-
genase pathway (Moncada et al., 1976). Release of PGI₂ 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). PGI₂ 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 PGI₂ in prevention of platelet aggregation and modulation of local perfusion in vivo (Moncada and Vane, 1977; Armstrong et al., 1978), more stable analogs of PGI₂ 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)-PGI₁ and (6S)-PGI₁ (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. PGE₂ and PGI₂ 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% CO₂ in O₂) Tyrode’s solution at 35°C. The hearts were electrically driven at a constant rate of 180 beats/min (Grass stimulator S 9, 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 (QO₂) was monitored polaro-
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 % CO2 in O2. 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), PGE2, (6R)-PGI1, (6S)-PGI1, PGI2-Na (Schering, Berlin), tyramine-hydrochloride (Merck, Darmstadt) were available for our investigations.

Stock solutions (1 mg/ml) of PGE2, (6R)-PGI1 and (6S)-PGI1 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. PGI2-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, PGE2, (6R)-PGI1 and (6S)-PGI1 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 PGI1 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 × 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 (± 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 PGE2 and PGI2 (3 × 10−9–3 × 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). PGE2 did also produce significant decrease in the LVP and QO2, whereas PGI2 did not (Table 1).

Cumulative application of (6S)-PGI1 (3 × 10−8–1 × 10−5 M) did also diminish the CPP for the same extent as seen with PGE2 and PGI2, 30 % of the initial value (P < 0.01). There was also a decrease in LVP and QO2 (P < 0.05). All these effects were reversible after a 20 min wash-out period. In contrast to this, (6R)-PGI1 (3 × 10−8–1 × 10−5 M) did not produce any significant change in LVP, CPP and QO2 (Table 1).

By comparing the relative potencies of PGE2, PGI2 and (6S)-PGI1 in decreasing the CPP, an approximately 20 times lesser potency of (6S)-PGI1 as compared to either PGE2 or PGI2 was found (Fig. 2).

Because this pattern of responses might indicate an inhibition of stimulus-induced noradrenaline release by (6S)-PGI1, some additional experiments were performed with reserpized animals.

Pretreatment of the hearts with reserpine + tyramine produced a decrease in LVP, QO2 and CPP, due