Br. J. Pharmac. (1985), **85**, 547–555

The cardiovascular and platelet actions of 9β-methyl carbacyclin (ciprostene), a chemically stable analogue of prostacyclin, in the dog and monkey

G. Allan, M.J. Follenfant, P. Lidbury*, P.L. Oliver & B.J.R. Whittle*

Departments of Pharmacology and Prostaglandin Research* Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS

1 9β-Methyl carbacyclin (9βMe; ciprostene) is a synthetic, chemically stable analogue of prostacyclin (PGI_2; epoprostenol). The platelet anti-aggregating and cardiovascular effects of 9βMe have been compared to PGI_2 in anaesthetized monkeys and dogs. In addition, their haemodynamic effects have been compared in open-chest anaesthetized dogs and conscious dogs.

2 Intravenous infusion of 9βMe and PGI_2 to the anaesthetized monkey resulted in a dose-dependent hypotension, tachycardia and inhibition of *ex vivo* ADP-induced platelet aggregation. 9βMe was 72 times less active than PGI_2 both as a hypotensive and anti-aggregating agent.

3 Intravenous infusion of 9βMe and PGI_2 to the anaesthetized beagle dog resulted in a qualitatively similar haemodynamic profile. Thus both substances induced a dose-dependent hypotension accompanied initially by a slightly increased heart rate, a dose-dependent increase in cardiac output, stroke volume and an increased peak LV dP/dt. At the higher doses studied, the initial increases in the parameters measured were succeeded by dose-dependent falls. 9βMe was 76 times less active than PGI_2 as a hypotensive agent.

4 In the anaesthetized greyhound, a dose-dependent anti-aggregating and hypotensive effect was seen with either drug, with 9βMe being 23 and 40 times less active than PGI_2, respectively.

5 Intravenous infusion of 9βMe and PGI_2 to the conscious beagle dog induced a dose-dependent hypotension and a variable effect on heart rate. 9βMe was 33 times less active than PGI_2 as an hypotensive agent.

6 The duration of the hypotensive response induced by 9βMe was not significantly different from that induced by PGI_2 in either monkey or beagle dog.

Introduction

Prostacyclin (epoprostenol, PGI_2) is an endogenous prostaglandin (Moncada *et al.*, 1976) with potent vasodilator and platelet anti-aggregating properties in animals (Whittle & Moncada, 1983) and in man (O'Grady *et al.*, 1980; Warrington *et al.*, 1980: Szczeklik *et al.*, 1980). Clinically, epoprostenol has been used to inhibit platelet activation during extracorporeal circulation (Bunting *et al.*, 1981; Gimson *et al.*, 1980) and as a substitute for heparin in renal dialysis (Smith *et al.*, 1982). Currently, epoprostenol is being evaluated in acute myocardial infarction, unstable angina, acute thrombotic stroke and ischaemic peripheral vascular disease. However, epoprostenol is an unstable compound which despite pharmaceutical formulation yields solutions of limited stability. 9β-Methyl carbacyclin [(5Z, 9*S*)-9-methyl-6a carba-prostaglandin I_2] (ciprostene, 9βMe; Figure 1) has been developed by the Wellcome Foundation Limited and the Upjohn Company as a chemically stable analogue of epoprostenol with similar biological properties.

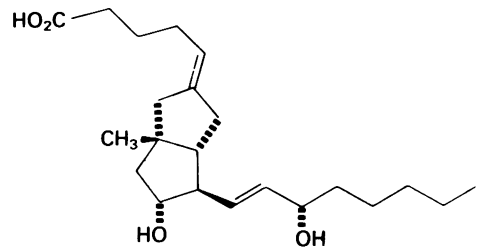

Figure 1 Chemical structure of 9β-methyl carbacyclin [(5Z, 9*S*)-9-methyl-6a carba-prostaglandin I_2].

We have studied the cardiovascular and platelet anti-aggregating effects of 9βMe in the dog and monkey as part of the pre-clinical pharmacodynamic evaluation. Results of the clinical volunteer study with 9βMe have been described separately (O'Grady *et al.*, 1984).

Methods

Anaesthetized monkey

Four adult female *Erythrocebus patas* monkeys (weighing 4–8.0 kg) were anaesthetized with sodium pentobarbitone (Sagatal, 10–20 mg kg^{-1} i.v.) following initial tranquillization with phencyclidine (Sernylan, 2 mg kg^{-1} i.m.). Maintenance doses of sodium pentobarbitone (2–5 mg kg^{-1} i.v.) were given as required. Rectal temperature was maintained at 38–39°C. Both femoral veins were cannulated to allow administration of additional anaesthetic into one and infusion of either prostanoid into the other. Both femoral arteries were cannulated to allow measurement of arterial blood pressure (Statham P23Gb or Kulite pressure transducer) from one artery and to allow sampling of arterial blood for platelet studies from the other. Heart rate was measured by integration (Beckman cardiotachometer) of either a Lead II ECG or of the arterial blood pressure pulse. Blood pressure and heart rate were recorded continuously on a Grass (Model 7) or Beckman (Type R611) polygraph. A 30–60 min equilibration period was allowed following completion of surgery.

Platelet aggregation *ex vivo* was measured as described previously (Whittle *et al.*, 1980) before and during infusion of PGI_2 or 9βMe. Platelet-rich plasma (PRP) was prepared from 3 or 4.5 ml blood samples and sufficient adenosine diphosphate (ADP) was used to produce a submaximal (4 μM) and a maximal (6 μM) aggregatory response. At least three control blood samples at about 10 min intervals, were taken before starting treatment.

9βMe and PGI_2 were administered by intravenous infusion (at a rate of 0.25 ml min^{-1} for 10–12 min) over the dose range 1.25–40 μg kg^{-1} min^{-1} and 0.02–0.64 μg kg^{-1} min^{-1}, respectively. Platelet aggregation was measured 10 min after the beginning of each infusion. There was a time delay of 15–30 s between the infusions, thus recovery of biological responses did not occur. On completion of infusions for one substance, a period of 30–40 min was allowed for recovery of biological responses before testing the other prostanoid. Doses were given in ascending order and the order of testing was alternated between the animals.

Anaesthetized dog (beagles)

Fifteen adult beagles of either sex and weighing 7.8–15.4 kg were used. Anaesthesia was induced with sodium thiopentone (20–30 mg kg^{-1} i.v.) and maintained with α-chloralose (initially 30–50 mg kg^{-1} i.v. with increments of 5–10 mg kg^{-1} as required) after cannulation of a femoral vein. Animals were placed on an operating table and rectal temperatures maintained at 38–39°C. A femoral artery was cannulated to measure pulsatile and mean arterial blood pressure (Kulite transducer). The trachea was cannulated and the lungs ventilated with room air using a Starling pump (rate 20 min^{-1} and tidal volume 150–250 ml). The chest was opened by splitting the sternum. A cuffed electromagnetic flow sensor (10–14 mm diameter) was placed around the ascending aorta to measure phasic aortic blood flow (Statham SP 2202 flowmeter). A heparin-saline filled polyethylene catheter was inserted into the apex of the left ventricle to record left ventricular pressures (LVP; Kulite transducer). A further femoral vein and artery were cannulated for drug administration and for blood sampling for pH and respiratory gas analysis. Lead II (ECG) was recorded via subdermal needle electrodes.

Stroke volume and cardiac output (less coronary flow) were derived by electrical integration of the phasic aortic flow. Maximum rate of change of left ventricular pressure (*dP/dt max*) was measured as an index of cardiac contractility by electrical differentiation of the LVP signal. Heart rate was obtained by integrating the arterial pulse pressure using a cardiotachometer. Total peripheral resistance (excluding coronary circulation) was approximated by dividing mean arterial pressure by the instantaneous cardiac output (mmHg/ml min^{-1} × 7997 = resistance in dyn cm^{-5} s) before and at the time of maximum hypotension during infusion.

Recordings were made on a Beckman Type R611 and RM Dynographs. Arterial blood pH and gas tensions were measured frequently (Corning model 175 Automatic Blood pH/Gas Analyser) and maintained in the physiological range. Preparations were allowed to stabilise for at least 40 min between completion of surgery and administration of the drugs.

The study was conducted in two parts. In the first, 9βMe and PGI_2 were evaluated separately (one prostanoid per dog; 5 or 6 dogs each) by intravenous infusion of a wide range of concentrations. Dogs were allocated treatment alternately and dose order randomly. In the second part of the study, 4 submaximally effective infusion concentrations of each substance were compared within the same dog ($n = 4$) to confirm their relative activity and to determine if there was any overt interaction. These were given in ascending dose order but alternating which agent was tested first. The

infusion rate was 1.0 ml min^{-1} for a 10 min period allowing recovery and/or stabilisation between infusions. Prior to giving the prostanoids, each dog received an infusion of the 1.25% $NaHCO_3$ vehicle as a control. Infusions were separated by 10–60 min intervals.

Anaesthetized dog (greyhounds)

Four adult greyhounds (weighing 18.5–22 kg) were anaesthetized essentially as for the beagles above and then maintained with sodium pentobarbitone (3 mg kg^{-1} s.c.). They were prepared for measurement of arterial blood pressure and *ex vivo* platelet aggregation essentially as described for the monkey above. PRP was prepared from 6 ml blood samples and sufficient ADP was used to produce a submaximal (5 and 10 μM) or near maximal (20 μM) aggregation. 9βMe and PGI_2 were tested by intravenous infusion (at 0.25 ml min^{-1}) over the dose range 0.2–20 μg kg^{-1} min^{-1} and 0.01–0.8 μg kg^{-1} min^{-1}, respectively.

Conscious dog

Four beagle dogs of either sex and weighing 8–13 kg were used. A cannula was surgically implanted, under aseptic conditions, into the aortic arch via a carotid artery of each dog. During a recovery period of not less than 10 days, animals were trained to sit quietly in restraint slings within the laboratory. On the day of use, records of arterial blood pressure (Statham pressure transducer) and Lead II ECG (intradermal needle electrodes) were made continuously on a Grass Model 7 Polygraph. Animals were allowed to stabilise for at least 60 min before starting treatment. The prostanoids were infused intravenously (via a percutaneous catheter in a cephalic or saphenous vein) at 0.1–1 ml min^{-1} (Braun Perfusor). Cumulative dose-response curves were constructed for each substance allowing 10 min per dose level. At least 60 min was allowed for recovery between dose-response curves.

Data analysis

Effects on platelet aggregation *ex vivo* were expressed as percentage inhibition of the mean pre-infusion value. Cardiovascular effects are given as the maximum changes (in absolute units) from the initial or pre-infusion levels. An estimate of the duration of the hypotensive response was obtained by measuring the time required for a 50% recovery ($t_{\frac{1}{4}}$) of the diastolic (DBP) or mean blood pressure following termination of the infusion.

Results are expressed as mean ± s.e.mean where (n) is the number of animals. The difference between means was evaluated where appropriate, using Students t test. Where appropriate, linear regression analysis was used to fit straight lines to the linear part of the log dose-response relationships and the regressions tested for parallelism. Additionally, the infusion dose for 50% inhibition of platelet aggregation (ID_{50}) and for a fall of 30 mmHg in diastolic blood pressure (ED_{30}) was calculated from the mean slope for the two regressions, providing there was no evidence of non-parallelism, and relative activities were calculated.

Drugs

9β-Methyl carbacyclin (9βMe) synthesized (Aristoff *et al.*, 1983) by the Upjohn Company was obtained as the calcium salt and kept as a stock solution of 10 mg ml^{-1} in ethanol at − 20°C. On the day of use, aliquots were taken at ambient temperature, dried under N_2 and resolubilised in ice cold 1.25% w/v $NaHCO_3$ (pH 8.5; isotonic). Epoprostenol (PGI_2) as the sodium salt, was

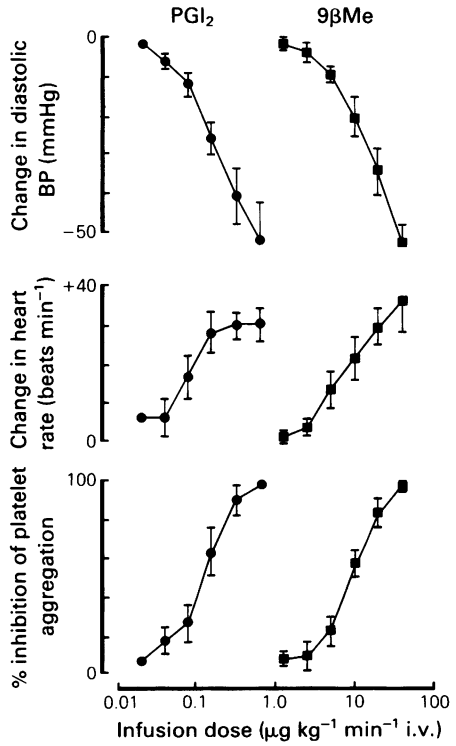

Figure 2 Cardiovascular and anti-aggregating effect of prostacyclin (PGI_2) and 9β-methyl carbacyclin (9βMe) in the pentobarbitone-anaesthetized monkey. Values are the mean of 3–4 animals; vertical lines show s.e.means. Intravenous infusion of PGI_2 (●) and 9βMe (■) resulted in dose-dependent hypotension, tachycardia and inhibition of ADP (6 μM)-induced platelet aggregation *ex vivo*.

Table 1 Comparison of the hypotensive and anti-aggregating effects of 9β-methyl carbacyclin (9βMe) and prostacyclin (PGI_2) in the patas monkey

Treatment	*Diastolic blood pressure*		*Platelet aggregation*	
	ED_{30}* (P0.95 limits)	Relative activity (P0.95 limits)	ID_{50}† (P0.95 limits)	Relative activity (P0.95 limits)
9βMe	14.3 (11.0–19.4) μg kg^{-1} min^{-1}	1.0	9.1 (7.1–11.5) μg kg^{-1} min^{-1}	1.0
PGI_2	0.20 (0.14–0.26) μg kg^{-1} min^{-1}	73 (50–114)	0.13 (0.10–0.16) μg kg^{-1} min^{-1}	71 (51–102)

*i.v. infusion dose required for a fall of 30 mmHg in DBP
†i.v. infusion dose required for a 50% inhibition of *ex vivo* platelet aggregation induced by ADP (6 μM).

freshly dissolved in 1M Tris buffer (pH 9.6) and stored on ice crystals. Aliquots were taken and diluted in ice cold 1.25% $NaHCO_3$ just before use. Infusion syringes were kept cold on the infusion pump (Sage model 355 or Braun Perfusor) with ice bags.

Results

Monkey

The resting blood pressures (systolic/diastolic) and heart rates (mean ± s.e.mean; $n = 4$) were 135 ± 7.4/85 ± 5.7 mmHg and 135 ± 7.4 beats min^{-1}, respectively.

Intravenous infusion of PGI_2 (0.04–0.64 μg kg^{-1} min^{-1}) and 9βMe (2.5–40 μg kg^{-1} min^{-1}) resulted in a dose-dependent depression of arterial blood pressure and an inhibition of *ex vivo* platelet aggregation (Figure 2).

Evaluation of the hypotensive and of the anti-aggregating effects for both prostanoids by linear regression analysis revealed highly significant regressions ($P < 0.001$) free of significant deviations from linearity. Comparisons of the regressions for either hypotensive or anti-aggregating activity did not find significant deviations from parallelism thus supporting relative activity comparisons. Thus 9βMe was 71–73 times less active than PGI_2 for either effect (Table 1). Following termination of the highest infusion rate, the recovery for the hypotension induced by 9βMe (− 67 ± 5.0 mmHg DBP; $t_{\frac{1}{2}}$: 5.3 ± 2.69 min) was not significantly different ($P > 0.05$) from that induced by PGI_2 (− 58 ± 12.9 mmHg; $t_{\frac{1}{2}}$: 2.2 ± 1.2 min).

An associated change in heart rate after administra-

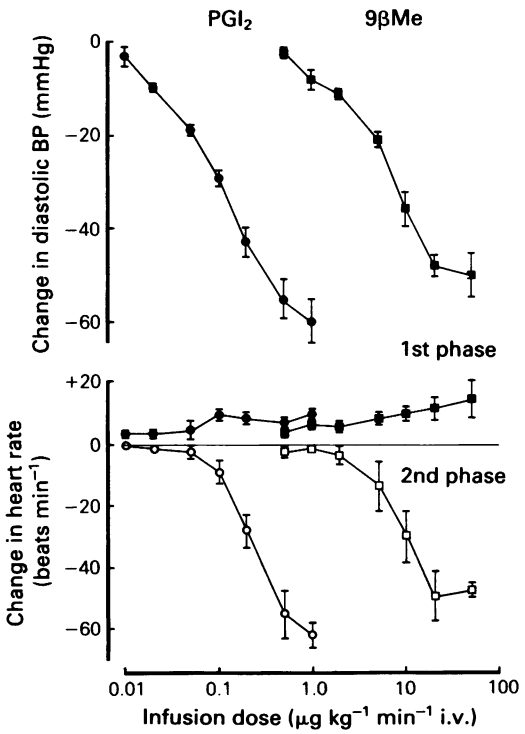

Figure 3 Effect of intravenous infusion of prostacyclin (PGI_2; ●, ○) and 9β-methyl carbacyclin (9βMe; ■, □) on diastolic blood pressure and heart rate of the anaesthetized beagle. Values are the mean of 3–6 animals; vertical lines show s.e.means. At the higher doses studied responses were generally biphasic with the initial increases (1st phase) in the measured variables being followed by dose-dependent falls (2nd phase).

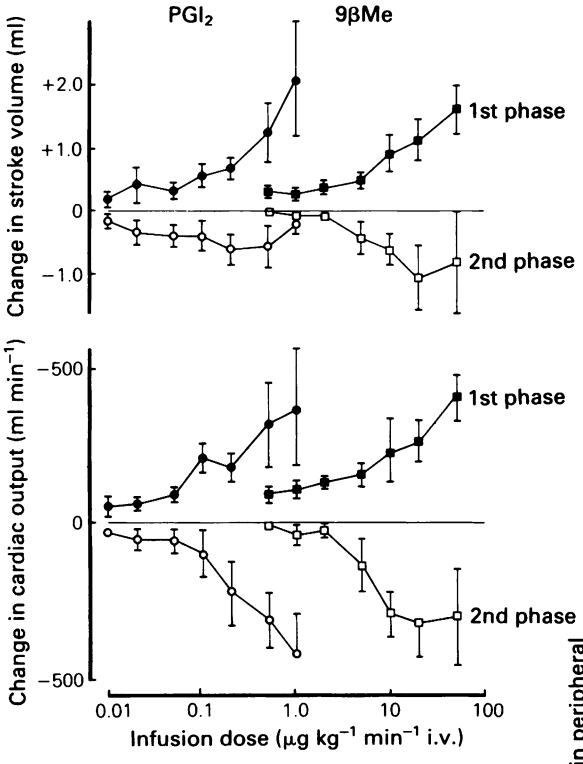

Figure 4 Effect of an i.v. infusion of prostacyclin (PGI_2; ●, ○) and 9β-methyl carbacyclin (9βMe; ■, □) on LV stroke volume and cardiac output (less coronary flow) of the anaesthetized beagle dog. Values are the mean of 3–6 animals; s.e.means shown by vertical lines.

tion of PGI_2 or 9βMe was generally a dose-related tachycardia (Figure 2). Individual data however revealed that the tachycardia following administration of epoprostenol in two animals was dose-related up to the highest dose tested (maximum increases of 22 and 38 beats min^{-1} with 0.32 and 0.64 $\mu g\,kg^{-1}\,min^{-1}$) whilst the other two reached their maxima at 0.16 $\mu g\,kg^{-1}\,min^{-1}$ (increases of 26 and 39 beats min^{-1}) and thereafter either showed no change with higher doses or exhibited a reduction in the existing tachycardia.

Anaesthetized dog (beagles)

The resting levels of the cardiovascular variables were arterial blood pressure (systolic/diastolic): 147 ± 3.8/91 ± 1.9 mmHg; heart rate: 216 ± 4 beats min^{-1}; stroke volume: 6.6 ± 0.43 ml; cardiac output: 1.36 ± 0.081 l min^{-1}; LVP: 132 ± 3.5 mmHg; LV*dP*/*dt*: 3680 ± 189 mmHg s^{-1} and peripheral resistance: 6720 ± 402 dyn cm^{-5} s (n = 15).

The haemodynamic effects of intravenous infusions of 9βMe (0.5–50 $\mu g\,kg^{-1}\,min^{-1}$) and PGI_2 (0.01–1.0 $\mu g\,kg^{-1}\,min^{-1}$) were qualitatively similar (see Figures 3–5). In all dogs arterial blood pressure was reduced in a dose-dependent manner, with the maximal effect occurring after 5 to 10 min of the infusion of either compound. During the early part of the infusions (0–3 min) cardiac output was increased in a dose-dependent manner but this increase was succeeded by a dose-dependent depression at higher doses (> 5 $\mu g\,kg^{-1}\,min^{-1}$ and > 0.1 $\mu g\,kg^{-1}\,min^{-1}$ of 9βMe and PGI_2 respectively). The increased cardiac output was mainly due to an increased stroke volume although slight increases in heart rate also occurred. The major effect of higher doses on heart rate was a dose-dependent bradycardia which succeeded the early slight tachycardia.

A small positive inotropic effect, as indicated by increased LVdP/*dt* was also observed in association with the increased cardiac output but this was also succeeded at high doses by a more marked depression

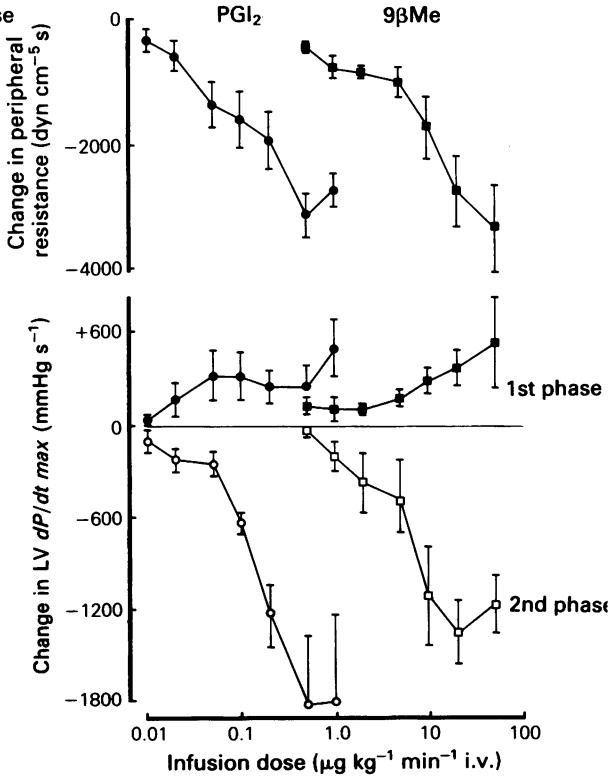

Figure 5 Effect of an i.v. infusion of prostacyclin (PGI_2; ●, ○) and 9β-methyl carbacyclin (9βMe; ■, □) on peripheral resistance and peak LV*dP*/*dt* of the anaesthetized beagle dog. Values are the mean of 3–6 animals; vertical lines show s.e.means.

Table 2 Inhibition of platelet aggregation by 9β-methyl carbacyclin (9βMe) and prostacyclin (PGI_2) when induced *ex vivo* by ADP in the anaesthetized greyhound

	% inhibition of aggregation		
	[5 μM]	[10 μM]	[20 μM]
9βMe ($\mu g\,kg^{-1}\,min^{-1}$)			
0.2	34 ± 8.6	35 ± 7.6	32 ± 9.8
0.4	77 ± 13.7	58 ± 11.4	60 ± 10.9
0.8	95 ± 4.0	89 ± 6.4	89 ± 6.4
PGI_2 ($\mu g\,kg^{-1}\,min^{-1}$)			
0.01	—	32 ± 0.6	35 ± 10.5
0.02	82 ± 13.6	71 ± 18.7	72 ± 9.6
0.04	89 ± 11.0	82 ± 10.7	89 ± 10.7

Results, shown as % inhibition of control platelet aggregation *ex vivo* induced by submaximal to near-maximal concentrations of ADP (5, 10 and 20 μM), are expressed as mean ± s.e.mean of 4 experiments.

(see Figure 5). Changes in peripheral resistance as calculated at the time of maximal hypotension and the instantaneous cardiac output suggested a dose-dependent peripheral vasodilatation (Figure 5).

Regressions relating the falls in diastolic blood pressure to log dose for either substance were highly significant ($P < 0.001$) and did not deviate significantly from linearity or parallelism. Relative hypotensive comparisons showed that 9βMe was 76 times (P 0.95 limits: 58–101) less active than PGI_2. The calculated ED_{30} (P 0.95 limits) values were 9βMe: 6.8(5.7–8.3) $\mu g\,kg^{-1}\,min^{-1}$ and PGI_2: 0.09(0.07–0.11) $\mu g\,kg^{-1}\,min^{-1}$.

A comparison of the $t_{\frac{1}{2}}$ for approximately equi-hypotensive doses of either prostanoid showed that the effect of 9βMe tended to last longer than that of PGI_2. This difference only became significant ($P < 0.05$) at the highest doses tested which caused a supramaximal hypotension. Thus, low doses of 9βMe (5 $\mu g\,kg^{-1}\,min^{-1}$) and PGI_2 (0.1 $\mu g\,kg^{-1}\,min^{-1}$) caused falls in mean arterial BP of 26 ± 3.0 and 31 ± 3.1 mmHg, respectively, with $t_{\frac{1}{2}}$ of 5.5 ± 1.03 and 3.0 ± 0.80 min, respectively ($P > 0.05$). The highest infusion doses of 9βMe (50 $\mu g\,kg^{-1}\,min^{-1}$) and PGI_2 (1.0 $\mu g\,kg^{-1}\,min^{-1}$) produced falls of 63 ± 6.7 and 70 ± 8.4 mmHg, respectively, with $t_{\frac{1}{2}}$ of 11.2 ± 2.04 and 4.2 ± 1.01 min, respectively ($P < 0.05$).

The haemodynamic effects of 9βMe and PGI_2 tested within the same dog ($n = 4$) were similar to those observed in the between-dog comparisons described above. Relative hypotensive activity comparisons revealed that the order of administration did not influence the evaluation. A relative activity comparison between the two substances showed that in this study 9βMe was 63 times (P 0.95 limits: 37–113) less active than PGI_2.

Anaesthetized dog (greyhound)

Intravenous infusion of either prostanoid resulted in a dose-dependent inhibition of *ex vivo* platelet aggregation (Table 2) and a dose-dependent hypotension. Relative activity comparisons (the regressions being linear and parallel) indicated that 9βMe was 23 or 40 times less active than PGI_2 as an anti-aggregating or hypotensive agent, respectively (Table 3). Whilst the difference between these relative activities could sug-

Table 3 Comparison of the hypotensive and anti-aggregating effects of 9β-methyl carbacyclin (9βMe) and prostacyclin (PGI_2) in the anaesthetized greyhound

Treatment	*Diastolic blood pressure*		*Platelet aggregation*	
	ED_{30}* (P 0.95 limits)	Relative activity (P 0.95 limits)	ID_{50}† (P 0.95 limits)	Relative activity (P 0.95 limits)
9βMe	1.9 (1.2–2.9) $\mu g\,kg^{-1}\,min^{-1}$	1.0	0.31 (0.22–0.41) $\mu g\,kg^{-1}\,min^{-1}$	1.0
PGI_2	0.05 (0.03–0.07) $\mu g\,kg^{-1}\,min^{-1}$	40 (23–73)	0.013 (0.009–0.015) $\mu g\,kg^{-1}\,min^{-1}$	23 (15–37)

*i.v. dose required for a fall of 30 mmHg in diastolic blood pressure.

†i.v. dose required for a 50% inhibition of *ex vivo* platelet aggregation induced by ADP (20 μM).

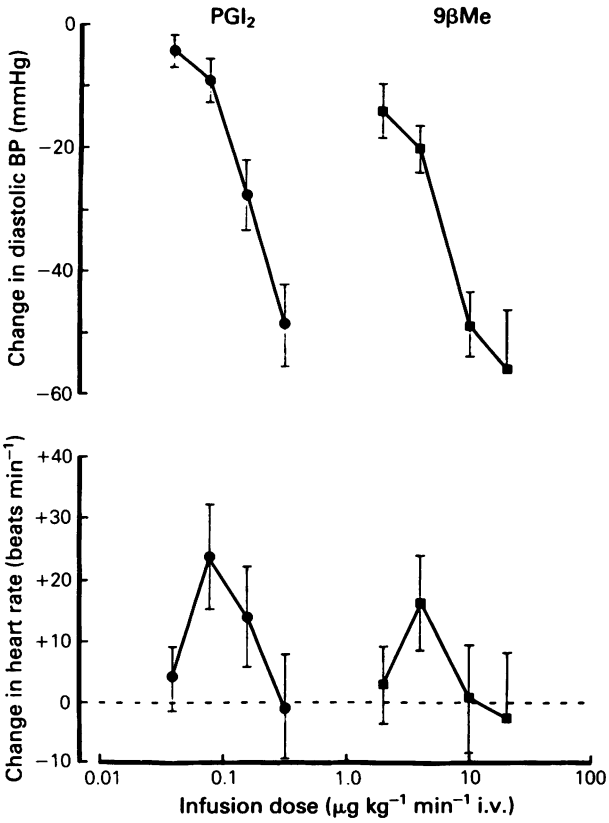

Figure 6 Cardiovascular action in the conscious dog. Values are the mean of 4 animals; vertical lines show s.e.means. Intravenous infusion of prostacyclin (PGI_2; ●) and 9β-methyl carbacyclin (9βMe; ■) resulted in dose-dependent hypotension.

gest a preferential effect of 9βMe on the platelet as compared with that on the blood pressure, there was however no significant difference between these values ($P > 0.05$; t test).

With either drug, and in particular for 9βMe, the anti-aggregating activity was apparent at dose levels having minimal effects on blood pressure (see ID_{50} and ED_{30}, Table 3). Thus, the ratio of the effective doses (ED_{30}/ID_{50}) was about 6.1 for 9βMe and 3.8 for PGI_2.

Conscious dog

The resting blood pressures (systolic/diastolic) and heart rates were $132 \pm 12.5/73 \pm 8.5$ mmHg and 109 ± 16.1 beats min^{-1} ($n = 4$), respectively.

Intravenous infusion of either 9βMe (2–20 μg kg^{-1} min^{-1}) or PGI_2 (0.08–0.32 μg kg^{-1} min^{-1}) induced dose-dependent falls in arterial blood pressure (Figure 6). Regressions relating the hypotensive response to log dose for either agent were highly significant ($P < 0.001$) and did not deviate significantly from linearity or parallelism. Relative hypotensive comparisons showed 9βMe to be 34 (P 0.95 limits: 19–53) times less active than PGI_2. The calculated ED_{30} (P 0.95 limits) values were 9βMe: 5.9(3.8–8.0) μg kg^{-1} min^{-1} and PGI_2: 0.17(0.12–0.24) μg kg^{-1} min^{-1}.

The duration of approximately equi-hypotensive doses of 9βMe and PGI_2 (20 and 0.32 μg kg^{-1} min^{-1} respectively) was comparable ($t_{\frac{1}{2}} = 1.9 \pm 0.4$ and 1.5 ± 0.1 min, respectively).

Heart rate, although not significantly ($P > 0.05$) changed during infusion with 9βMe and PGI_2, tended to be increased (mean change 4–27%) with low doses (2–5 and 0.04–0.08 μg kg^{-1} min^{-1}, respectively). At higher doses, the tachycardia was less pronounced and in the occasional dog, heart rate was slightly depressed (12–18%).

Discussion

9β-Methyl carbacyclin (ciprostene; 9βMe) is a chemically stable analogue of prostacyclin (epoprostenol; PGI_2) which is currently undergoing clinical evaluation (O'Grady *et al.*, 1984). The cardiovascular effects of 9βMe and PGI_2 following intravenous administration to anaesthetized monkeys and anaesthetized and conscious dogs are now described.

Qualitatively, the cardiovascular effects of 9βMe and PGI_2 are similar. Thus, in the experiments described here, intravenous infusion of either agent resulted in a dose-dependent hypotension with 9βMe being 73, 40–76 or 34 times less potent than PGI_2 in the monkey, anaesthetized dog or conscious dog, respectively. In the anaesthetized beagle, the hypotension was associated with a dose-dependent fall in peripheral resistance accompanied initially by a slightly increased heart rate, a dose-dependent increase in cardiac output, stroke volume and an increased peak $LVdP/dt$. At the higher doses studied these initial increases in the parameters measured were succeeded by dose-dependent falls (Figures 3–5). Heart rate in the conscious dog, although variably affected, did tend to increase with low doses and to decrease with higher doses (Figure 6). In the monkey, however, heart rate was increased in a dose-dependent manner by either substance (Figure 2).

Hypotension associated with injection of vasodilator substances is generally accompanied by an increased heart rate due to stimulation of baroreflex mechanisms. However, PGI_2 is a potent hypotensive agent which can also produce bradycardia when administered intravenously, this response being associated with the stimulation of a vagal reflex which is sufficient to overcome the normal baroreflex (Hin-

tze et al., 1979; Chapple et al., 1980). A recent study using intracoronary artery administration of PGI_2 and arachidonic acid has attributed the reflex effects to activation of receptors located predominantly in the posterior wall of the left ventricle (Hintze & Kaley, 1984). Chiavarelli et al. (1982) have demonstrated that in anaesthetized dogs the predominating effect of PGI_2 on heart rate is dependent on the basal rate of the animal under study, with drug-induced tachycardia (hence baroreceptor reflex stimulation) occurring at low basal rates, whilst drug-induced bradycardia (vagal afferent activation) predominates at high basal rates. The opposing effects on heart rate observed in our anaesthetized preparations could be a feature of the resting heart rate (i.e. monkey, 135 beats min^{-1}; dog, 216 beats min^{-1}). However, the conscious dog study described here, despite having low resting heart rates (mean 109 beats min^{-1}) did not consistently show tachycardia and occasionally exhibited bradycardia, an effect consistent with a previous report using PGI_2 (Hintze et al., 1981). The usual effect on heart rate in man, following the administration of PGI_2, is tachycardia (Fitzgerald et al., 1979; Warrington et al., 1980; O'Grady et al., 1980; Szczeklik et al., 1980; Fish et al., 1982) although bradycardia and other signs of vagal activity have also been observed (Pickles & O'Grady, 1982; Hassan et al., 1982). Administration of 9βMe to man also results in increased heart rate (O'Grady et al., 1984).

The dose-dependent fall in cardiac output demonstrated following administration of 9βMe or PGI_2 to the anaesthetized dog is probably a result of the marked bradycardia, since stroke volume is only slightly decreased (Figure 3). The predominant effect of both drugs on $LV\frac{dP}{dt}$ was a reduction, suggesting a depression of myocardial contractility. However, since this could be attributable to a reduction in afterload, (Mason, 1969) or a reduction in contractility via vagal activation (Randall & Armour, 1974) or a fall in heart rate (Zucker & Cornish, 1981), the effect of the two agents on myocardial contractility cannot be accurately assessed from our studies.

Platelet aggregation, assessed *ex vivo* using ADP, was inhibited in a dose-dependent manner by intravenous infusions of either agent to the anaesthetized monkey or greyhound dog. In the monkey, 9βMe, relative to PGI_2, was equally effective on the platelet and on the blood pressure; thus 9βMe was 71–73 times less effective than PGI_2 both as a hypotensive and an anti-aggregating agent. In the dog study, there was apparently some slight selectivity for the effect of 9βMe on the platelet as compared with that on the blood pressure (i.e. 9βMe was 23 or 40 times less active than PGI_2 as an anti-aggregating or hypotensive agent, respectively) but this difference did not achieve statistical significance.

The *in vivo* biological half-life of 9βMe tended to be longer than that of PGI_2 in both species, as indicated by the $t_{\frac{1}{2}}$ for recovery of the hypotensive response following administration of approximately equi-hypotensive doses of both agents. However, this tendency only achieved statistical significance in the anaesthetized dog with the highest dose tested when the hypotensive response was supramaximal (Figure 3). Nevertheless the duration of the cardiovascular action of either compound was relatively brief.

In conclusion, 9β-methyl carbaçyclin (ciprostene) is a chemically stable analogue of epoprostenol with a cardiovascular and platelet anti-aggregating profile qualitatively similar to epoprostenol and is 34–70 times less active. The initial clinical evaluation in volunteers of 9β-methyl carbacyclin has confirmed that this agent is also qualitatively similar to epoprostenol in man and is about 100 times less potent (O'Grady et al., 1984).

References

ARISTOFF, P.A., JOHNSON, P.D. & HARRISON, A.W. (1983). Synthesis of 9-substituted carbacyclin analogues. *J. Org. Chem.*, **43**, 5341–5348.

BUNTING, S., O'GRADY, J., FABIANI, J.N., TERRIER, E., MONCADA, S., VANE, J.R. & DUBOST, Ch. (1981). Cardiopulmonary bypass in man: effects of prostacyclin. In *Clinical Pharmacology of Prostacyclin*. ed. Lewis, P.J. & O'Grady, J. pp. 181–193. New York: Raven Press.

CHAPPLE, D.J., DUSTING, G.J., HUGHES, R. & VANE, J.R. (1980). Some direct and reflex cardiovascular actions of prostacyclin (PGI_2) and prostaglandin E_2 in anaesthetised dogs. *Br. J. Pharmac.*, **68**, 437–447.

CHIAVARELLI, M., MONCADA, S. & MULLANE, K.M. (1982). Prostacyclin can either increase or decrease heart rate depending on the basal state. *Br. J. Pharmac.*, **75**, 243–249.

FISH, A., HASSAN, S., O'GRADY, J., PICKLES, H. & WARRINGTON, S. (1982). On the mechanism of the tachycardia caused by epoprostenol (prostacyclin) in normal human subjects. *J. Physiol.*, **325**, 19P.

FITZGERALD, G.A., FRIEDMAN, L.A., MIYAMORI, I., O'GRADY, J. & LEWIS, P.J. (1979). A double blind placebo controlled crossover study of prostacyclin in man. *Life Sci.*, **25**, 665–672.

GIMSON, A.E.S., HUGHES, R.D., MELLOR, P.J., WOODS, H.F., LANGLEY, P.G., CANALESE, J., WILLIAMS, R. & WESTON, M.J. (1980). Prostacyclin to prevent platelet activation during charcoal haemoperfusion in fulminant hepatic failure. *Lancet*, **i**, 173–175.

HASSAN, S., PICKLES, H.P., FISH, A., BURKE, C., WARRINGTON, S. & O'GRADY, J. (1982). The cardiovascular and platelet effects of epoprostenol (prostacyclin, PGI_2) are unaffected by β-adrenoceptor blockade in man. *Br. J. clin. Pharmac.*, **14**, 369–377.

HINTZE, T.H., MARTIN, E.G., MESSINA, E.J. & KALEY, G. (1979). Prostacyclin (PGI_2) elicits reflex bradycardia in dogs: evidence for vagal mediation. *Proc. Soc. exp. Biol. Med.*, **162,** 96–100.

HINTZE, T.H., PANZENBECK, M.J., MESSINA, E.J. & KALEY, G. (1981). Prostacyclin (PGI_2) lowers heart rate in the conscious dog. *Cardiovasc. Res.*, **15,** 538–546.

HINTZE, T.H. & KALEY, G. (1984). Ventricular receptors activated following myocardial prostaglandin synthesis initiate reflex hypotension, reduction in heart rate, and redistribution of cardiac output in the dog. *Circulation Res.*, **54,** 239–247.

MASON, D.T. (1969). Usefulness and limitations of the rate of rise of intraventricular pressure (dP/dt) in the evaluation of myocardial contractility in man. *Am. J. Cardiol.*, **23,** 516–527.

MONCADA, S., GRYGLEWSKI, R.J., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263,** 663–665.

O'GRADY, J., WARRINGTON, S., MOTI, M.J., BUNTING, S., FLOWER, R., FOWLE, A.S.E., HIGGS, E.A. & MONCADA, S. (1980). Effects of intravenous infusion of prostacyclin (PGI_2) in man. *Prostaglandins*, **19,** 319–332.

O'GRADY, J., HEDGES, A., WHITTLE, B.J.R., AL-SINAWI, L.A-H., MEKKI, Q.A., BURKE, C., MOODY, S.G., MOTI, M.J. & HASSAN, S. (1984). A chemically stable analogue, 9β-methyl carbacyclin, with similar effects to epoprostenol (prostacyclin, PGI_2) in man. *Br. J. clin. Pharmac.*, **18,** 921–933.

PICKLES, H. & O'GRADY, J. (1982). Side effects occurring during administration of epoprostenol (prostacyclin PGI_2) in man. *Br. J. clin. Pharmac.*, **14,** 177–185.

RANDALL, W.C. & ARMOUR, J.A. (1974). Regional vagosympathetic control of the heart. *Am. J. Physiol.*, **227,** 444–452.

SMITH, M.C., DANVIRAYASSYS, K., CROW, J.W., CATO, A.E., PARK, G.D., HASSID, A. & DUNN, M.J. (1982). Prostacyclin substitutions for heparin in long term haemodialysis. *Am. J. Med.*, **73,** 669–678.

SZCZEKLIK, J., SZCZEKLIK, A. & NIZANKOWSKI, R. (1980). Haemodynamic changes induced by prostacyclin in man. *Br. Heart. J.*, **44,** 254–258.

WARRINGTON, S.J., SMITH, P.R., & O'GRADY, J. (1980). Non invasive assessment of the cardiovascular effects of prostacyclin (PGI_2) in man. *Eur. J. Cardiol.*, **12,** 73–80.

WHITTLE, B.J.R., MONCADA, S., WHITING, F. & VANE, J.R. (1980). Carbacyclin – a potent stable prostacyclin analogue for the inhibition of platelet aggregation. *Prostaglandins*, **19,** 605–627.

WHITTLE, B.J.R. & MONCADA, S. (1983). Pharmacological interactions between prostacyclin and thromboxanes. *Br. med. Bull.*, **39,** 232–238.

ZUCKER, I.H. & CORNISH, K.G. (1981). The Bezold-Jarisch reflex in the conscious dog. *Circulation Res.*, **49,** 940–948.

(Received December 20, 1984.
Revised January 28, 1985.
Accepted January 30, 1985.)