Responses of baboon cerebral and extracerebral arteries to prostacyclin and prostaglandin endoperoxide in vitro and in vivo

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SUMMARY The responses of baboon cerebral and extracerebral arteries to prostaglandin endoperoxide (PGH₂) and prostacyclin (PGI₂) were investigated on isolated arteries and in vivo by serial angiography. Both PGH₂ and PGI₂ could produce dose-dependent contraction or relaxation of isolated arteries. PGH₂ induced relaxation was indicative of prostacyclin synthetase activity, the enzyme which converts PGH₂ to PGI₂. In isolated arteries tested one to four hours post mortem only the vertebral artery showed prostacyclin synthetase activity. Thus PGH₂ induced contraction of cerebral arteries may be indicative of a physiological function. Vasomotor tone may in part be the result of a balance between PGH₂ constriction and PGI₂ dilatation. In vivo PGI₂ infusion caused pronounced and prolonged dilatation of cerebral arteries, which lasted longer than the cardiovascular changes. As PGI₂ is the most potent cerebral vasodilator drug tested, it may be of clinical use in the treatment of cerebral vasospasm.

Prostacyclin is a recently discovered prostaglandin with potent vasodilator effects on peripheral and central blood vessels. In addition prostacyclin is one of the most potent inhibitors of platelet aggregation known. The potential therapeutic applications of the use of this drug or more stable analogues is currently under study (Moncada and Vane, 1978).

In view of its potent relaxing actions on human and baboon cerebral arteries (Boullin *et al.*, 1979) prostacyclin may be of use in the prevention or reversal of cerebral arterial vasospasm which commonly occurs after the rupture of aneurysms of the major cerebral arteries.

Before any clinical studies it is of course necessary to examine the actions of prostacyclin and precursors (prostaglandin endoperoxides, PGG₂, PGH₂) upon the cerebral vasculature of animals.

For some years we have been using the baboon as a model for studying the aetiology of human vasospasm (see Boullin *et al.*, 1977, 1978).

Accordingly it was relevant to test the effects of prostacyclin and PGH₂ on this system.

In this paper we describe their effects on baboon arteries in vitro and in vivo after systemic administration and visualisation by carotid angiography.

In addition to confirming the previous reports of the vasodilator effects of prostacyclin itself, we now find that both PGH₂ and prostacyclin can produce constrictor actions in appropriate dosage.

Methods

ISOLATED ARTERIES

Male baboons, body weight 6-14 kg, were killed by intravenous injection of pentobarbitone sodium (140 mg/kg). The basilar, vertebral, middle cerebral (MCA), anterior cerebral (Ant CA), abdominal aorta, femoral, and superior mesenteric arteries were removed within one hour of death and placed in Krebs solution (Starling *et al.*, 1975) at 4°C. Thereafter the tissues were refrigerated for up to four days post mortem. To test the effects of drugs the arteries were dissected spirally and mounted in a 10 ml isolated organ bath. Further

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details are given in previous papers (Starling *et al.*, 1975; Boullin *et al.*, 1976, 1977, 1978). Isotonic contractions of the muscles were amplified by transducer and recorded as previously described.

RESPONSES OF BABOON ARTERIES IN VIVO

The method for studying drug actions on the calibre of baboon cerebral arteries in vivo is described in detail by Boullin *et al.* (1978). Animals were anaesthetised with halothane and the major cerebral arteries visualised by sequential carotid angiography using 60% Urografin 290 (sodium and meglumine diatrizoate). Angiography was performed before and after drug administration. The calibre of the major cerebral arteries was measured before and after drug administration, and the changes expressed as a percentage of the initial values. Drugs were given either intra-arterially via a catheter inserted into the common carotid artery or by intravenous injection. Further details are given in Results.

DRUGS

5-Hydroxytryptamine (5-HT) and noradrenaline (NA) were obtained from Sigma London Chemical Company, Poole, Dorset; dopamine (DM) (as Intropin) from Arnar-Stone Laboratories Ltd, London SW7. These compounds were dissolved in 0.9% NaCl solution. Prostaglandin endoperoxide (PGH_2) and prostacyclin (PGI_2) are very unstable in aqueous solution (Moncada *et al.*, 1976). PGH_2 as the free acid was stored as a stock solution in dry acetone at -20°C . Small aliquots were diluted with 50 mM Tris/HCl buffer pH 7.5 and used immediately. Synthetic PGI_2 sodium salt was dissolved in 1 M Tris/HCl buffer pH 8.5 and further diluted with 50 mM Tris/HCl buffer pH 8.5 as required. All drug solutions were kept in ice.

Results

EFFECTS OF PROSTAGLANDIN ENDOPEROXIDE (PGH_2)

Isolated arteries

Previous workers have found variable effects of PGH_2 on arteries from various species. Whereas some vessels are contracted by PGH_2 , others including coronary and abdominal arteries relax (Moncada *et al.*, 1976; Needleman *et al.*, 1977). We have also observed variable effects on baboon cerebral arteries here. Because PGH_2 relaxations are believed to be due to conversion of PGH_2 to PGI_2 by the enzyme prostacyclin synthetase, we studied the actions of PGH_2 at varying times post mortem; we expected PGH_2 relaxations to be

seen only shortly after death. In fact relaxations were only seen with the vertebral artery. All other cerebral arteries produced contractions to PGH_2 even when tested one to four hours post mortem as described below.

With regard to PGH_2 induced relaxations on the vertebral artery, we made five tests within 48 hours of death and PGH_2 caused relaxation each time. Tests made on day 3 produced contraction.

On successive days post mortem typical results are shown in Fig. 1. On day 1, 14–16 hours post mortem the responses to two doses of PGH_2 were triphasic: an initial very rapid contraction, then relaxation which gradually was reversed and followed by sustained contraction (Fig. 1, day 1, left record).

At this time cumulative doses of PGI_2 produced the typical responses of relaxation with low doses. As will be described below, high concentrations of PGI_2 caused contraction (Fig. 1, day 1, right record). On day 2 when the protocols with PGH_2 and PGI_2 were repeated early in the day (38–40 hours post mortem, Fig. 3, record A) the responses to both PGH_2 and PGI_2 were qualitatively similar to those on day 1 but quantitatively greater. Our experience with both human and baboon cerebral arteries is that their responses to drugs are maximal on the second day.

One hour later on day 2 (Fig. 1, record B) however, PGH_2 no longer caused relaxation but merely a two phase contraction. At this time PGI_2 responses on the other hand were not altered (records not shown). The pattern of responses of day 2 was repeated on day 3; again PGH_2 produced only contraction. Of the peripheral arteries only the femoral artery produced a triphasic response to PGH_2 similar to that shown in Fig. 1.

We conclude from the data shown in Fig. 1 that the contractile effects of PGH_2 are a direct action on the arterial muscle, while secondary relaxation is mediated by conversion of PGH_2 to PGI_2 by prostacyclin synthetase present in the walls of the arterial vessels (See Discussion). Therefore it was surprising to find that all other arteries only contracted to PGH_2 even when tested within four hours of death. PGH_2 caused dose-dependent contractions of all cerebral arteries in concentrations from 0.3 to 100 nmol/l with no evidence of responses reaching a maximum (Fig. 2). Higher concentrations could not be tested because of shortage of material. Figure 2 shows cumulative dose/response curves for PGH_2 on the basilar, vertebral, MCA, and Ant. CA, abdominal aorta, femoral, and superior mesenteric arteries.

Fig. 1 Prostacyclin synthetase activity of baboon isolated vertebral arteries removed one hour post mortem and tested for activity to PGH_2 and PGI_2 at the following times post mortem: day 1 (14–16 hours); day 2 (38 hours, A; 40 hours, B); day 3 (64 hours). Transducer amplified contractions or relaxations of arterial muscle where 10mm contraction is equivalent to 50 mV recorder response (arterial response, mm, ordinate) are plotted against duration of experimental recording (time, min, abscissa). The results were obtained from one artery tested on successive days. PGH_2 and PGI_2 were added to the isolated arteries in the following doses as indicated by the dots. Doses were cumulative for both drugs. (a) Day 1: PGH_2 (ng/ml), 7 and 20. PGI_2 doses producing relaxation (ng/ml): 0.1, 0.2, 0.7, 2, 7, 20, and 70, followed by doses causing contraction ($\mu\text{g/ml}$) 0.2, 0.7, 2, and 7. (b) Day 2 A: PGH_2 7 ng/ml; PGI_2 1, 2, and 7 ng/ml; B: PGH_2 7 ng/ml. (c) Day 3: PGH_2 2 and 7 ng/ml; PGI_2 1, 2, 7, and 20 ng/ml.

It must be emphasised that on vertebral arteries also, PGH_2 produced dose-dependent contractions and that threshold doses caused contraction and not relaxation (Fig. 3); it was not the other way round. Concentrations of PGH_2 above 10 nmol/l produced triphasic responses: an initial transient contraction which was rapidly followed by a relaxation. The last phase of response was a contraction; the second and third phases were dose-dependent.

Arterial calibre in situ

PGH_2 was tested in only one experiment because of shortage of material. Doses of 4 and 10 μg were injected intra-arterially and angiograms per-

formed 4–5 min thereafter. There was no significant change in the calibre of the cerebral vessels examined.

EFFECTS OF PROSTACYCLIN (PGI_2)

Isolated arteries

Isolated arteries responded to PGI_2 in two ways: low concentrations between 0.3 to 800 nmol/l caused dose-dependent relaxations; very high concentrations (over 0.8 $\mu\text{mol/l}$), probably outside the physiological range (see Discussion), produced dose-dependent contractions. Both types of response are shown in Fig. 1; dose-response curves for PGI_2 on various cerebral and extracerebral arteries are shown in Fig. 4a and b.

Fig. 2 Dose-response relationships for PGH_2 contractions of baboon arteries. Values are mean $\pm$ SEM of three to five experiments with cerebral arteries (solid lines) and single experiments with peripheral vessels (interrupted lines). Contractile responses (ordinate) are plotted against log PGH_2 concentration (nmol/l, abscissa). $\bullet$ — $\bullet$ Basilar artery, $\blacksquare$ — $\blacksquare$ vertebral artery, $\blacktriangle$ — $\blacktriangle$ anterior cerebral artery, $\blacktriangledown$ — $\blacktriangledown$ middle cerebral artery, $\circ$ — $\circ$ femoral artery, $\square$ — $\square$ abdominal artery, $\triangle$ — $\triangle$ superior mesenteric artery.

Prostacyclin (PGI_2) in vivo

The actions of PGI_2 on the blood pressure, heart rate, and calibre of cerebral blood vessels were studied in four baboons by sequential angiography 10 min to three hours after administration.

Five experiments were made as one animal was used twice with an interval of two months between experiments. In four experiments PGI_2 was administered systematically and once intracranially into the subarachnoid space using the procedure described by Boullin *et al.* (1978).

PGI_2 was given by intra-arterial infusion in two experiments over 2–10 min in doses of 68 ng/kg and 29 $\mu\text{g/kg}$ (180 pmol/kg and 77 nmol/kg). These doses produced profound changes in the cardiovascular system (severe hypotension and bradycardia) lasting 20–40 min but there was no change in the calibre of the major cerebral arteries.

We also gave PGI_2 by intravenous infusion (femoral vein) in another two experiments. The doses were 4.5 and 13 $\mu\text{g/kg}$ in one experiment and 83 $\mu\text{g/kg}$ in another (12, 35, and 221 nmol/kg).

Fig. 3 Responses of baboon isolated vertebral artery to various doses of PGH_2 . Recordings as Fig. 1. The preparation was washed three times between each dose (records not shown).

The results of an experiment involving intravenous administration are given in Fig. 5. The upper record shows that there was pronounced dilatation of the major intracranial vessels (internal carotid; middle and anterior cerebral arteries). In contrast, the only extracranial artery measured (internal maxillary) showed profound vasoconstriction. This probably occurred as a consequence of the cardiovascular changes observed. These are illustrated in the lower record of Fig. 5. The first dose of PGI_2 which caused the most substantial changes in cerebral arterial calibre caused a moderate fall in pulse pressure and bradycardia; the second dose of PGI_2 which caused little further cerebral arterial dilatation, did produce severe hypotension and compensatory tachycardia, lasting 10 minutes. Thus arterial dilatation after intravenous PGI_2 occurred in the absence of severe changes in blood pressure or heart rate.

The effects of PGI_2 given intracisternally were also studied in one baboon; PGI_2 was given through a needle inserted into the cisterna magna. PGI_2 31 $\mu\text{g/kg}$ was administered by a slow infusion (0.6 ml/1.5 min). The effects on cerebral arterial calibre and the cardiovascular system are shown in Fig. 6 (upper and lower records respectively).

Immediately before PGI_2 infusion the cerebral arteries showed some change in calibre in comparison with the baseline angiogram (time 0, upper records). A second angiogram 10 min after infusion showed dilatation of all cerebral vessels, but this dilatation was not sustained. Thereafter substantial constriction developed in all intracranial vessels. These were a pronounced and prolonged bradycardia with slowly developing hypotension (Fig. 6 lower records).

Fig. 4 Dose-response relationships for effects of PGI₂ on baboon isolated cerebral and extracerebral arteries. Upper record, relaxation responses; lower record, contractile responses. Recordings as Fig. 2 except relaxations plotted as relaxation (mm). For cerebral vessels values are the mean of three to five observations for relaxation and three to four observations for contraction. For extracerebral vessels the values were obtained in single experiments. Key to symbols as in Fig. 2.

Fig. 5 Changes in calibre of baboon cerebral arteries and cardiovascular effects in vivo produced by two intravenous infusions of PGI_2 ($4.5 \mu\text{g/kg}$ and $13 \mu\text{g/kg}$) as indicated. The experiment was made in an anaesthetised baboon. Methods of recording responses are given in Methods and Boullin *et al.* (1978a). Upper records: changes in cerebral arterial calibre (% change ordinate) were calculated from the baseline angiograms made at zero time (hours, abscissa). Four subsequent angiograms were made at the times indicated by points on the graph. Lower records: heart rate (●—● (beats/min) and systolic/diastolic blood pressure were recorded at the times indicated by values for heart rate (●). The stippled area represents the systolic/diastolic blood pressure (mm Hg) recorded at the same time as heart rate. Upper and lower records were obtained from the same animal; the duration of recording of cardiovascular changes was longer than the period of angiographic recordings. Symbols used to identify arteries are given in the legend to Fig. 6.

RESPONSES OF INDIVIDUAL ARTERIES IN VITRO

The relative potencies of PGH_2 and PGI_2 and concentrations producing equivalent responses upon the individual arteries are given in Table 1. The vertebral artery is the most sensitive to the effects of both PGH_2 and PGI_2 . Otherwise there is little variation in potency on the cerebral vessels. The concentrations of PGH_2 in causing contraction are somewhat similar to the concentrations of PGI_2 causing relaxation (see Discussion).

COMPARISON WITH OTHER DRUGS

We have previously reported the effects of dopamine and 5-HT on human and baboon isolated cerebral arteries (Boullin *et al.*, 1977) and these will not be described in detail here.

Dopamine causes dose-dependent relaxation in low concentrations and contraction in high concentrations, while 5-HT produces only dose-dependent contractions.

Table 2 gives a comparison of the effects of PGH_2 and PGI_2 with dopamine (DM), 5-hydroxytryptamine (5-HT), and noradrenaline (NA). PGI_2 was 15 to 30 times more potent than DM in relaxing or contracting the anterior cerebral and vertebral arteries. On the other hand NA was much more potent than PGI_2 in its contractile effects (eight to four times from Table 2). Finally Table 2 shows that PGH_2 and 5-HT were of approximately equal potency as vasoconstrictors.

Discussion

Although PGH_2 has been shown by other workers to contract peripheral arteries in vitro including rabbit aorta, pig coronary artery, and human umbilical artery (Hamborg *et al.*, 1975; Ellis *et al.*, 1976; Tuvemo *et al.*, 1976), there did not appear to be any previous reports other than our own of PGH_2 contracting cerebral arteries in vitro (Boullin *et al.*, 1979). Although our previous

Fig. 6 Effect of intracisternal PGI_2 on cerebral arterial calibre (upper records) and blood pressure and heart rate (lower records). At the arrow PGI_2 $31 \mu\text{g/kg}$ was infused through a needle inserted into the cisterna magna. Infusion of 0.6 ml was made in 90 seconds. The results of upper and lower records were obtained in a single animal. Data were recorded as described in Fig. 5 except that the systolic/diastolic blood pressure is indicated by bars attached to solid circles (●). The following symbols are used to identify various portions of intracranial and extracranial arteries: MC₁, main trunk of middle cerebral; C₂, intracranial carotid; AC₁, main trunk of anterior cerebral; C₃, termination of intracranial internal carotid; MC₂, branch of MC₁; MC₃, branch of a branch of MC, (ie branch of MC₂); C₁, extracranial internal carotid; IM, internal maxillary.

Table 1 Concentrations and relative potencies of PGH_2 and PGI_2 causing equivalent responses of baboon isolated arteries

Artery	PGH_2 contraction*	PGH_2 relaxation† (nmol/l)	PGI_2 contraction*
Vertebral	8 ± 2.5 (11.2)	12 ± 3.6 (5.8)	1300 ± 550 (6.2)
Basilar	32 ± 1.8 (2.8)	45 ± 9.0 (1.6)	2200 ± 440 (3.6)
MCA	17 ± 2.0 (5.3)	55 ± 11.4 (1.3)	2200 ± 930 (3.6)
Ant CA	21 ± 8.3 (4.3)	70 ± 17.2 (1.0)	4400 ± 870 (1.8)
Femoral	13 (6.9)	60 (1.2)	3500 (2.3)
Superior mesenteric	90 (1.0)▲	18 (3.9)	8000 (1.0)

▲ Potency=1 (highest concentration causing recorded response). Values are the mean $\pm$ SEM of three to five experiments; unqualified values are from one or two experiments.

* Produced by 100mm contraction.

† 50% maximum response.

results indicate that baboon cerebral and extra-cerebral arteries possess prostacyclin synthetase activity, the conversion rates for PGH_2 to PGI_2 were very slow except for the vertebral and middle cerebral vessels (see Boullin *et al.*, 1979). As the arteries were tested in vitro only a short while after death, and only the vertebral and femoral arteries showed evidence of relaxation by PGH_2 it seems very likely that the constrictor effects of PGH_2 represent a physiological effect and as such likely to occur in the intact animal. If this is true, endoperoxide formation may play a direct role in the maintenance of vasoconstrictor tone of arteries.

From the in vitro studies it appears that prostacyclin synthetase activity persists for only a short while post mortem in most baboon cerebral arteries. The exception is the vertebral artery which converted PGH_2 to PGI_2 up to 40 hours post mortem. Previously Boullin *et al.* (1979) found that the enzyme was also viable in the human basilar artery at this time, and in a variety of baboon arteries half to three hours after death. However, the percentage conversion of PGH_2 to PGI_2 was low in all arteries except the vertebral

and middle cerebral arteries. The failure to find sufficient conversion of PGH_2 to PGI_2 to relax any baboon cerebral artery except the vertebral in the current work, reinforces the view that prostaglandin endoperoxides may exert direct effects on cerebral vessels. An alternative possibility is that, in the absence of prostacyclin synthetase, prostaglandin endoperoxides are transformed rapidly to PGE_2 which is a potent constrictor of human and baboon cerebral arteries (Boullin *et al.*, 1976, 1977). It is not possible to determine the significance of prostacyclin synthetase in relation to responses of cerebral arteries without an extended study in different species.

The relaxing actions of PGI_2 on isolated arteries have been described earlier by Moncada *et al.* (1977) for human abdominal vessels and by Boullin *et al.* (1979) for human and baboon cerebral vessels. In addition to confirming these results, we now show that intravenous PGI_2 is a potent cerebral vasodilator. Doses in the nmol/kg range produced dramatic and prolonged dilatation of all cerebral arteries measurable on angiograms. The effects were extremely prolonged (more than one and a quarter hours, Fig. 5) and occurred in the absence of severe changes in blood pressure and heart rate. These vasodilator actions were greater than in other experiments when there was severe hypotension and compensatory tachycardia. It is possible that the rather surprising failure of intra-arterial prostacyclin to cause cerebral arterial dilatation was the result of the systemic hypotension. Du Boulay *et al.* (1972, 1973) showed that systemic hypotension resulted in as much as 20% constriction of the major cerebral arteries. The dilatation seen here in the face of hypotension, therefore, was remarkable in view of the vasoconstriction which could be predicted.

Intracisternal prostacyclin also caused transient vasodilatation but this was followed by constriction. The haemodynamic changes were different from those seen with systemic prostacyclin, in that hypotension was not accompanied by tachycardia, but rather bradycardia. These effects

Table 2 Comparative effects of PGH_2 , PGI_2 , DM, NA, and 5-HT

Drug Response	PGI_2 ↓**	PGI_2 ↑*	DM ↓**	DM ↑*	PGH_2 ↑**	NA ↑*	5-HT ↑**
Drug concentration producing effect nmol/l							
Anterior cerebral	70	4400	1100	100,000	21	500	14
Vertebral	12	1300	400	40,000	8	35	10

↓ = contraction; ↑ = relaxation; * = response of 100mm contraction; ** = 50% maximum response.

could have been due to actions on the hypothalamus or other brain regions and would need much further study.

PGI₂ is 15–30 times more potent than dopamine in relaxing cerebral arteries. As dopamine has been tested in subarachnoid haemorrhage patients with cerebral arterial vasospasm (Boullin *et al.*, 1977), there is the possibility that prostacyclin or more stable analogues may be of value in the treatment of spasm.

The constrictor effects of high concentrations of PGI₂ seem unlikely to have any physiological significance because the rates of PGI₂ synthesis by arteries and the heart are in the ng/g/min (pmol/g/min) range (De Deckere *et al.*, 1977; Moncada *et al.*, 1977; Boullin *et al.*, 1979). There was no vasoconstrictor effect of PGI₂ in situ, which probably reflects the rapid rate of hydrolysis of PGI₂ to 6-oxo-PGF_{1α} (Moncada and Vane, 1977).

As regards the cause of vasospasm after subarachnoid haemorrhage, there is now evidence for the presence of unidentified vasoconstrictor factors in CSF of subarachnoid haemorrhage which are related to the severity of spasm and mortality (Blaso, 1978; Blaso and Boullin, 1978; Boullin and Blaso, 1978). Moreover in these same patients who died with angiographic evidence of vasospasm, Schianchi and Hughes (1978) have reported pathological changes in the endothelium of cerebral arteries known to have been in spasm during life (confirmed in experimental animal models by Tanake *et al.*, 1978). As the endothelium is the site of prostacyclin synthetase, there is reason to implicate PGI₂ in the aetiology of vasospasm after subarachnoid haemorrhage. Consequently vasospasm might involve defective prostacyclin synthetase leading to PGI₂ deficiency, allowing prostaglandin endoperoxide (PGH₂) constrictor actions to become dominant. These defects in prostaglandin actions would be additional to the actions of the unidentified vasoconstrictor factors in CSF.

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