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).

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
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 intrararterially 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 (PGH2) and prostacyclin (PGI2) are very unstable in aqueous solution (Moncada et al., 1976). PGH2 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 PGI2 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 (PGH2)
Isolated arteries
Previous workers have found variable effects of PGH2 on arteries from various species. Whereas some vessels are contracted by PGH2, 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 PGH2 relaxations are believed to be due to conversion of PGH2 to PGI2 by the enzyme prostacyclin synthetase, we studied the actions of PGH2 at varying times post mortem; we expected PGH2 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 PGH2 even when tested one to four hours post mortem as described below.

With regard to PGH2 induced relaxations on the vertebral artery, we made five tests within 48 hours of death and PGH2 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 PGH2 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 PGI2 produced the typical responses of relaxation with low doses. As will be described below, high concentrations of PGI2 caused contraction (Fig. 1, day 1, right record). On day 2 when the protocols with PGH2 and PGI2 were repeated early in the day (38–40 hours post mortem, Fig. 3, record A) the responses to both PGH2 and PGI2 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, PGH2 no longer caused relaxation but merely a two phase contraction. At this time PGI2 responses on the other hand were not altered (records not shown). The pattern of responses of day 2 was repeated on day 3; again PGH2 produced only contraction. Of the peripheral arteries only the femoral artery produced a triphasic response to PGH2 similar to that shown in Fig. 1.

We conclude from the data shown in Fig. 1 that the contractile effects of PGH2 are a direct action on the arterial muscle, while secondary relaxation is mediated by conversion of PGH2 to PGI2 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 PGH2 even when tested within four hours of death. PGH2 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 PGH2 on the basilar, vertebral, MCA, and Ant. CA, abdominal aorta, femoral, and superior mesenteric arteries.
Responses of baboon cerebral and extracerebral arteries

Fig. 1 Prostacyclin synthetase activity of baboon isolated vertebral arteries removed one hour post mortem and tested for activity to PGH₂ and PGI₂ 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 were 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. PGH₂ and PGI₂ were added to the isolated arteries in the following doses as indicated by the dots. Doses were cumulative for both drugs. (a) Day 1: PGH₂ (ng/ml), 7 and 20. PGI₂ doses producing relaxation (ng/ml): 0.1, 0.2, 0.7, 2, 7, 20, and 70, followed by doses causing contraction (ng/ml) 0.2, 0.7, 2, and 7. (b) Day 2 A: PGH₂ 7 ng/ml; PGI₂ 1, 2, and 7 ng/ml; B: PGH₂ 7 ng/ml. (c) Day 3: PGH₂ 2 and 7 ng/ml; PGI₂ 1, 2, 7, and 20 ng/ml.

It must be emphasised that on vertebral arteries also, PGH₂ produced dose-dependent contractions and that threshold doses caused contraction and not relaxation (Fig. 3); it was not the other way round. Concentrations of PGH₂ 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
PGH₂ was tested in only one experiment because of shortage of material. Doses of 4 and 10 µg were injected intra-arterially and angiograms performed 4–5 min thereafter. There was no significant change in the calibre of the cerebral vessels examined.

EFFECTS OF PROSTACYCLIN (PGI₂) Isolated arteries
Isolated arteries responded to PGI₂ in two ways: low concentrations between 0.3 to 800 nmol/l caused dose-dependent relaxations; very high concentrations (over 0.8 µmol/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 PGI₂ on various cerebral and extracerebral arteries are shown in Fig. 4a and b.
The actions of PGI₂ 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 PGI₂ was administered systematically and once intracranially into the subarachnoid space using the procedure described by Boullin et al. (1978). PGI₂ was given by intra-arterial infusion in two experiments over 2–10 min in doses of 68 ng/kg and 29 µg/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 PGI₂ by intravenous infusion (femoral vein) in another two experiments. The doses were 4.5 and 13 µg/kg in one experiment and 83 µg/kg in another (12, 35, and 221 nmol/kg).

The results of an experiment involving intravenous administration are given in Fig. 5. The preparation was washed three times between each dose (records not shown).

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 PGI₂ which caused the most substantial changes in cerebral arterial calibre caused a moderate fall in pulse pressure and bradycardia; the second dose of PGI₂ which caused little further cerebral arterial dilatation, did produce severe hypotension and compensatory tachycardia, lasting 10 minutes. Thus arterial dilatation after intravenous PGI₂ occurred in the absence of severe changes in blood pressure or heart rate.

The effects of PGI₂ given intracranially were also studied in one baboon; PGI₂ was given through a needle inserted into the cisterna magna. PGI₂ 31 µg/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 PGI₂ 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 PG\textsubscript{12} 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.
RESPONSES OF INDIVIDUAL ARTERIES IN VITRO
The relative potencies of PGH₂ and PGI₂ 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 PGH₂ and PGI₂. Otherwise there is little variation in potency on the cerebral vessels. The concentrations of PGH₂ in causing contraction are somewhat similar to the concentrations of PGI₂ 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 PGH₂ and PGI₂ with dopamine (DM), 5-hydroxytryptamine (5-HT), and noradrenaline (NA). PGI₂ 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 PGI₁ in its contractile effects (eight to four times from Table 2). Finally Table 2 shows that PGH₂ and 5-HT were of approximately equal potency as vasoconstrictors.

Discussion
Although PGH₂ has been shown by other workers to contract peripheral arteries in vitro including rabbit aorta, pig coronary artery, and human umbilical artery (Hamberg 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 PGH₂ contracting cerebral arteries in vitro (Boullin et al., 1979). Although our previous

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Fig. 5 Changes in calibre of baboon cerebral arteries and cardiovascular effects in vivo produced by two intravenous infusions of PGI₂ (4.5 μg/kg and 13 μg/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.
Fig. 6  *Effect of intracisternal PGI₂ on cerebral arterial calibre (upper records) and blood pressure and heart rate (lower records).* At the arrow PGI₂ 31 μg/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 PGH₂ and PGI₂ causing equivalent responses of baboon isolated arteries

<table>
<thead>
<tr>
<th>Artery</th>
<th>PGH₂ contraction*</th>
<th>PGH₂ relaxation† (nmol/l)</th>
<th>PGI₂ contraction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebral</td>
<td>8±2.5 (11.2)</td>
<td>12±3.6 (5.8)</td>
<td>1300±550 (6.2)</td>
</tr>
<tr>
<td>Basilar</td>
<td>32±1.8 (2.8)</td>
<td>45±9.0 (1.6)</td>
<td>2200±440 (3.6)</td>
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<tr>
<td>MCA</td>
<td>17±2.0 (5.3)</td>
<td>55±11.4 (1.3)</td>
<td>2200±930 (3.6)</td>
</tr>
<tr>
<td>Ant CA</td>
<td>21±8.3 (4.3)</td>
<td>70±17.2 (1.0)</td>
<td>4400±870 (1.8)</td>
</tr>
<tr>
<td>Femoral</td>
<td>13 (6.9)</td>
<td>60 (1.2)</td>
<td>3500 (2.3)</td>
</tr>
<tr>
<td>Superior mesenteric</td>
<td>90 (1.0)</td>
<td>18 (3.9)</td>
<td>8000 (1.0)</td>
</tr>
</tbody>
</table>

*Potency =1 (highest concentration causing recorded response). Values are the mean ± SEM of three to five experiments; unqualified values are from one or two experiments. *Produced by 100nm contraction." †+50% maximum response.

results indicate that baboon cerebral and extra-cerebral arteries possess prostacyclin synthetase activity, the conversion rates for PGH₂ to PGI₂ 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 PGH₂ it seems very likely that the constrictor effects of PGH₂ 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 PGH₂ to PGI₂ 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 PGH₂ to PGI₂ was low in all arteries except the vertebral and middle cerebral arteries. The failure to find sufficient conversion of PGH₂ to PGI₂ 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 PGE₂ 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 PGI₂ 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 PGI₂ is a potent cerebral vasodilator. Doses in the nmol/kg range produced dramatic and prolonged dilatation of all cerebral arteries measurable on angiography. 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 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 PGH₂, PGI₂, DM, NA, and 5-HT

<table>
<thead>
<tr>
<th>Drug Response</th>
<th>PGI₂</th>
<th>PGI₂</th>
<th>DM</th>
<th>DM</th>
<th>DM</th>
<th>PGI₂</th>
<th>NA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior cerebral</td>
<td>70</td>
<td>4400</td>
<td>1100</td>
<td>100,000</td>
<td>21</td>
<td>500</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Vertebral</td>
<td>12</td>
<td>1300</td>
<td>400</td>
<td>40,000</td>
<td>8</td>
<td>35</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

↓ =contraction; ↑ =relaxation; * =response of 100nmmm contraction; ** =50% maximum response.
Responses of baboon cerebral and extracerebral arteries

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

PGL₂ 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-PGF₁α (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 PGL₂ deficiency, allowing prostaglandin endoperoxide (PGH₃) 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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