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RESPONSES OF HUMAN AND BABOON ARTERIES TO PROSTAGLANDIN ENDOPEROXIDES AND BIOLOGICALLY GENERATED AND SYNTHETIC PROSTACYCLIN: THEIR RELEVANCE TO CEREBRAL ARTERIAL SPASM IN MAN

D.J. BOULLIN,[1] S. BUNTING,[2] W.P. BLASO,[1] T.M. HUNT[1] & S. MONCADA[2]

[1]M.R.C. Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE
and
[2]Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

1 Isolated strips of human or baboon basilar, middle cerebral, vertebral or common carotid arteries were set up in an isolated organ bath or in a superfusion cascade system.

2 These arteries relaxed to prostacyclin but contracted to prostaglandin endoperoxide (PGH_2).

3 Human and baboon isolated arteries also generated prostacyclin from exogenous endoperoxide (PGH_2).

4 Human arteries generated prostacyclin 36 h post-mortem but not 40 h post-mortem. The biologically generated prostacyclin relaxed the basilar artery and overcame the contractile effects of PGH_2.

5 Thromboxane A_2-like activity generated during human platelet aggregation by arachidonic acid caused contractions of the human basilar artery.

6 Prostacyclin reversed contractions of human basilar arteries caused by an unidentified vasoconstrictor factor in cerebrospinal fluid obtained from patients with cerebral arterial vasospasm after subarachnoid haemorrhage following rupture of cerebral arterial aneurysms.

7 The above vasospasm may be due at least in part to disordered physiological control of the calibre of cerebral arteries caused by diminished synthesis of prostacyclin.

Introduction

Recently a new prostaglandin has been discovered and its structure elucidated (Moncada, Higgs & Vane, 1977; Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976; Moncada, Gryglewski, Bunting & Vane, 1976). This substance was named prostacyclin (PGI_2); it is generated by blood vessels, prevents platelet aggregation and relaxes arterial smooth muscle in animals (Moncada *et al.*, 1976). Moncada *et al.* (1977) have postulated a physiological role for prostacyclin in preventing platelets from clumping on to vessel walls. They have suggested that inhibition of prostacyclin synthesis may play a role in diseases such as atherosclerosis.

Cerebral arterial spasm (CAS) commonly follows subarachnoid haemorrhage (SAH) after rupture of cerebral arterial berryaneurysms. This disorder involves arterial damage, platelet aggregation and formation of thrombii (Simeone, Ryan & Cotter, 1968; Zervas, Kuwayama, Rosoff & Salzman, 1973). Release of 5-hydroxytryptamine (Buckell, 1964; Allen, Henderson, Chou & French, 1974), prostaglandins (Pennink, White, Crockarell & Robertson, 1972; Robertson, 1973) and other vasoactive substances have all been implicated as causing CAS (Kapp, Mahaley & Odom, 1968; Bohr & Sobieski, 1968; Odom, 1975; Boullin, Mohan & Grahame-Smith, 1976; Allen, Gross, French & Chou, 1976). Nevertheless, no comprehensive explanation for CAS has been put forward and no effective therapy exists.

The discovery of prostacyclin and the ability of human arteries to generate this substance, suggests that variations in prostacyclin synthesis might be involved in the prolonged CAS which follows SAH. Changes in the synthesis of a highly potent vasoactive substance could account for arterial constriction lasting several weeks.

We have shown that prostacyclin has potent effects on human and baboon cerebral and extracerebral arteries. The endoperoxide precursors have opposite actions to prostacyclin on these tissues. We also show that the cerebral vessels are themselves capable of synthesising prostacyclin.

As the baboon is used as a model for investigating

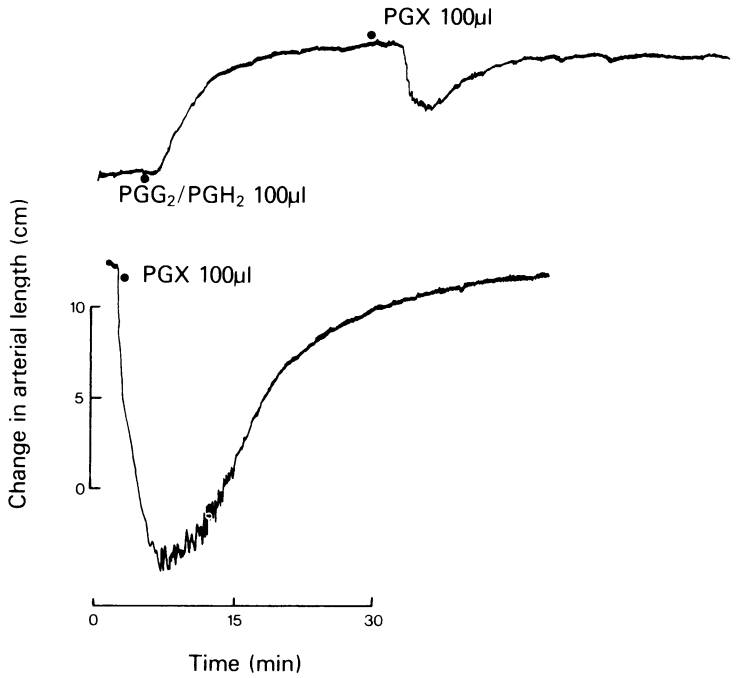

Figure 1 Responses of human basilar artery to prostaglandin endoperoxides and prostacyclin (PGX). Prostaglandin endoperoxides generated biologically as described in **Methods** were added to a human isolated basilar artery in a 10 ml organ bath in Krebs solution at 37°C as described (see text). Contractions were amplified by transducer and recorded isotonically (amplified change in arterial length, ordinate; time, min, abscissa). 100 µl of PGG_2/PGH_2 solution is equivalent to 1 µg assuming 100% conversion of arachidonic acid. 100 µl PGX is equivalent to 200 ng PGI_2 assuming 100% conversion of prostaglandin endoperoxides.
Upper record: PGG_2/PGH_2 induced contraction is partly and transiently reversed by prostacyclin (PGX).
Lower record: Prostacyclin relaxes the artery.

the aetiology of CAS (Boullin, Adams, Mohan, Green, Hunt, Du Boulay & Rogers, 1977; Boullin, Du Boulay & Rogers, 1978), we have also used baboon arteries, obtaining essentially similar results to those described for human arteries.

Methods

Human basilar, vertebral and middle cerebral arteries were obtained at autopsy 14–50 h after death from patients dying from causes other than subarachnoid haemorrhage. Various cerebral and extracerebral arteries were also obtained from 6–12 kg male baboons within 20 min of killing by intravenous phenobarbitone and/or air embolism. The arteries were cut spirally and suspended in a 10 ml isolated organ bath in Krebs' solution at 37°C (Starling, Boullin, Grahame-Smith, Adams & Gye, 1975; Boullin *et al.*, 1976) or in the superfusion cascade system of Vane (1969). Contractions of the arterial smooth muscle were recorded as described previously (Starling *et al.*, 1975). Rat stomach fundus preparations were also used under similar conditions after preparation according to Vane (1957).

Blood was collected from normal healthy subjects of either sex who had not taken aspirin for at least 14 days. Nine volumes of blood was mixed with 1 volume of 3.8% sodium citrate. Platelet rich plasma (PRP) was prepared and platelet aggregation recorded with a Corning-EEL model 169 platelet aggregometer as described previously (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975; Boullin, Green & Price, 1972).

Prostaglandin endoperoxides (PGG_2, PGH_2) were generated biologically (Moncada *et al.*, 1977). Synthetic prostacyclin (PGI_2) sodium salt was dissolved in 1 M Tris buffer pH 8.4 as a stock solution and then further diluted in 50 mM Tris buffer pH 7.5 and used immediately.

Thromboxane A_2-like activity was obtained from PRP aggregated with arachidonic acid: aliquots of the solution were removed when the rate of aggregation was maximal (Ellis, Oelz, Roberts, Payne, Sweetman, Nies & Oates, 1976) and tested on arteries. Prostacyclin synthetase activity was measured in the basilar and vertebral arteries from the same subject by the following procedure: the vertebral arteries

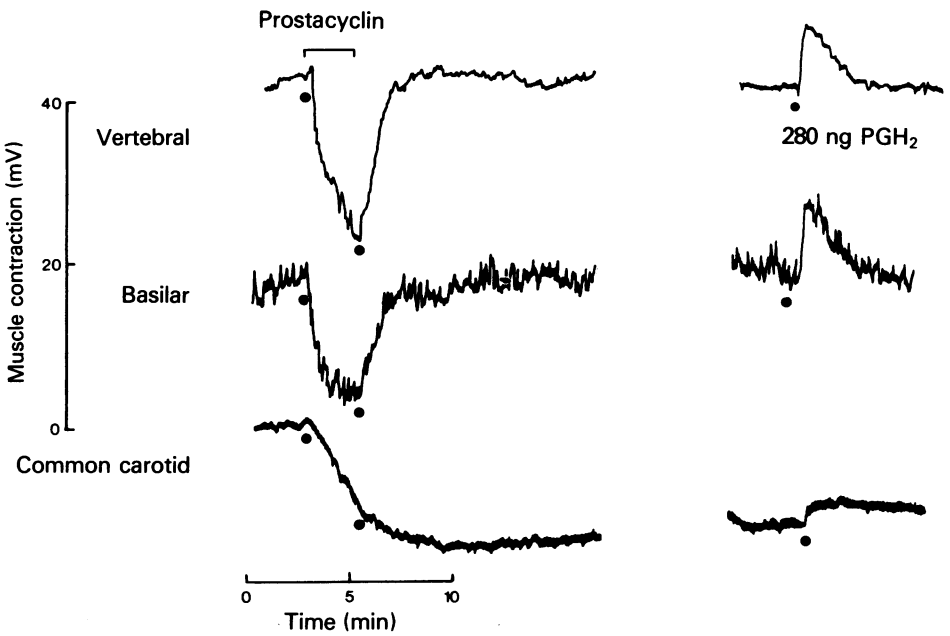

Figure 2 Responses of baboon cerebral and extracerebral arteries to prostacyclin and PGH_2. Arteries from a 12 kg baboon were superfused in cascade. Transducer amplified recordings of auxotonic muscle contractions (ordinate) in relation to time (abscissa). Prostaglandin endoperoxides (PGH_2) and synthetic prostacyclin (PGI_2) were prepared as described (see text).

Left records: When prostacyclin was infused at the rate of 100 ng/min (between closed circles as indicated), the vertebral, basilar and common carotid arteries relaxed. Note the relaxation of the common carotid artery was sustained after the termination of prostacyclin infusion.

Right records: All arteries were contracted by a single dose of 280 ng PGH_2. Note the relative sensitivity of the cerebral arteries in comparison to the common carotid artery.

(approximately 50 mg) were chopped into 1 ml 0.05 M Tris buffer pH 7.5 and prostacyclin synthetase activity was measured by incubation for 3–11 min at 20°C with 250 ng/ml PGH_2. Prostacyclin formation was assayed by inhibition of ADP-induced platelet aggregation using human platelet rich plasma.

With basilar arteries prostacyclin synthetase activity was assessed as follows: PGH_2 contracts cerebral arteries by a direct effect (see **Discussion**) whereas PGI_2 caused relaxation, thus, the response of basilar arteries to PGH_2 of either pure relaxation or biphasic relaxations and contractions gave a qualitative indication of the conversion of endoperoxides to prostacyclin. Similar experiments were performed using a variety of baboon cerebral and extracerebral arteries.

Results

Prostacyclin (PGI_2) relaxed and prostaglandin endoperoxides (PGG_2, PGH_2) contracted human and baboon cerebral and extracerebral arteries (Figures 1 and 2). Figure 1 shows a relaxation lasting about 30 min when prostacyclin was added to an isolated organ bath containing a human basilar artery. Similar effects were seen with the middle cerebral artery (not shown).

Baboon arteries set up in the superfusion cascade system were also relaxed by prostacyclin (Figure 2). The relaxation reversed shortly after cessation of prostacyclin application to baboon cerebral arteries (vertebral and basilar) but persisted with the extracranial common carotid artery.

In contrast to the effects of prostacyclin, the prostaglandin endoperoxides contracted the human basilar artery (Figure 1) and the baboon arteries (Figure 2). In other experiments the responses of baboon basilar, middle cerebral and anterior cerebral arteries to PGH_2 were examined 45–90 min after death. These arteries were extremely sensitive to PGH_2 producing dose-dependent contractions with concentrations of 0.1–1.0 ng/ml. Although these baboon arteries were invariably relaxed by PGI_2, PGH_2 produced variable effects. In some instances a biphasic contraction followed by relaxation was observed while in others only contraction was seen (see below). Arteries examined within 3 h of death were able to synthesise prostacyclin from exogenous PGH_2

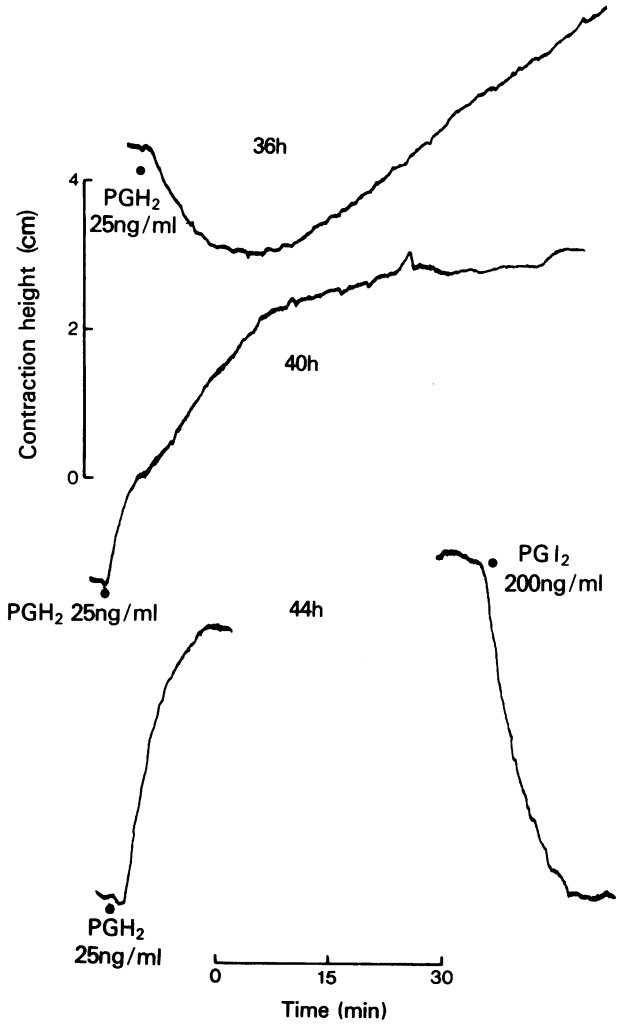

Figure 3 Post mortem basilar artery responses to PGH_2 and synthetic prostacyclin (PGI_2). Transducer amplified recordings of isotonic muscle contractions as described in Figure 1.
Left records: Responses of the same basilar artery to PGH_2 36, 40 and 43 h after death.
Right records: Response to PGI_2 at 44 h (identical effects were seen at 36 and 40 h, not shown).
There was no change in arterial tone during the intervening periods between tests.

(Table 1).

The prostacyclin synthetase activity of human basilar and vertebral arteries was examined in tissues taken from eight subjects 14–50 h after death from various causes but not involving any head injury. Tissues were contracted by PGH_2 (10–50 ng/ml) or 6-oxo-PGF_1 (10–80 ng/ml). In seven out of nine experiments PGH_2 caused only contraction of the basilar artery, and the vertebral arteries did not generate prostacyclin when tested as described above. However, in one case there was sufficient prostacyclin synthetase activity in both basilar and vertebral arteries for prostacyclin synthesis to be determined.

The responses obtained where prostacyclin synthetase activity was investigated 36–44 h after death are shown in Figures 3 and 4. Figure 3 shows the effects of PGH_2 and PGI_2. When PGH_2 was first applied to the isolated basilar artery 36 h after death there was an immediate relaxation lasting about 30 min followed by sustained contraction (upper record, Figure 3). When PGH_2 was applied 40–44 h after death, the artery contracted without any relaxation (middle and left lower records, Figure 3). On the other hand, PGI_2 caused only relaxation at all times (lower right record, Figure 3).

The results obtained after 36 h showed that the

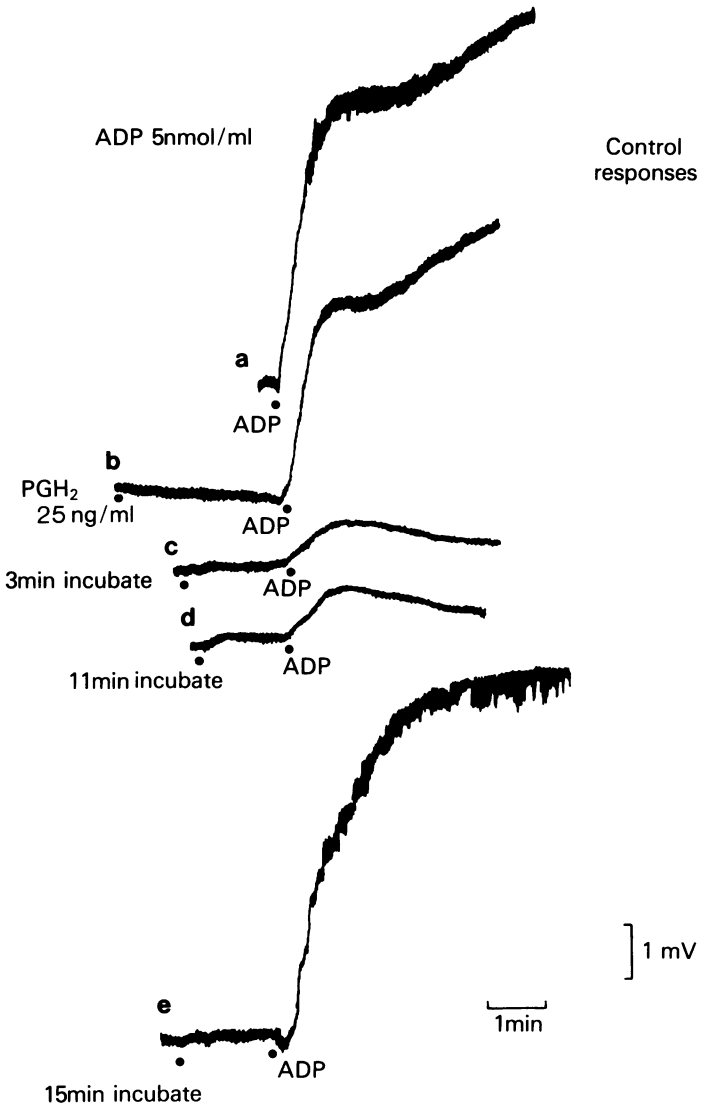

Figure 4 Prostacyclin synthetase activity of human cerebral arteries obtained 36 h after death. Synthetase activity was assessed by measuring the inhibitory activity of PGI_2 upon ADP-induced platelet aggregation following incubation of 50 mg of finely chopped arteries with 250 ng/ml PGH_2 for 3–15 min at 20 or 37°C as shown.

Records are of platelet aggregation (change in optical density of platelet rich plasma, (mV) ordinate in relation to time (min) abscissa.

Records top to bottom:

a. Control response to ADP
b. Control response to PGH_2 (25 ng/ml)
c. 50 mg chopped vertebral artery in 1 ml 50 mM Tris pH 7.4 incubated for 3 min at 20°C. 100 µl aliquot tested on PRP (equivalent to 25 ng/ml PGH_2 tested in record 2)
d. As c incubated for 11 min at 20°C
e. As c incubated for 15 min at 37°C. Note complete disappearance of inhibitory effect of PGI_2.

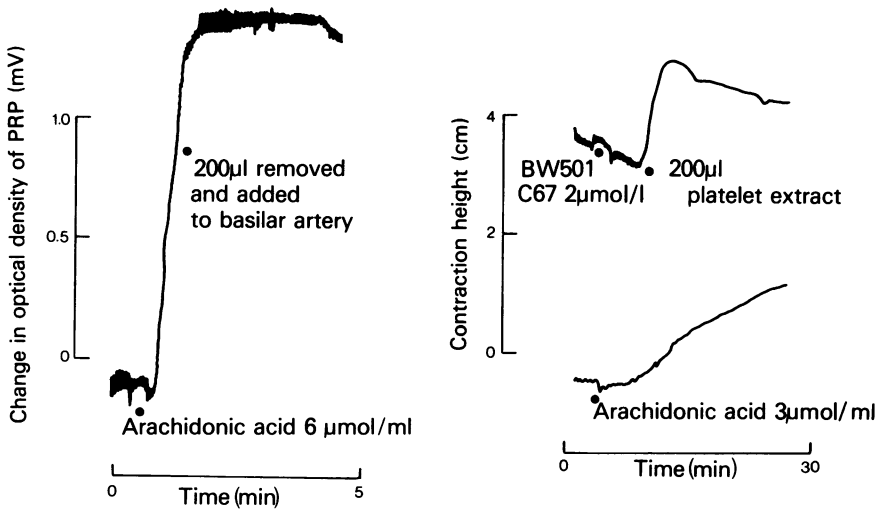

Figure 5. Thromboxane-like activity generated by platelets contracts the basilar artery.
Left record: 1000 µl of human platelet rich plasma (PRP) stirred at 1000 rev/min at 37°C. Arachidonic acid 6 µmol/ml caused irreversible aggregation (change in optical density of PRP, mV ordinate). At the solid circle, 200 µl aggregating PRP was removed and immediately applied to a human isolated basilar artery (right record: see below).
Right record: Human isolated basilar artery: upper trace: BW501C67, a potent 5-hydroxytryptamine antagonist, was applied to the artery followed by the 200 µl fraction of aggregating platelets described above. This caused a sustained contraction of the basilar artery. The control experiment showed that arachidonic acid produced only a slowly developing contraction (lower trace).

basilar artery possessed adequate prostacyclin synthetase activity to produce sufficient PGI_2 from PGH_2 to cause an immediate relaxation.

The results of one experiment are shown in Figure 4 which give the effects of PGH_2 alone or PGH_2 added to incubates of chopped vertebral artery for various times on ADP-induced human platelet aggregation. PGH_2 (25 ng/ml) alone produced slight inhibition of ADP-induced aggregation (second record from top). In two tests the mean inhibition was 23.0%. The inhibitory effect of low concentrations of PGH_2 (25–100 ng/ml) was greatly enhanced by incubation with chopped vertebral arteries at 20°C for 3–11 min or 37°C for 15 min. The third and fourth records (from top) in Figure 4 illustrate this. The bottom record shows that all prostacyclin-like inhibitory activity was

Table 1 Prostacyclin synthetase activity of baboon cerebral arteries

Artery	*Conversion of PGH_2 to PGI_2* *% conversion*	*Synthesis rate (ng g^{-1} min^{-1})*
Vertebral	33.3	12.5
Middle cerebral	36	10.1
Anterior communicating	12	28
Basilar	6.2	20
Internal + external carotid	5.4	3.5
Common carotid	5.3	4.8
Aorta	7.0	4.8
Superior mesenteric	3.0	2.7

Prostacyclin synthetase activity was measured in 10–50 mg of chopped artery by the method of Bunting *et al.* (1976). Synthesis was measured 0.5–3 h post mortem. Values are the mean of three experiments.

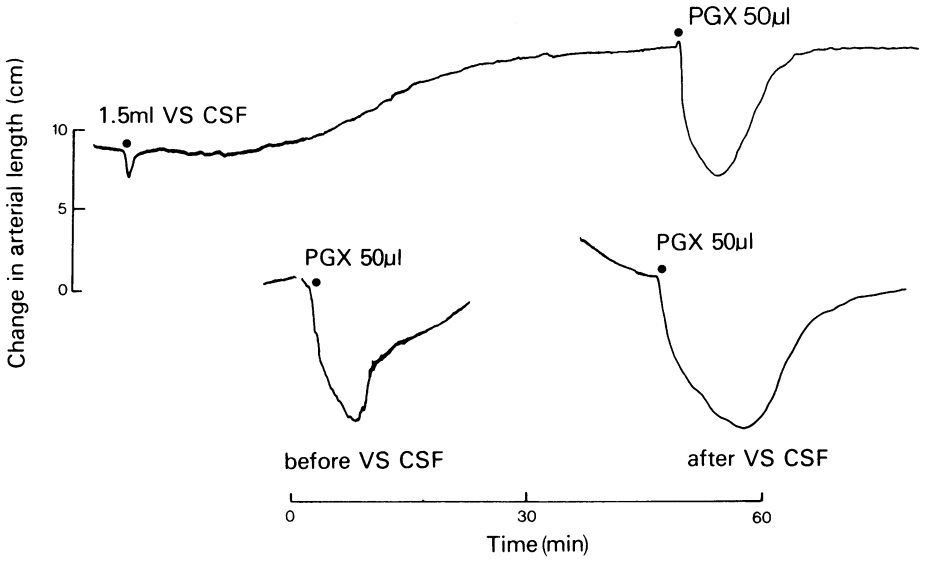

Figure 6 Antagonism of vasospastic CSF induced contraction of the human basilar artery by biologically generated prostacyclin (PGX).

Recordings as Figure 1.

Upper record: CSF 1.5 ml (VS CSF) from a 46 year old female patient with SAH applied to the basilar artery. PGX (50 µl, equivalent to 100 ng prostacyclin, see Figure 1) caused a transient relaxation when added to the organ bath in the continued presence of CSF.

Lower record: responses to 100 ng prostacyclin before (left) and after (right) application of CSF as shown in upper record. The preparation was washed between drug administrations and these recordings are not shown.

There was no change in arterial tone between left and right records. Intervening records of tone between tests are not shown.

abolished by incubation for 15 min at 37°C.

The conversion of PGH_2 to PGI_2 was 11% in this experiment. This low conversion could be due to the prolonged interval between death and the obtention of the arteries. On the other hand, other tests confirmed that prostacyclin synthetase activity was short-lived *in vitro*, since there was no inhibitory action against ADP-induced aggregation using the same arteries tested 4 hours later (40 h after death).

To investigate the actions of thromboxane A_2 on the arterial strips, we used aliquots of PRP in which arachidonic acid had induced platelet aggregation. When irreversible platelet aggregation was induced by arachidonic acid (Figure 5 left) removal of an aliquot of the PRP caused contraction of the isolated human basilar artery which had been pretreated with BW501C67 2 µmol/l, a potent 5-HT antagonist (Figure 5 right, upper record). The pattern of this contraction was quite different from the slowly developing contraction produced by arachidonic acid alone (Figure 5, right, lower record). The thromboxane A_2-like activity disappeared when the platelets were allowed to stand for 5 min at 20°C.

CSF from SAH patients with CAS contains an unidentified vaso-active substance which contracts human and animal cerebral arteries (Boullin *et al.*, 1976; Allen *et al.*, 1976). Prostacyclin relaxes the arteries producing a transient relaxation lasting 15–30 min in the presence of this vasoconstrictor factor. This relaxation is of comparable duration to that shown in Figure 1 where prostacyclin decreased spontaneous arterial tone (lower record) or reversed PGG_2/PGH_2 induced contractions (upper record). Figure 6 also shows that the responses of the arteries to prostacyclin were not changed during or after prolonged contact of the arteries with CSF. This indicated that the CSF vasoconstrictor factor did not alter the effects of prostacyclin.

Discussion

Our results extend the earlier work of Moncada *et al.* (1977), who demonstrated that human gastrointestinal arteries generated prostacyclin, an extremely potent inhibitor of platelet aggregation. We now show that prostacyclin can be generated by human cerebral arteries and that prostacyclin induces relaxation of human and baboon isolated cerebral and extracerebral vessels.

The percentage conversion of prostaglandin endoperoxide by human vertebral arteries was much less than that reported by Moncada *et al.* (1977) for gastrointestinal arteries tested within 3 h of removal. This difference may be due to the long interval (14–50 h) between death and testing. Under these conditions prostaglandin endoperoxides generally contracted arteries; biphasic relaxation/contraction rarely occurred. This was probably due to defective prostacyclin synthesis with minimal conversion of PGH_2 to PGI_2. Using human mesenteric and coeliac arteries, Bunting, Gryglewski, Moncada & Vane (1976) showed that after application of the prostacyclin synthetase inhibitor 15-hydroperoxy arachidonic acid (15-HPAA), PGH_2 only produced contraction.

While prostacyclin relaxes arteries, thromboxane A_2-like activity generated from platelets incubated with arachidonic acid always produced contractions.

Baboon intracranial arteries studied very soon after death (30–180 min) converted 6–36% of added PGH_2 to prostacyclin; these vessels were extremely sensitive to PGH_2, contracting to 0.1 to 1.0 ng/ml but less sensitive to PGI_2 than human cerebral vessels. Therefore the effects of PGH_2 predominated. These data indicate that prostaglandin endoperoxides, like PGI_2, may both play a physiological role in the maintenance of cerebral arterial tone. While PGH_2 may cause contraction directly, rapid conversion to PGI_2 will lead to relaxation. There may be species variations in prostacyclin synthetase activity and in sensitivity to prostacyclin and its precursor.

In our experiments contractions with human cerebral arteries could be due to a mixture of prostaglandin endoperoxide intermediates and thromboxane A_2 (Moncada *et al.*, 1977) generated during platelet aggregation. Previous work has shown that platelet aggregation by arachidonic acid is due to conversion to endoperoxides with subsequent formation of thromboxane A_2.

In the case of CAS after SAH, the prolonged spasm observed lasting 3 weeks or more can be visualised as a disturbance in a dynamic balance between synthesis and release of prostaglandin endoperoxides, other contractile substances and synthesis and release of prostacyclin. Prolonged CAS could imply prostacyclin deficiency so that contractile effects of prostaglandin endoperoxides became paramount. An alternative explanation would involve diversion of prostaglandin endoperoxide catabolism along the thromboxane pathway.

In this regard it is of interest that the effects of prostacyclin on the baboon common carotid artery were much more prolonged than on the intracranial arteries (Figure 2). Because prostacyclin can reverse contractions of cerebral arteries produced by the unidentified constrictor factor(s) in CSF (Figure 6) variations in the intensity and duration of the spasm may be due to alterations in prostacyclin formation.

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