Arterial eicosanoid production following chronic exposure to a periarterial haematoma

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SUMMARY Cisternal and lumbar cerebrospinal fluid obtained some days following a subarachnoid haemorrhage contains abnormally large quantities of various prostanoids; some may be partly the result of abnormal production by the cerebral arteries. The extra-arterial and intra-arterial production of 6 oxo PGF_{1α} (prostaglandin metabolite), PGE₂, PGF_{2α} and TXB₂ were measured in perfused rabbit common carotid arteries taken both from normal rabbits and from rabbits in which the arteries had been ensheathed by blood clot *in vivo* for 7 days using two techniques. Prostaglandin production by control arteries was highest during the first hour of perfusion but declined or increased marginally (PGE₂) during the succeeding three hours. Arteries exposed to a periarterial haematoma for 7 days produced prostaglandins at a high rate throughout the 4 hours of study, and there was a progressive and marked increase in PGE₂ production. The disproportionate increase in the cerebral vasoconstrictor PGE₂ may reflect the inflammatory response which occurred in the adventitia of the vessels. Increased prostanoid production by cerebral arteries probably does contribute to the increased levels in CSF after subarachnoid haemorrhage.

Although blood will induce immediate contraction of cerebral arteries *in vitro*, angiography in patients following subarachnoid haemorrhage has shown that cerebral vasospasm is seldom seen in the first 48 hours after a bleed and is maximal at around 7 days. The use of early CT scanning has revealed that vasospasm tends to develop in those arteries which have been enveloped in a significant periarterial haematoma.^{1,2} Hence some blood component is involved in the development of cerebral vasospasm, and this phenomenon seems to reflect an exaggerated contractile response to vasoactive agents released where clot and arterial wall are in close apposition. The prostaglandin hypothesis of cerebral vasospasm supposes that an excessive production intracranially of the cerebral vasoconstrictors Thromboxane A₂ (TXA₂), prostaglandin (PG) F_{2α}, or PGE₂, or defective synthesis of the vasodilator prostacyclin, might be implicated in the cerebrovas-

cular changes in subarachnoid haemorrhage (see ref 3 for review). In parallel studies of dogs and man it was found that subarachnoid haemorrhage results in a marked increase in PGE₂, PGF_{2α}, 6 oxo PGF_{1α} (the prostacyclin metabolite) but little TXB₂ (the TXA₂ metabolite) in lumbar and cisternal CSF examined 3 to 7 days after subarachnoid haemorrhage (see ref 4 for review of the literature). Proportionately, the increase in PGE₂ was greatest in both species. Although prostanoid production by extravasated blood cells in the subarachnoid space undoubtedly accounted for some of the increase, this would not have explained the marked increase in PGE₂ observed. It was suggested on the basis of *in vitro* synthesis results that some of this might derive from increased production by cerebral arteries.

The objects of the present investigation were to determine: (1) whether PGE₂, F_{2α}, 6 oxo F_{1α} and TXB₂ diffuse outwards from arterial walls into a bathing fluid such as CSF; (2) how quickly an altered arterial production of these prostanoids is reflected in the bathing fluid; (3) whether the extra-arterial release of prostanoids is modified by ensheathing the artery with blood clot *in vivo* for 7 days, in an attempt to mimic events in subarachnoid haemorrhage.

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Received 19 December 1983.

Accepted 30 January 1984.

Materials and methods

Materials Standard prostaglandins (PG) E_2 , 6 oxo F $_{1\alpha}$, F $_{2\alpha}$ (tromethamine salt) and TXB $_2$ were gifts from Dr John Pike, the Upjohn Co., Kalamazoo, Michigan, USA; 3H PGE $_{2\alpha}$, 3H 6 oxo PGF $_{1\alpha}$, and 3H PGF $_{2\alpha}$ were from the Radiochemical Centre, Amersham. 3H TXB $_2$ was from New England Nuclear, Boston, Mass. USA. Antisera to PGE $_2$, TXB $_2$, and 6 oxo PGF $_{1\alpha}$ were from Institut Pasteur Production, Marnes-La Coquette, France. Antiserum to PGF $_{2\alpha}$ was a gift from Dr Keith Hillier, University of Southampton, England.⁴ Indomethacin and Bovine Serum Albumin (Cohn Fraction V Powder) were from Sigma Chemical Co., St. Louis, USA. 14C indomethacin (13.5 Ci/mol) was a gift from Merck, Sharp & Dohme. Krebs-Ringer-Bicarbonate buffer containing 1 mmol/l glutathione (KRB) was used in the perfusion studies (per litre: NaCl 7.24 g, KCl 0.372 g, KH $_2$ PO $_4$ 0.163 g, NaHCO $_3$ 2.184 g, glucose 1.8 g, MgSO $_4$ 7H $_2$ O 0.32 g, CaCl $_2$ 2H $_2$ O 0.184 g, glutathione 0.3072 g; pH adjusted to 7.4 after equilibrating with 95% O $_2$ /5% CO $_2$ for 15 min. 0.1 mol/l phosphate buffer pH 7.5 was used in the radioimmunoassays.

Methods

(1) **Basal perfusion studies** New Zealand white rabbits premedicated with droperidol 0.75 mg/kg im were anaesthetised with Althesin (0.9% Alphaxolone + 0.3% Alphadolone (1 ml/kg I/V) and heparinised. 2–3 cm of both common carotid arteries were removed quickly. Each artery was cannulated at both ends and submerged in 4 ml of Krebs-Ringer-Bicarbonate buffer pH 7.4 (KRB) gassed with 95% O $_2$ /5% CO $_2$ at 37° in an organ bath. Following a 10 min perfusion to wash out prostaglandins and free fatty acids, KRB containing 40 g/l bovine serum albumin at 37° was perfused continuously through the arterial lumen for 4 hours using a peristaltic pump, flow rate 1 ml min $^{-1}$, perfusing volume 4–4.5 ml. The perfusing and bathing buffers were collected and replaced by fresh buffer after each 60 min of perfusion. PGs and TXB $_2$ in the fluids were extracted and assayed by radioimmunoassay.⁴ Slices were taken from the ends (before perfusion) and the middle (after perfusion) of the arteries, fixed in formalin and examined histologically. Albumin was added to the internal perfusing fluid to mimic physiological circumstances more closely. Preliminary experiments had shown that inclusion of albumin in the buffer did not influence arterial prostaglandin production significantly.

(2) **Perfusion with indomethacin** 10 μ l of a 15 mg/ml solution of indomethacin in ethanol was added to 10 ml KRB containing 40 g/l albumin, stirring vigorously. Eight arteries were perfused under basal conditions for 60 min as above. Buffer containing indomethacin (15 μ g/ml) was perfused intra-arterially during the second 60 min perfusion period, the arteries being bathed externally with KRB. Perfusion with KRB + albumin (without indomethacin) was resumed for the 3rd and 4th hours. In one further experiment 0.3 μ g/ml of ^{14}C indomethacin (25 $\times$ 10 3 dpm) was perfused intra-arterially, together with 15 μ g/ml of the unlabelled compound. The labelled material recovered in the perfusates at the end of the 2nd, 3rd, and 4th hours of perfusion was measured by scintillation counting.

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(3) **Perfusion of arteries surrounded by blood clot for 7 days in vivo** In a preliminary experiment, blood simply injected around a carotid artery had been entirely resorbed at the end of one week, leaving no residual haematoma and normal arterial histology. Two techniques were used to maintain clot *in situ*: (i) Four rabbits were anaesthetised and the common carotid arteries exposed. Fresh blood was injected around one artery and enclosed in a loose sheath of polyvinyl chloride to hold the clot in apposition to the arterial wall. The animals recovered. Seven days later the arteries were removed and perfused *in vitro* under "basal" conditions. Arteries from seven further rabbits were studied which had been surrounded by a polyvinyl chloride sheath *without* blood clot for 7 days. (ii) In order to avoid the need for polyvinyl chloride sheaths to contain the clot, five rabbits were dosed with the antifibrinolytic agent tranexamic acid (TEA) (500 mg orally daily) for 10 days. On the third day, the carotid arteries were exposed, blood injected around one artery, the other acting as a control, and the neck closed. Both arteries were removed 7 days later and perfused. Sections of the arteries were examined histologically.

Results

PROSTAGLANDIN/TXB $_2$ RELEASED FROM ARTERIES PERFUSED UNDER BASAL CONDITIONS All the prostaglandins measured and TXB $_2$ were released into both the intraluminal perfusing fluid and the extra-arterial bathing buffer (table 1). Production of PGE $_{2\alpha}$, 6 oxo PGF $_{1\alpha}$, and TXB $_2$ was highest during the first hour but declined to lower levels during the succeeding 3 hours. Production of PGF $_2$ was maintained at a more-or-less constant level throughout perfusion, although a small increase was apparent by the fourth hour.

Intraluminally the prostacyclin metabolite 6 oxo PGF $_{1\alpha}$ was released in highest quantity (median 48.7 pg per mg wet wt of artery in the first 60 min), followed by PGE $_2$ and PGF $_{2\alpha}$ in much lower and roughly similar amounts, with a small contribution from TXB $_2$ (medians 7.1, 6.5, and 2.4 pg per mg wet wt respectively). Four arteries were examined histologically after 4 hours of perfusion, and all had lost around 80–95% of the endothelial cells. In two further arteries examined after 10 and 40 min of perfusion, the endothelium showed "occasional defects" and 25% loss respectively. Endothelial loss undoubtedly contributed to the decline of 6 oxo PGF $_{1\alpha}$ noted after the first hour. 6 oxo PGF $_{1\alpha}$ was also the most abundant prostanoid in the *extra-arterial* fluid (median 218.0 pg/mg wet wt of artery in the first 60 min) followed by PGE $_2$ (median 47.9 pg/mg wet wt) with smaller amounts of PGF $_{2\alpha}$ and TXB $_2$ (medians 20.0 and 11.5 pg/mg wet wt respectively). An endothelial origin for the prostacyclin metabolite found extra-arterially was unlikely: in a study in which 3H 6 oxo PGF $_{1\alpha}$ was perfused intra-arterially

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Table 1 Prostaglandin and thromboxane B₂ release from carotid arteries perfused under basal conditions

Collection period	No. of samples	Prostaglandins released (pg per mg wet wt of artery in 60 min)							
		PGF _{2α}		PGE ₂		6oxoPGF _{1α}		TXB ₂	
		Median	Range†	Median	Range	Median	Range	Median	Range
Intra-arterial									
1st hour	18	6.5	2.0-23.7	7.1	1.1-43.3	48.7	7.0-124.1	2.4	0.9-4.0
2nd	10	4.1	2.2-8.2	5.8	1.0-26.1	20.4	3.3-53.5	2.3	0.6-4.2
3rd	10	6.2	1.9-19.5	7.9	3.8-67.5	28.7	7.6-83.5	1.7*	0.7-9.2
4th	7	5.9	1.7-7.7	9.3	3.4-36.8	25.1	6.2-47.3	2.2	0.5-4.2
Extra-arterial									
1st hour	24	20.0	11.1-74.6	47.9	13.7-116.5	218.0	88.4-420.4	11.5	5.2-57.6
2nd	13	17.5	9.2-49.1	42.6	12.1-161.3	140.1	57.1-234.8	5.5	1.6-17.6
3rd	10	18.7	8.7-36.0	55.2	17.4-151.2	106.1	22.6-231.9	4.0	1.2-8.4
4th	7	16.0	7.3-30.6	58.1	21.9-137.0	83.7	22.0-138.0	2.7	1.0-5.5

*9 samples

†Observed range

Table 2 Effect of indomethacin perfusion on arterial release of prostaglandins and TXB₂

Collection period	No. of samples	Prostaglandins released (pg per mg wet wt of artery in 60 min)							
		PGF _{2α}		PGE ₂		6oxoPGF _{1α}		TXB ₂	
		Median	Range	Median	Range	Median	Range	Median	Range
Intra-arterial									
1st hour	8	5.5	2.0-23.7	5.0	1.1-25.2	44.2	21.0-124.1	1.54	1.2-2.9
2nd†	8	3.0	2.0-11.4	6.6	2.1-29.0	19.4	13.0-39.1	Insuff.	—
3rd	7	2.1	1.5-5.4*	3.4	1.4-17.9*	11.9	5.2-11.9*	„	—
4th	7	1.8	1.2-5.0*	2.4	1.4-16.2	11.3	4.0-28.5*	„	—
Extra-arterial									
1st hour	8	26.7	11.1-49.0	42.6	14.6-116.5	222.3	88.6-333.6	11.4	5.2-34.8
2nd	8	22.3	10.3-41.4	44.6	21.9-114.5	117.4	60.7-185.0	3.5	2.0-13.2
3rd	7	11.4	5.1-26.4	34.0	15.9-103.4	56.7	36.6-103.9	1.5	0.9-11.3
4th	7	10.5	3.6-21.1	20.7	12.6-107.6	59.6	15.3-86.0	1.4	0.7-4.8

*Significantly lower values ($p < 0.05$) than samples collected from normal arteries basally during the corresponding collection period (table 1) (Mann Whitney U test)†During the second hour indomethacin was perfused intraluminally: conc. 15 μ g per ml.

(two arteries), 3.0% and 1.9% of label was recovered from outside the artery in four hours, compared with 95.2% and 98.7% respectively from inside.

EFFECTS OF INDOMETHACIN PERFUSION

Eight arteries were perfused intraluminally during the second hour of study with indomethacin at a therapeutic concentration for man (15 μ g/ml). The objective was to manipulate prostanoid production within the arterial wall, and to determine how closely the change was reflected in the external bathing buffer. Indomethacin decreases prostaglandin and TXA₂ synthesis by inhibiting cyclo-oxygenase in the biosynthetic pathway. Because prostaglandin production by control arteries decreased progressively after the first hour (see above), results for indomethacin-perfused arteries must be compared with those of controls for the same collection period. Indomethacin caused a decrease in all intra- and

extra-arterial prostanoids, but this was significant only for intra-arterially released PGF_{2α}, PGE₂, and 6 oxo PGF_{1α} (table 2). When ¹⁴C indomethacin was infused with the unlabelled compound intra-arterially (one artery), 97.8% of the labelled material was recovered from intraluminal perfusates by the end of the fourth hour and only 0.7% extra-arterially. Thus very little indomethacin penetrated through the arterial wall.

IN VITRO PROSTAGLANDIN/TXB₂ RELEASE BY ARTERIES WHICH WERE ENSHEATHED BY BLOOD CLOT IN VIVO FOR 7 DAYS(i) Using a polyvinyl chloride sheath to hold blood clot *in situ*

This first method of maintaining clot adjacent to the arterial wall was employed in four animals. After 7 days the ensheathed arteries were removed and perfused *in vitro*. Prostaglandin production during the first hour was not increased in comparison to control

Table 3 Prostaglandin and TXB₂ release from arteries which were ensheathed with blood clot for 7 days in vivo (with a cuff)

Collection period	No. of samples	Prostaglandins released (pg per mg wet wt of artery in 60 min)							
		PGF _{2α}		PGE ₂		6oxoPGF _{1α}		TXB ₂	
		Median	Range	Median	Range	Median	Range	Median	Range
Intra-arterial									
1st hour	4	2.3	2.2-2.8	5.2	3.4-20.6	17.9	3.1-36.2	1.7	1.2-2.7
2nd	4	2.3	1.1-3.4	10.9	8.5-13.5	16.8	4.6-22.4	1.8	1.3-4.4
3rd	4	3.3	1.5-5.3	27.2	15.4-31.7	14.7	6.8-36.6	1.7	1.1-5.8
4th	4	6.1	3.8-9.7	62.2	46.7-188.3†	43.1	9.4-56.8	2.6	1.6-12.4
Extra-arterial									
1st hour	4	12.2	10.1-16.0	79.3	38.5-1444.8	163.1	20.9-288.6	22.6	14.9-28.2
2nd	4	11.2	5.3-21.7	141.8	54.4-285.0	86.5	27.2-220.5	12.7	6.2-39.3*
3rd	4	18.1	12.7-31.5	320.4	183.1-1097.1†	179.1	23.9-258.5	16.2	12.9-68.5†
4th	4	27.7	26.4-49.4*	524.9	244.4-987.5†	286.3	37.8-408.5	35.0	19.2-144.4†

* and †Significantly higher than samples collected from normal arteries during the corresponding collection periods:

*p < 0.05; †p < 0.01 (Mann Whitney U test)

Table 4 Prostaglandin and Thromboxane B₂ release from 5 carotid arteries ensheathed by blood clot for 7 days (TEA model) compared with release from arteries surrounded by polyvinyl chloride cuff+clot, or polyvinyl chloride cuff only

Collection period	Prostaglandins released (pg per wet wt of artery in 60 min)											
	PGF _{2α}			PGE ₂			6 oxo PGF _{1α}			TXB ₂		
	TEA + Clot*	†Cuff + Clot	Cuff only	TEA	Cuff + Clot	Cuff only	TEA	Cuff + Clot	Cuff only	TEA	Cuff + Clot	Cuff only
Intra-arterial												
1st hour	3.9 2.2-14.0	2.3	2.9	4.7 1.5-15.7	5.2	5.2	64.1 23.2-214.9	17.9	42.4	2.9 1.2-7.4	1.7	3.4
2nd	4.6 3.4-8.5	2.3	3.1	10.9 6.3-16.6	10.9	19.6	72.6 45.9-132.9	16.8	45.2	3.0 1.3-6.2	1.8	3.4
3rd	4.7 2.6-8.0	3.3	4.7	13.5 7.2-20.3	27.2	21.7	88.6 21.1-101.7	14.7	74.9	2.5 1.8-5.6	1.7	4.9
4th	6.7 3.2-9.3	6.1	4.9	24.1 7.5-33.9	62.2	31.6	105.7 39.2-212.0	43.1	73.7	2.5 1.3-6.8	2.6	2.9
Extra-arterial												
1st hour	24.4 16.3-75.3	12.2	24.2	52.3 21.0-169.4	79.3	140.5	470.3 270.4-1656.9	163.1	335.0	47.1 22.3-148.8	22.6	41.2
2nd	22.6 14.2-69.3	11.2	28.1	87.7 34.5-214.9	141.8	417.3	285.1 214.2-1140.2	86.5	430.8	26.9 16.7-130.4	12.7	66.8
3rd	23.3 14.1-77.1	18.1	33.9	111.5 50.8-320.0	320.4	430.3	280.3 235.3-1072.7	179.1	409.2	24.5 14.7-150.9	16.2	75.0
4th	30.0 15.7-55.1	27.7	37.8	174.2 63.8-286.3	524.9	647.3	252.1 190.7-1430.6	286.3	426.1	25.7 13.5-88.2	35.0	45.2

*Median and range for 5 arteries

†Median for 4 arteries (cuff + clot) or 7 arteries (cuff only)

arteries (tables 3 and 1). Whereas prostaglandin production by the controls decreased during perfusion (PGF_{2α}, 6 oxo PGF_{1α} and TXB₂) or increased only marginally (PGE₂), a progressive increase of all prostaglandins was observed for the arteries with clot, both intra- and extra-arterially. The most dramatic increase was in PGE₂ production: by the 4th hour the median concentration intra-luminally was twelve times higher than in the first, and PGE₂ production was significantly greater than that of control arteries (p < 0.01 Mann Whitney U test). A similar progressive increase occurred in PGE₂ release extra-arterially, to values significantly higher than those of control arteries (p < 0.01). By the 4th

hour the median value for PGE₂ showed a nine-fold increase above that for normal arteries. Extra-arterial release of TXB₂ and of PGF_{2α} was also significantly increased above normal (p < 0.01 and p < 0.05 respectively) but the production achieved was considerably less than that for PGE₂ (4th Hour medians 35.0 (TXB₂), 27.7 (PGF_{2α}) and 524.9 (PGE₂) pg per mg wet wt of artery h⁻¹). Production of 6 oxo PGF_{1α} increased during perfusion but not significantly.

When examined histologically, a marked inflammatory response was observed in the periaortic adventitia of arteries surrounded by clot and polyvinyl chloride sheath. Numerous macrophages were pres-

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ent together with some red blood cells. It was probable that a large part of the inflammatory response was attributable to the presence of the sheath rather than to trapped blood clot. Seven arteries were studied *without* entrapped blood clot. There was variation in the individual arterial responses, but overall prostaglandin production increased during perfusion *in vitro*, and the responses were not significantly different from those of arteries with polyvinyl chloride sheaths and clot. Again the most dramatic increase was in PGE₂ production, and there was a significant increase in TXB₂ ($p < 0.01$). The arteries were markedly inflamed.

(ii) Using antifibrinolytic treatment (TEA) to maintain the clot *in situ*

An alternative method of maintaining clot close to the arteries for 7 days was sought which avoided the introduction of foreign material. In some preliminary experiments (to be reported in detail elsewhere) this proved to be possible by treating the rabbits with the anti-fibrinolytic agent TEA (see methods). At the time of sacrifice 7 days after injecting blood into the neck, the carotid artery was thickened. Microscopically an inflammatory response was observed in the adventitia with macrophages, but this was less gross than in the case of polyvinyl chloride-ensheathed arteries. Release of prostanoids increased throughout the 4 hours of *in vitro* perfusion, and production was significantly increased in comparison to the paired control arteries. The response was less exaggerated than that observed with the cuffed arteries (table 4).

Discussion

This study has shown that prostaglandins and thromboxane do diffuse out from the rabbit common carotid arteries into extra-arterial bathing fluid under basal conditions of perfusion *in vitro*. 6 oxo PGF_{1 α} was the most abundant, followed by PGE₂. Since hardly any ³H 6 oxo PGF_{1 α} perfused intraluminally diffused through the arterial wall, it can be concluded that the large quantities of this metabolite found extra-arterially derived from prostacyclin production in the outer layers of the artery, and not from the endothelial lining. Moncada *et al*⁵ demonstrated that although the ability to produce prostacyclin is greatest at the intimal surface of the rabbit aorta, the smooth muscle layer also produces a substantial amount. Medial smooth muscle cells in tissue culture produce significant quantities of prostacyclin⁷ together with PGE₂ and PGF_{2 α} . Thromboxane synthesis has been reported for human umbilical artery⁸ and rabbit pulmonary artery.⁹ The extra-arterial PGs detectable in our studies probably

derive mainly from cells of the outer layers of the arterial wall, and in the light of current knowledge are likely to have been produced mostly by cells of the tunica media.

When indomethacin at a therapeutic concentration was perfused through arteries for 60 minutes, the extra-arterial release of all the prostanoids during the following 2 hours fell below that of control arteries, although the fall was not statistically significant. A more striking decrease was observed in the intra-arterial levels. The rabbit common carotid artery has a thick wall, and it is probable that the concentrations of indomethacin achieved in the outer layers of the arteries were too low to effect significant inhibition of prostanoid synthesis there. We found that minimal amounts of (radiolabelled) indomethacin permeated right through the wall into the extra-arterial fluid. These experiments demonstrate that changes in prostaglandin production in the outer arterial layers will be reflected in the prostanoid concentrations of a bathing fluid.

A prime reason for embarking on this study was to discover whether prostanoid production and release by arteries chronically enveloped by blood clot is altered. Our initial findings with the polyvinyl chloride model were exciting in that they demonstrated a progressive increase in prostaglandin production throughout 4 hours of perfusion *in vitro*, with a very exaggerated increase in PGE₂. However in studies using a polyvinyl chloride cuff only without entrapped blood, very similar findings emerged, and it seems that much of the increased production must be attributed to the presence of the cuff around the artery *in vivo*. This induced a marked inflammatory response whether clot was present or absent. This criticism cannot be lodged against our other model using TEA. Although our observations are still preliminary, they show that prostaglandin production by the arteries was increased in much the same way as in the polyvinyl chloride model, but to a less marked degree. The arteries were inflamed, and further experiments with electron-microscopy are underway.

It seems likely that the exaggerated response must reflect in some way the inflammatory reaction in the arterial wall. Remarkably similar observations have been reported for a perfused hydronephrotic kidney model in the rabbit. Nishikawa *et al*¹⁰ found that unilateral ureteric obstruction for 3 days resulted in a marked facilitation of PGE₂ release from an isolated perfused rabbit kidney basally, and in response to the vasoactive peptides bradykinin and angiotensin II. In addition, the perfused hydronephrotic kidney, unlike the normal kidney, has been shown to release TXA₂; TX biosynthesis has been "unmasked" in other models of renal damage in the

rabbit (for review see¹¹). Recently, Okegawa *et al*¹¹ confirmed an enhanced release of PGE₂ and TXA₂ by the isolated perfused rabbit hydronephrotic kidney in response to bradykinin, and observed a sustained release in response to endotoxin. The responses were reduced 3–10 days after reversing the ureteric obstruction. Histologically, proliferation of fibroblast-like cells was observed in the cortex of the kidney together with mononuclear cells. With relief of obstruction, the monocytes decreased but not the fibroblasts. This group therefore concluded that the mononuclear cells had a critical involvement in the exaggerated arachidonic acid metabolism, and queried whether the close contact which they observed between mononuclear cells and the processes of cortical fibroblasts might have important metabolic consequences.

There are at least three ways in which inflammatory cell infiltration, particularly of monocytes/macrophages, could account for the observed increases in PGE₂ and TXB₂. Macrophages themselves might be the source. These cells synthesise PGE₂, TXA₂ and prostacyclin,^{12–14} and PGE₂ is a major arachidonic acid metabolite of activated macrophages.¹⁵ In our own preliminary studies (unpublished) incubated monocytes isolated from normal whole blood produced PGE₂ and TXB₂ in roughly similar amount with some, but much less, PGF_{2α} and 6 oxo PGF_{1α}. Activation of macrophages by endotoxin¹¹ or by “handling” of the inflamed tissue *in vitro* (present experiments) might have stimulated increased PGE₂ and TXB₂ release. There are two points against this being the whole mechanism for the changes observed here however: first the levels of PGE₂ greatly exceeded those of TXA₂. Secondly a significant increase in *intraluminal* PGE₂ but not TXB₂ occurred, and it is difficult to relate this finding to macrophage production directly.

A second possibility is that lysosomal proteases or perhaps highly reactive oxygen-free radicals released from activated macrophages¹⁶ may have damaged the membranes of adjacent arterial smooth muscle cells. Membrane damage is known to increase arachidonic acid liberation from membrane phospholipids and, by increasing substrate availability, to increase prostaglandin production. This explanation could readily account for exaggerated release of PGE₂ and TXB₂ which are both products of normal medial smooth muscle cells.

The third possibility is that upon activation, macrophages release a factor which stimulates PGE₂ production by adjacent connective tissue cells. Recent studies support the concept that products of lymphocytes and monocytes play a role in connective tissue cell activation. There have been several reports that conditioned media obtained from

adherent monocytes stimulate fibroblast and/or synovial cell production of PGE₂ markedly (50–200 fold).^{11,17} Nolan *et al*¹⁸ observed that a “proteinaceous” factor in medium from activated macrophages stimulated PGE₂ production by chondrocytes, and (perhaps relevant to the present observations), that stimulation was detectable after a 4 hour exposure to the factor and was maximal at around 8 hours.

Do the observations from this study have any relevance to the cerebrovascular disturbances after subarachnoid haemorrhage? Because of practical considerations it was necessary to work with a large conducting artery, and it would be unwise to speculate too far until it has been shown that arteries from the base of the brain show similar behaviour in our experiments. It does seem however that prostaglandins produced in the outer arterial layers could contribute to the “pool” of these agents in the CSF. From our own observations (unpublished), on CSF collected from patients with a variety of intracranial pathology, there seems little doubt that CSF circulating in the subarachnoid space has a higher prostaglandin content than that secreted into the ventricles. Blood vessels crossing the subarachnoid space could well be one source of the “added” prostanooids, and the results of the indomethacin experiments indicate that changes in vascular production are likely to be reflected in altered CSF levels. This fits with some preliminary observations in dogs⁴ in which a marked fall in prostaglandins in cisternal CSF occurred within 30 min of intravenous injection of indomethacin. In view of the speed of the response, a decrease in vascular production in relatively thin walled vessels seemed likely.

Against this background, our observation of a disproportionate increase in PGE₂ in CSF after subarachnoid haemorrhage could well reflect increased production by cerebral arteries as we have suggested. Furthermore the experiments described here indicate that such an increase might result from an inflammatory reaction in the walls of those arteries enveloped by blood clot. The timing of vasospasm in man parallels closely the clearance of red cells from CSF, mainly by lysis with subsequent phagocytosis.¹⁹ Post-mortem studies in man have revealed that where death occurs at least three weeks after subarachnoid haemorrhage, there may be subendothelial cellular infiltration with lymphocytes and macrophages followed by subendothelial fibrosis located in the segment of arteries formerly shown to be in spasm on angiography. Where death occurs earlier, there may be evidence only of some inflammatory changes within the adventitia and possible some necrosis within the tunica media. Controversy has arisen over whether such structural changes are reproducible in animals or not. A consensus is

emerging that simple cisternal instillation of blood does not produce histological change at least up to one week, unlike rupture of an artery²⁰⁻²¹ except for the presence of macrophages and red cells in the adventitia.

It has been proposed²² that the increased thromboxane production of the rabbit hydronephrotic kidney (see above) contributes to renal vasoconstriction and the reduced glomerular filtration rate that occurs with hydronephrosis. Perhaps an exaggerated release of the cerebral vasoconstrictors PGE₂ and TXA₂ in and around the walls of large arteries ensheathed in blood coagulum similarly contribute to the vasospasm after subarachnoid haemorrhage.

This work was supported by Action Research. Our preliminary findings have been presented to the Medical Research Society, July 1983 (*Clinical Science* 1983;65:68p).

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