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AETIOLOGY OF CEREBRAL ARTERIAL SPASM FOLLOWING SUBARACHNOID HAEMORRHAGE: EVIDENCE AGAINST A MAJOR INVOLVEMENT OF 5-HYDROXY-TRYPTAMINE IN THE PRODUCTION OF ACUTE SPASM

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1 Acute cerebral arterial spasm was produced in baboons by intracisternal injection of blood or 5-hydroxytryptamine (5-HT). Blood induced spasm was not antagonized by the potent 5-HT antagonist BW501C67, but 5-HT induced cerebral arterial spasm was largely antagonized.

2 Blood platelets from baboons were isolated and aggregated *in vitro* with adenosine diphosphate (ADP) and 5-HT. 5-HT induced platelet aggregation was abolished by BW501C67 *in vitro*. It was also abolished after intracisternal administration of the drug. Thus it is concluded that BW501C67 antagonized 5-HT induced cerebral arterial spasm.

3 BW501C67 also failed to antagonize contractions produced by human cerebrospinal fluid, serum and platelet extracts on the isolated human basilar artery, the isolated rat fundus and aorta.

4 These results suggest that the role of 5-HT in the aetiology of human cerebral spasm may be minimal.

Introduction

When a cerebral arterial aneurysm ruptures blood is lost into the subarachnoid space. Following haemorrhage, there is often prolonged constriction of the cerebral arteries. This cerebral arterial spasm (CAS) is associated with a high morbidity and mortality (Adams, Loach & O'Laoire, 1976).

Although there is no clinical evidence it is a commonly held view that the CAS following subarachnoid haemorrhage can be divided into early and late phases (Wilkins, 1976). The former is considered to develop within minutes and to last for 1–2 days; the latter develops later and may last for several weeks (for references see Boullin, Adams, Mohan, Green, Hunt, Du Boulay & Rogers, 1977). On the other hand such biphasic spasm has been produced in experimental animals (Brawley, Strandness & Kelly, 1968; Wilkins 1976).

In any event, the cause of CAS is not established but a variety of chemicals possess potent constrictor activity upon cerebral arteries, and have been proposed as primary or secondary causative agents. These include 5-HT (Allen, Gold, Chou & French, 1974; Allen, Henderson, Chou & French, 1974a, b) catecholamines such as dopamine (Wurtman, Lavyne & Zervas, 1974), prostaglandins and similar substances (La Torre, Patrono, Fortuna & Grossi-Belloni, 1974).

In addition there is an unidentified vasoconstrictor substance in the cerebrospinal fluid (CSF) of CAS patients (Boullin, Mohan & Grahame-Smith, 1976; Allen, Gross, French & Chou, 1976; Boullin & Mohan, 1977).

This paper is concerned with the spasm which occurs in baboons following intracisternal injection of autologous blood to produce a rapidly developing CAS (Boullin *et al.*, 1977). We present evidence here against 5-HT playing a major causative role in the generation of this aspect of CAS. We show that in experiments using the baboon model for human CAS, a potent 5-HT antagonist (BW501C67 (α-anilino-*N*-2-chlorphenoxypropyl-acetamidine HCl monohydrate), Wellcome Laboratories, Beckenham, Kent) fails to antagonize spasm produced by blood, whereas 5-HT induced spasm is largely antagonized.

This absence of antagonism of CAS occurred at a time when baboon platelet aggregation responses to 5-

HT were completely absent. Further evidence for the specificity of action of BW501C67 were obtained in experiments with human isolated basilar arteries and rat isolated extracerebral tissues.

Methods

Anaesthetized baboons

Baboons (8–11 kg) were anaesthetized with 0.5% halothane in oxygen after tranquilization with phencyclidine, 5–10 mg/kg. Anaesthesia having been established, the tidal volume of the gas was adjusted in the Oxford ideal ventilator (C.F. Palmer Ltd). The tidal volume was kept constant. The temperature of the baboons was maintained at 35–37°C throughout the experiments; $PaCO_2$, blood pH, PO_2 arterial B.P. and haematocrit were monitored. One of the common carotid arteries was catheterized percutaneously from the femoral artery using a tapered polyethylene PE 60 catheter with a single end hole. The above parameters were monitored and sufficient time was left (0.5–1 h) for stabilization. Less than 2 ml of contrast medium (Urografin 60%; a mixture of sodium diatrizoate and meglumine diatrizoate) was used during catheterization to visualize the position of the catheter tip. The BP was in the range 90–110 mm; pH 7.2–7.4; $PaCO_2$ 30–45. In any given animal the $PaCO_2$ did not vary by more than ± 5 mm Hg; PO_2 was in the range 300–600 mm Hg. Patency of the catheter was maintained with heparinized Ringer lactate solution BP, pH 7.4 by a perfusion pump delivering 1.5 ml/min.

Angiograms were obtained at times appropriate to the experimental protocol but never less than 30 min apart to avoid interference with the transient vasodilator action of Urografin 60% (Du Boulay, Kendall, Symon, Pasztor, Crockard, Belloni & Sage, 1975). For angiography 5 ml Urografin 60% was injected at a standard pressure (400 mm Hg) through the carotid catheter using a Cordis injector. Eight films were automatically exposed by the same switch that fired the injector at a speed of 3/s in a Puck 25×30 cm serial changer (Siemens). Two times magnification angiography was used employing a Siemens Bi 125/3/50R X-ray tube focussed to 0.2×0.2 mm and driven by a Tridoros 53 automatic six pulse phase generator (Siemens Elema A.B., Solna, Sweden).

When the experiment had been completed all angiograms were numbered, randomized and the calibres of vessels were measured blind as previously described (Du Boulay & Symon, 1971).

The catheter used for angiography was also employed for recording blood pressure and heart rate using a Statham BB23 transducer coupled to a Devices M2 polygraph. Arterial blood samples were obtained for determination of haematocrit, platelet count (Coulter Counter), arterial pH and $PaCO_2$ using the capillary pH electrode (Siggaard Andersen & Engel, 1960) and the Severinghaus-Clark electrode assembly (Severinghaus & Bradley, 1958). Blood or 5-HT and other drugs were administered into the subarachnoid space via a needle or catheter introduced into the *cisterna magna*.

Isolated tissues

Human basilar artery: This was obtained at autopsy 12 h to 5 days after death as described by Boullin *et al.* (1976).

Rat aorta and stomach fundus: Sprague-Dawley male rats (250–300 g) were killed by decapitation and the aorta removed and dissected into a strip (Furchgott & Bhadrakom, 1953). The stomach fundus strip was prepared according to Vane (1957).

Saline solutions

The preparations were set up in a 10 or 13 ml isolated organ bath containing Krebs solution (mmol/l: NaCl 118.2; KCl 4.7; $CaCl_26H_2O$ 2.52; $MgSO_4.7H_2O$ 1.18; KH_2PO_4 1.18; $NaHCO_3$ 25.0; glucose 11.1) at 37°C. The solution was gassed with 5% carbon dioxide in oxygen. The pH was 7.4.

Drugs

5-HT, noradrenaline (NA), adrenaline, dopamine were obtained from Sigma Chemical Company, Kingston, Surrey. BW501C67 was kindly donated by Wellcome Research Laboratories Ltd, Beckenham, Kent. The structural formula and 5-HT antagonistic activity of this compound are described by Mawson & Whittington (1970) and Fraser (1970).

All drugs were dissolved in ascorbic acid (1 mg/ml). Concentrations are expressed in terms of molarities of the base and represent the concentration of drug present in the organ bath. The volumes of drugs added varied from 10–250 µl into a 10 ml organ bath.

Recording of isolated tissue responses

These were recorded electrically using the isotonic transducer system of Boullin *et al.* (1976) without further modification.

Platelet aggregation

Blood was withdrawn from baboon femoral arteries into 3.8% (w/v) sodium citrate. Platelet rich plasma (PRP) was prepared, and platelet aggregation induced with 5-HT, adenosine diphosphate (ADP) as described by Boullin, Green & Price (1972) and Boullin & Grimes (1976).

Platelet extracts

PRP was prepared and 10 ml aliquots were centrifuged at 15,000 g/15 min at 2°C in a refrigerated centrifuge to prepare a platelet pellet. The supernatant plasma solution was discarded. The pellet was then disrupted in 1.0 ml 0.9% NaCl at 2°C by ultrasonic impulses as described by Starling, Boullin, Grahame-Smith, Adams & Gye (1975). The sonicate was then centrifuged at 100,000 *g*/60 min in a Beckman L3-50 ultracentrifuge. The resulting pellet was redissolved in 1 ml 0.9% NaCl. Aliquots of this fraction (resuspended platelet extract) and the supernatant solution (supernatant from platelet extract) were tested as described in **Results**.

Cerebrospinal fluid (CSF)

This was obtained from subarachnoid haemorrhage (SAH) patients prior to surgery for clipping or cyanoacrylate wrapping of ruptured berryaneurysms. The subjects were divided into two groups on the basis of evidence for preoperative spasm obtained with angiograms made within 4–10 h of CSF collection: CSF from patients with preoperative spasm was termed VSCSF. CSF from similar patients with no angiographic evidence of spasm was termed NVSCSF. We also obtained 'normal' CSF from other subjects undergoing myelography for suspected prolapsed intravertebral disc. The effects of VSCSF and NVSCSF on the various isolated preparations were compared with responses to normal CSF obtained as described above. CSF was frozen at −20°C immediately after collection and was subsequently thawed for testing on the isolated tissues. In some instances the freezing, thawing and testing was repeated several times (see **Results**).

Further experimental details are given in earlier publications (Starling *et al.*, 1975; Boullin *et al.*, 1976).

Serum

Blood was collected from volunteer laboratory personnel of both sexes (aged 18–46 years) and allowed to clot. The serum fluid was removed and either used immediately or kept at −20°C until required.

Number of experiments

The results were obtained from sixteen experiments with eleven baboons; five animals were used twice. The interval between experiments in these cases was not less than 4 weeks, and BW501C67 was always administered in the second experiment. The data shown in the results are based on a single experiment, except where stated.

The observations described for isolated tissues are based upon results obtained in three to six experiments.

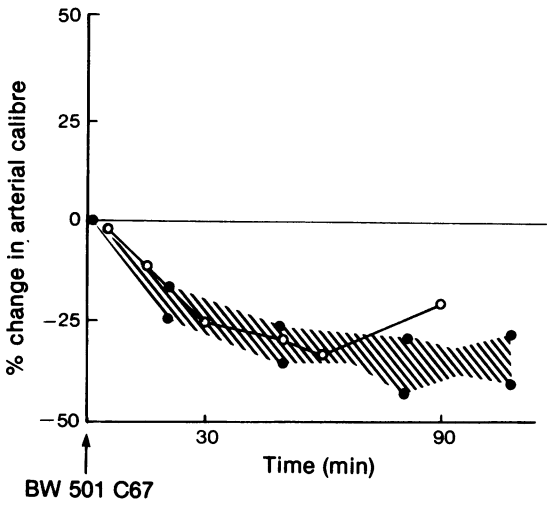

Figure 1 Failure of BW501C67 to antagonize blood induced cerebral arterial spasm (CAS) in baboon.

Diameter of major cerebral arteries (intracranial internal carotid, middle anterior cerebral and pericallosal arteries) was measured as described in **Methods**. The changes in arterial calibre are plotted as % change (ordinate) in comparison with basal observations (not shown) obtained 10 min prior to beginning the experiments.

O Shows the time course of CAS after intracisternal injection of 2 ml autologous blood in six animals. The values are the mean observations with the above cerebral arteries.

The shaded area represents the range of calibre of the cerebral vessels in a single experiment. BW501C67 2 μmol/kg was injected at zero time.

Results

Baboon: spasm induced by intracisternal blood

Series of eight angiograms were made at 30 min intervals following completion of catheterization when the $PaCO_2$ had been stabilized (see **Methods**). Approximately 10 min after the first angiogram 2 ml arterial blood was withdrawn from the common carotid artery through the arterial catheter. CSF (2 ml) was slowly removed over 2 min through the cannula inserted into the *cisterna magna*. This was then replaced by the 2 ml arterial blood slowly injected over a similar period.

Development of CAS after blood

In three experiments following blood injection CAS developed involving the internal carotid, middle and anterior cerebral arteries. As shown in Figure 1, CAS appeared within 15 min reaching a peak after about

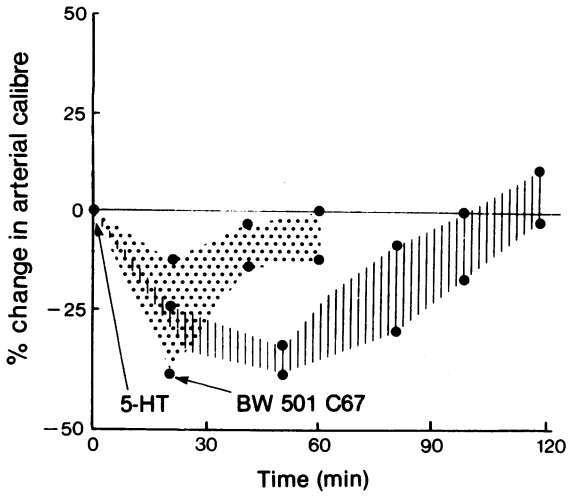

Figure 2 Reversal of 5-HT induced cerebral arterial spasm in the baboon by BW501C67.

Diameter of major cerebral vessels were measured (see **Figure 1** and **Methods**) and plotted as % change (ordinate) in comparison with basal observations (not shown) obtained at zero time.

Drugs were administered as indicated at the arrows.

|||| shows the results obtained when 5-HT 100 μg/kg was injected intracisternally.

«« shows the results obtained in a second experiment where 6.5 mol/kg BW501C67 was given after 5-HT (5-HT was given in both experiments).

|||| and «« give the range of changes of the diameter of the major cerebral vessels (see **Figure 1**) observed during the course of the experiments.

60 min and showing a decline after 90 min (see also Symon, Du Boulay, Ackerman, Dorsch & Shah, 1973; Boullin *et al.*, 1977).

Effect of BW501C67 on blood induced CAS

Figure 1 also illustrates the effects of intracisternal injection of 2 μmol/kg BW501C67 10 min after blood. This drug failed to influence the time course of the development of CAS in the major cerebral vessels. The result was obtained in three experiments.

Development of CAS after 5-HT

When a similar experimental protocol was used with four baboons, except that CAS was induced by intracisternal injection of 100–500 μg/kg (247–1235 nmol/kg) 5-HT in 2 ml autologous CSF or saline, the time course and duration were similar to that seen with blood as shown in Figure 2 which should be compared to Figure 1.

Effect of BW501C67 in 5-HT induced CAS

When the injection of intracisternal 5-HT was followed 15 min later by intracisternal 6.5 μmol/kg BW501C67, the 5-HT induced CAS was rapidly reversed (Figure 2).

The above data show that BW501C67 effectively antagonized 5-HT induced CAS, yet failed to block CAS induced by blood. Before ascribing the antagonism of 5-HT induced CAS to a specific blockade of 5-HT receptors we thought it necessary to see the effects of BW501C67 in two different experimental situations. First, we observed the responses of baboon platelets to 5-HT and adenosine diphosphate (ADP) after BW501C67 was added to baboon platelet rich plasma (PRP) *in vitro*. Secondly, we examined the responses of the platelets to 5-HT and ADP removed from the circulation before and after intracisternal injection of BW501C67. If 5-HT responses of the platelets were blocked under the latter conditions then it would be reasonable to assume that 5-HT receptors in the cerebral blood vessels would also be blocked. BW501C67 is a potent and specific inhibitor of 5-HT induced human platelet aggregation (Boullin & Glenton, 1978).

Platelet aggregation

First, baboon platelet aggregation was investigated in normal platelets removed from the circulation prior to administration of blood or any drug. The upper record of Figure 3 shows both 5-HT and ADP induced aggregation. Then the middle record shows that when BW501C67 is added to baboon PRP (still taken prior to intracisternal administration of BW501C67), the antagonist completely blocks the response to 5-HT, while that produced by ADP remains undiminished. This demonstrates the relative specificity of BW501C67 in this preparation.

Finally, BW501C67 was administered intracisternally and 20 min later platelet aggregation responses to 5-HT-ADP were reassessed. The lower record of Figure 3 shows the results obtained with both 5-HT and ADP aggregation. Responses of both aggregation agents were affected after intracisternal administration of 2 μmol/kg BW501C67. The 5-HT response is abolished while that to ADP is diminished. This type of result was observed three times, although the doses of 5-HT and ADP required to induce aggregation varied between 10 and 80 μmol/l.

Regarding the diminished ADP response after BW501C67, we found that such diminution did occur during the course of experiments involving serial angiography without giving BW501C67. The ADP response in Figure 3 shows no greater diminution in size than that normally seen in our experiments. Consequently, we concluded that as BW501C67 abolished 5-HT induced platelet aggregation after intracisternal administration, the antagonism of 5-HT

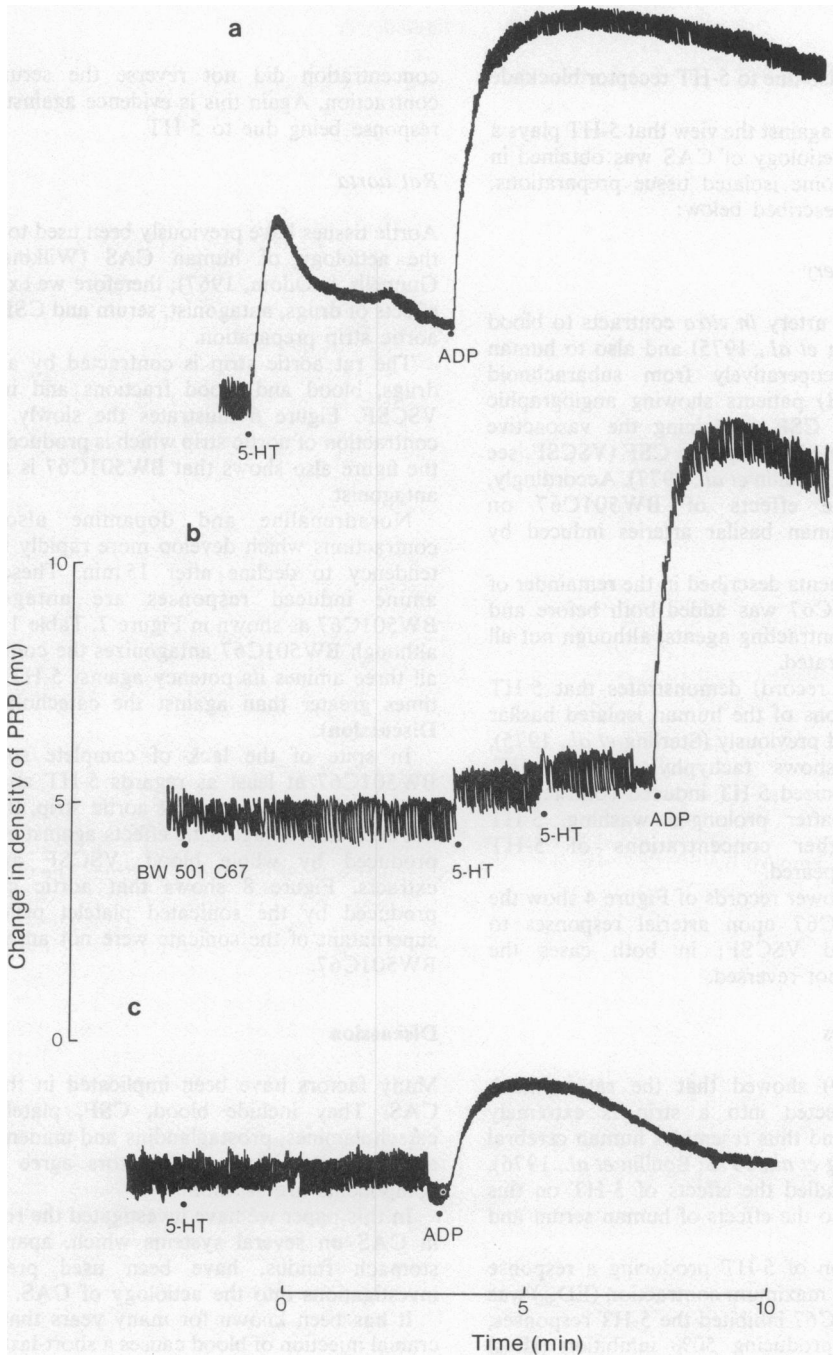

Figure 3 Effect of intracisternal injection of BW501C67 and BW501C67 added to platelet rich plasma (PRP) on 5-HT and ADP induced baboon platelet aggregation.

Records show platelet aggregation responses as change in density of PRP (mV, ordinate) in relation to time (min, abscissa).

(a) Control responses to 5-HT (50 μmol/l) and ADP (50 μmol/l). Both doses were added to the same sample of PRP.

(b) Same experimental conditions; BW501C67 (0.2 μmol/l) was added to PRP 5 min prior to other drugs. Note that only the 5-HT response is abolished.

(c) Responses 20 min after BW501C67 (2 μmol/kg) given intracisternally. Note abolition of 5-HT response; ADP response shows no greater diminution than that seen in the absence of BW501C67 administration (see text).

induced CAS was also due to 5-HT receptor blockade (see **Discussion**).

Further evidence against the view that 5-HT plays a major role in the aetiology of CAS was obtained in experiments with some isolated tissue preparations. These results are described below:

Human basilar artery

The human basilar artery *in vitro* contracts to blood and serum (Starling *et al.*, 1975) and also to human CSF obtained preoperatively from subarachnoid haemorrhage (SAH) patients showing angiographic evidence of CAS. CSF containing the vasoactive factor has been termed vasospastic CSF (VSCSF, see Boullin *et al.*, 1976; Boullin *et al.*, 1977). Accordingly, we examined the effects of BW501C67 on contractions of human basilar arteries induced by VSCSF and serum.

In all the experiments described in the remainder of this paper, BW501C67 was added both before and after the various contracting agents, although not all responses are illustrated.

Figure 4 (upper record) demonstrates that 5-HT produced contractions of the human isolated basilar artery. As described previously (Starling *et al.*, 1975), this preparation shows tachyphylaxis to 5-HT. BW501C67 antagonized 5-HT induced contractions, but subsequently after prolonged washing, 5-HT responses to higher concentrations of 5-HT (5–50 μmol/l) reappeared.

The middle and lower records of Figure 4 show the effects of BW501C67 upon arterial responses to human serum and VSCSF; in both cases the contractions were not reversed.

Rat stomach fundus

Vane (1957, 1969) showed that the rat stomach fundus when dissected into a strip is extremely sensitive to 5-HT and thus resembles human cerebral arteries (see Starling *et al.*, 1975; Boullin *et al.*, 1976). Accordingly we studied the effects of 5-HT on this preparation and also the effects of human serum and CSF.

The concentration of 5-HT producing a response equal to 50% of the maximum contraction (ED_{50}) was 0.1 μmol/l. BW501C67 inhibited the 5-HT responses, the concentration producing 50% inhibition (ID_{50}) being 0.8 nmol/l. The ID_{50} for inhibition of 5-HT induced human platelet aggregation is 34 nmol/l (Boullin & Glenton, 1978). Thus BW501C67 is a potent inhibitor of 5-HT actions on several cellular systems.

The effects of BW501C67 on a fundus contraction produced by 200 μl fresh human serum are shown in Figure 5; the rapid contraction which followed was partly but only transiently antagonized and a higher concentration did not reverse the serum induced contraction. Again this is evidence against the serum response being due to 5-HT.

Rat aorta

Aortic tissues have previously been used to investigate the aetiology of human CAS (Wilkins, Wilkins, Gunnells & Odom, 1967); therefore we examined the effects of drugs, antagonist, serum and CSF on the rat aortic strip preparation.

The rat aortic strip is contracted by a variety of drugs, blood and blood fractions and normal and VSCSF. Figure 6 illustrates the slowly developing contraction of aortic strip which is produced by 5-HT; the figure also shows that BW501C67 is an effective antagonist.

Noradrenaline and dopamine also produce contractions which develop more rapidly but show a tendency to decline after 15 min. These catecholamine induced responses are antagonized by BW501C67 as shown in Figure 7. Table 1 shows that although BW501C67 antagonizes the contractions of all three amines its potency against 5-HT is 50–233 times greater than against the catecholamines (see **Discussion**).

In spite of the lack of complete specificity of BW501C67 at least as regards 5-HT and catecholamine responses on the rat aortic strip, the drug did not exert any antagonistic effects against contractions produced by whole blood, VSCSF and platelet extracts. Figure 8 shows that aortic contractions produced by the sonicated platelet pellet and the supernatant of the sonicate were not antagonized by BW501C67.

Discussion

Many factors have been implicated in the cause of CAS. They include blood, CSF, platelets, 5-HT, catecholamines, prostaglandins and unidentified vasoactive agents. Most investigators agree that blood components are essential.

In this paper we have investigated the role of 5-HT in CAS on several systems which, apart from the stomach fundus, have been used previously in investigations into the aetiology of CAS.

It has been known for many years that the intracranial injection of blood causes a short-lasting spasm, and it is indeed remarkable in the baboon that the extent and time course of experimental CAS induced with intracisternal blood is so faithfully mimicked by 5-HT as shown in Figure 1. This might well have led to the erroneous conclusion that 5-HT was the cause of the blood induced CAS but for the dramatic effects of the 5-HT antagonist BW501C67 in reversing the 5-HT induced spasm to the extent of about 65% (overall change in magnitude and duration; calculated from

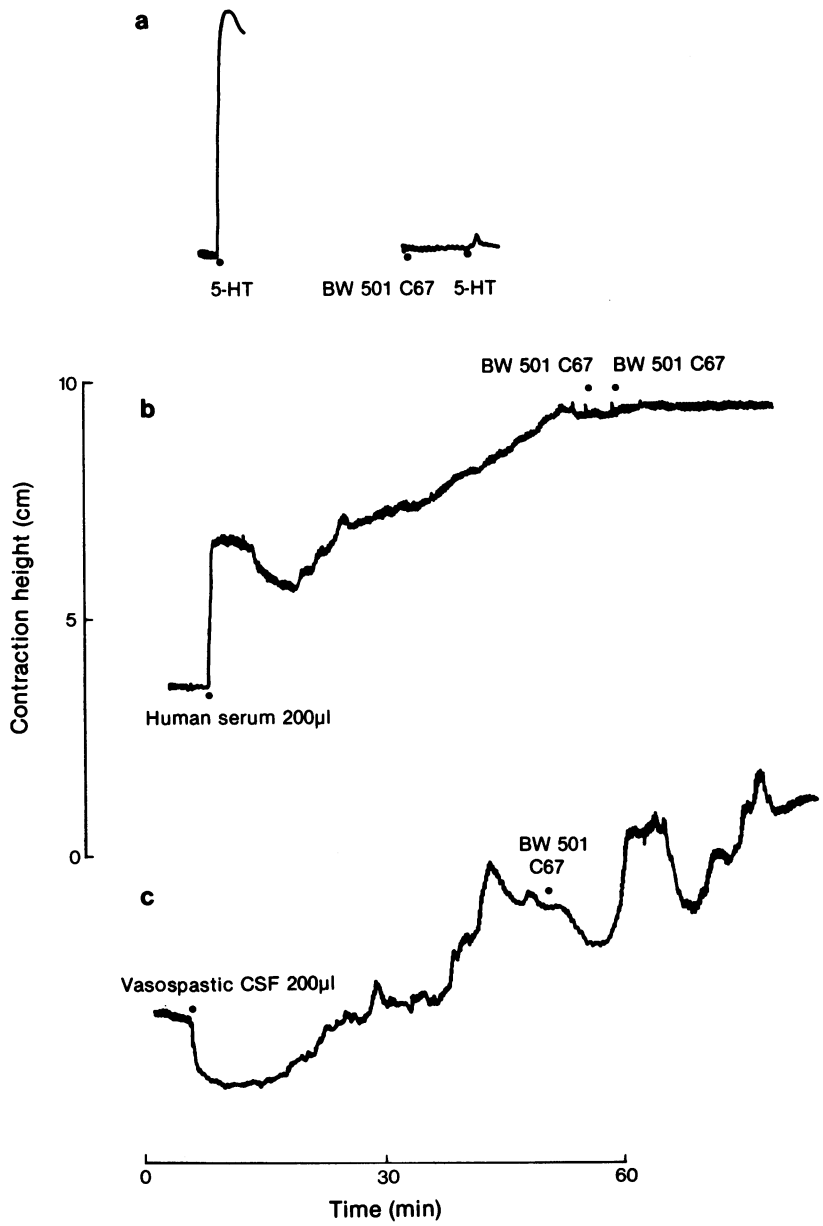

Figure 4 Effects of BW501C67 on 5-HT human serum and vasospastic CSF-induced contractions of the human isolated basilar artery.

Change in length of arterial smooth muscle (contraction height, cm ordinate) is plotted against time (min, abscissa). Isotonic record (see **Methods**).

(a) Contraction produced by 5-HT (0.1 µmol/l) (left); complete inhibition of 5-HT contraction when BW501C67 (0.1 µmol/l) was administered just prior to 5-HT (right).

(b) Human serum (200 µl) contracted the artery; two doses of BW501C67 (1 µmol/l or 10 µmol/l) had no antagonistic effect.

(c) Contraction of basilar artery produced by 200 µl lyophilized vasospastic CSF (see Boullin *et al.*, 1976 and **Methods**). Subsequent administration of BW501C67 (10 µmol/l) did not reverse the contraction. Recordings between drug and CSF doses are not shown; the responses were all obtained with the same artery.

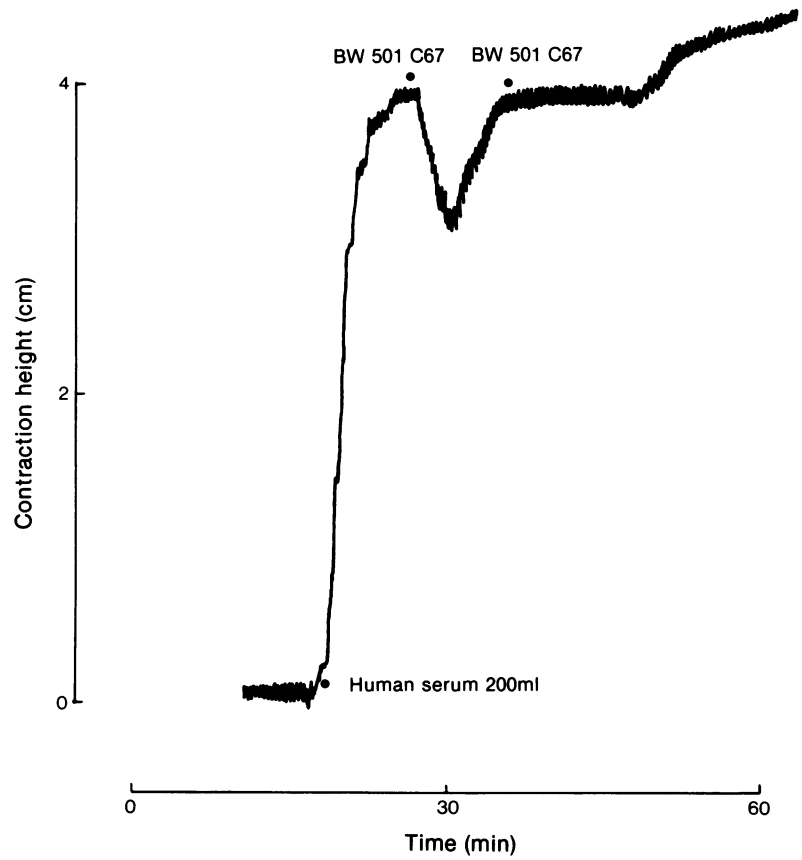

Figure 5 Effect of BW501C67 on a contraction of the rat stomach fundus preparation.
Experimental and recording conditions as Figure 4. Fresh human serum (200 µl) contracted the fundus to a maximum in about 8 min. Then BW501C67 (1 µmol/l) produced partial (23%) but transient (8 min) reversal of the contraction. A higher concentration of BW501C67 (10 µmol/l) thereafter had no antagonistic effect.

Figure 1), but having no effect whatsoever on blood induced CAS.

The lack of involvement of 5-HT in experimental CAS is further supported by the demonstration that 5-HT induced platelet aggregation was abolished by BW501C67 after intracisternal administration of the drug. Thus the antagonism of 5-HT induced CAS was accompanied by a specific antagonism of 5-HT induced aggregation.

It could be argued that the antagonism of 5-HT induced CAS following intracisternal BW501C67 involved some action of the drug unrelated to 5-HT receptor antagonism. However, as the drug concentrations in plasma were sufficient to effectively abolish 5-HT induced aggregation without abolishing ADP induced aggregation, this seems unlikely. Nevertheless the specificity of BW501C67 as a 5-HT antagonist is not absolute at least in regard to the rat aortic strip. Table 1 shows that although BW501C67 is 50–233 times more potent as an antagonist of 5-HT, than dopamine or noradrenaline, the drug does have anti-catecholamine activity at quite low concentrations.

As BW501C67 antagonized 5-HT induced CAS by 65% we conclude that the role of 5-HT in simulated CAS in baboons is at the most minimal.

We used the aortic strip in our studies as it has been used earlier for studies in relation to human CAS (Wilkins *et al.*, 1967) and shows greater sensitivity to the catecholamines than 5-HT. We used the rat stomach fundus for the converse reason, namely preferential sensitivity to 5-HT.

The experiments with these tissues show that CSF, serum and platelet extracts all produced contractions of varying magnitude and duration. Yet in only one

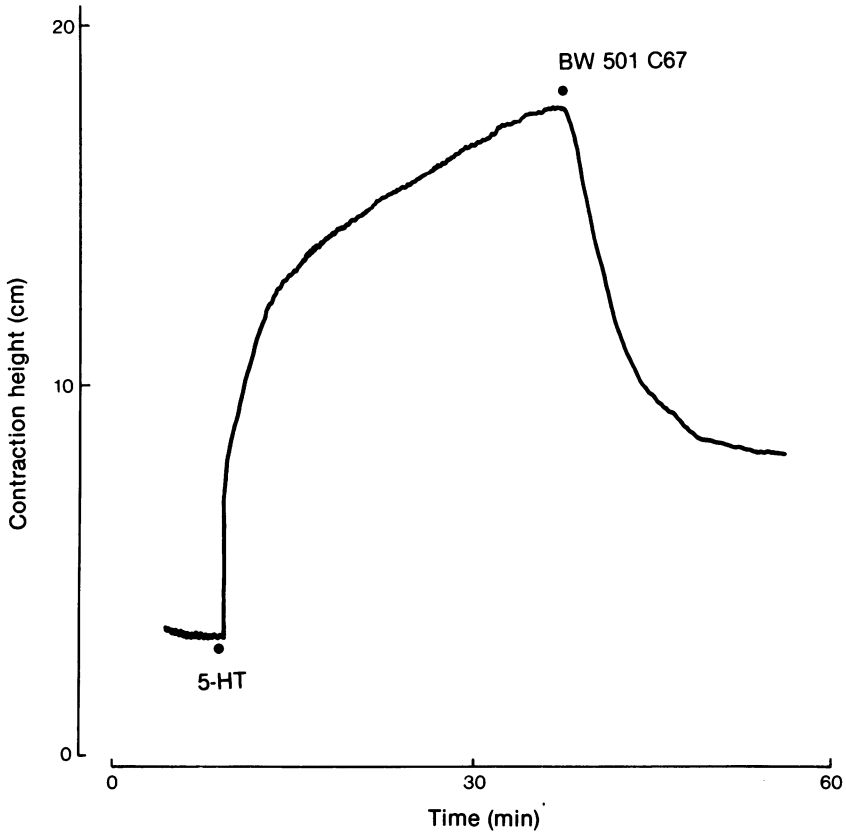

Figure 6 Antagonism of 5-HT (2 μmol/l) induced contraction of the rat aortic strip by BW501C67 (0.2 μmol/l). Experimental and recording conditions as Figure 4.

instance did BW501C67 either prevent these contractions from developing, alter their rate of development or reverse them once they were developed. The exception was that the 5-HT antagonist produced a transient reversal of serum induced contraction of the fundus (Figure 5). This reversal was to be predicted since human serum is known to contain 5-HT (Erspamer, 1966); however, only a small component of the serum induced contraction could be attributed to 5-HT since even high concentrations of BW501C67 failed to antagonize the sustained contractions caused by serum.

All 5-HT in blood is contained in the platelets

Table 1 Potency of BW501C67 as an antagonist of 5-HT, dopamine and noradrenaline induced contractions of the rat aortic strip.

	5-HT	*Dopamine*	*Noradrenaline*
ID_{50} (μmol/l)	0.015 ± 0.0013	0.8 ± 0.007	3.5 ± 1.22
Relative potency (noradrenaline = 1)	233	4.3	1

Contractions of the aortic strip were induced with 2 μmol/l 5-HT, 0.5 μmol/l dopamine and 0.1 μmol/l noradrenaline.

The results are the mean ± s.e. mean of four experiments with each tissue. BW501C67 was added to the organ bath 6 min prior to the contracting drug.

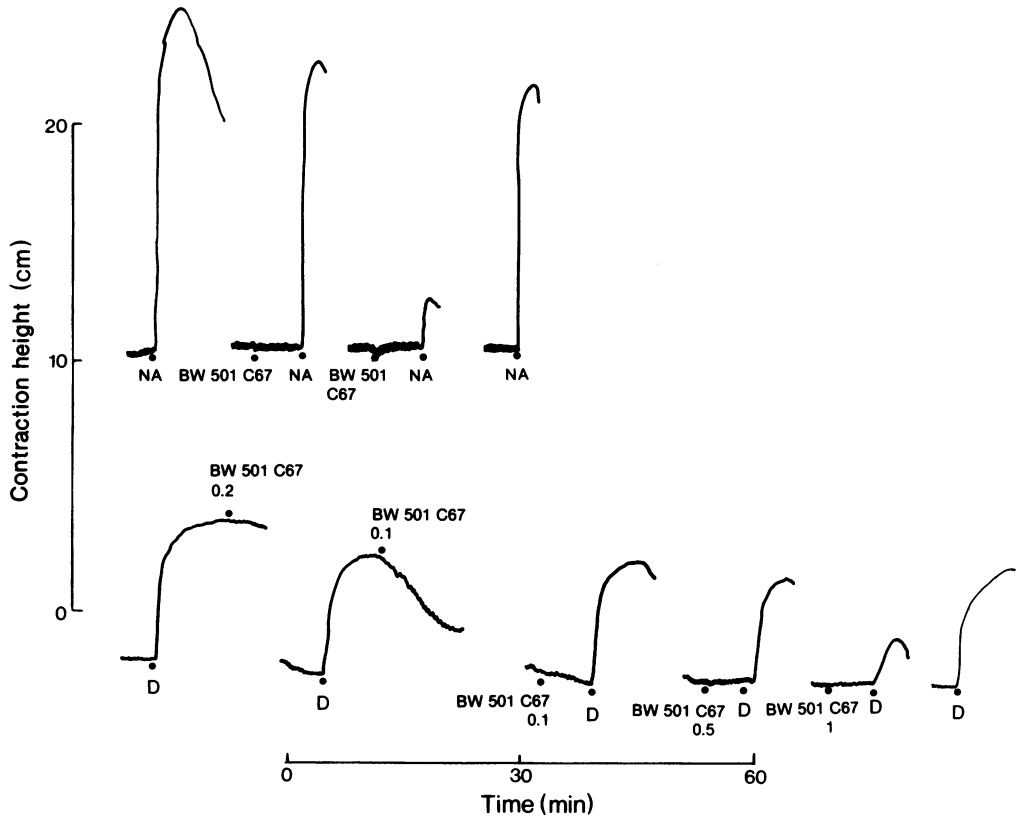

Figure 7 Blockade of noradrenaline (0.1 μmol/l) and dopamine (D 0.5 μmol/l) induced contractions of the rat aortic strip by BW501C67 (concentrations in μmol/l). Experimental and recording conditions as Figure 4. Traces show drug responses only; intervening traces showing effects of washing out drugs and return of responses to the baseline are not shown. All data were obtained in a single experiment over a 4 h period.

(Stacey, 1966), therefore we investigated the effects of platelet extracts on the aortic strip. Figure 8 shows that platelets cause contractions of the aortic strip which are not due to 5-HT. They may well be caused by prostaglandins (Yamamoto, Feindel, Wolfe, Katoh & Hodge, 1972; Pelofsky, Jacobson & Fisher, 1972; White, Heaton & Denton, 1971) or thromboxane (Bunting, Moncada & Vane, 1976; Needleman, Moncada, Bunting & Vane, 1976; Ellis, Oelz, Roberts, Payne, Sweetman, Nies & Oates, 1976; Dusting, Lattimer, Moncada & Vane, 1977).

Thus, although BW501C67 is not an entirely specific inhibitor of 5-HT reactions on the tissues used here, it effectively antagonizes 5-HT responses. Moreover, in every instance the effects of the factors associated with CAS mentioned at the beginning of the discussion (blood and extracts, CSF and platelets) remain virtually unchanged by BW501C67. These conclusions even apply to the platelets which clearly can synthesize or release other agents than 5-HT that contract tissues.

Consequently we believe that our data are compatible with the view that experimentally induced CAS in baboons by intracisternal injection of blood is not due to 5-HT. A similar conclusion may be drawn from the actions of human serum and VSCSF on the tissues studied, including the isolated human basilar artery. Therefore it is very unlikely that 5-HT plays a major role in the aetiology of human CAS.

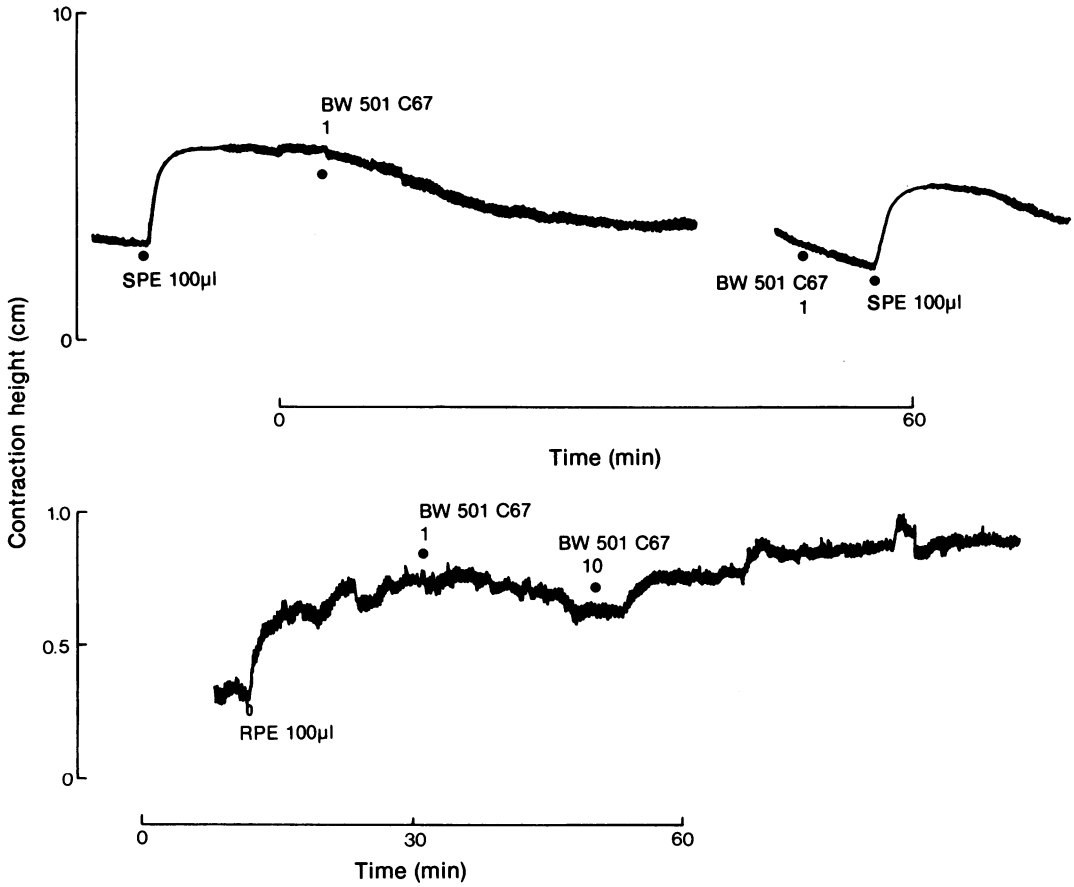

Figure 8 Failure of BW501C67 to reverse contractions of the rat aortic strip induced by platelet extracts. Experimental and recording conditions as Figure 4. For preparation of platelet extracts see **Methods**.

(a) Left: contraction induced by supernatant from platelet extract from (SPE) not antagonized by BW501C67 (1 µmol/l).

Right: prior treatment with BW501C67 (1 µmol/l) failed to prevent the subsequent response to platelet extract. Note that the platelet extract responses were transient and this transience was not altered by BW501C67.

(b) BW501C67 (1 and 10 µmol/l) fails to reverse a contraction produced by 100 µl resuspended platelet pellet extract (RPE).

Experimental recording and conditions as Figure 5. 100 µl of platelet extract (see **Methods**) was applied as indicated.

References

ADAMS, C.B.T., LOACH, A.B. & O'LAOIRE, S.A. (1976). Intracranial aneurysms: analysis of results of microneurosurgery in 100 consecutive patients. *Br. med. J.*, **2**, 607–609.

ALLEN, G.S., GOLD, L.H.A., CHOU, S.N. & FRENCH, L.A. (1974). Cerebral arterial spasm. Part 3: *In vivo* intracisternal production of spasm by serotonin and blood and its reversal by phenoxybenzamine. *J. Neurosurg.*, **40**, 451–458.

ALLEN, G.S., GROSS, C.J., FRENCH, L.A. & CHOU, S.N. (1976). *In vitro* contractile activity of vasoactive agents including human CSF on human basilar and anterior cerebral arteries. *J. Neurosurg.*, **44**, 594–600.

ALLEN, G.S., HENDERSON, L.M., CHOU, S.N. & FRENCH,

L.A. (1974a). Cerebral arterial spasm. Part 1: *In vitro* contractile activity of vasoactive agents on canine basilar and middle cerebral arteries. *J. Neurosurg.*, **40**, 433–441.

ALLEN, G.S., HENDERSON, L.M., CHOU, S.N. & FRENCH, L.A. (1974b). Cerebral arterial spasm. Part 2: *In vitro* contractile activity of serotonin in human serum and CSF on the canine basilar artery, and its blockage by methysergide and phenoxybenzamine. *J. Neurosurg.*, **40**, 442–450.

BOULLIN, D.J., ADAMS, C.B.T., MOHAN, J., GREEN, A.R., HUNT, T.M., DU BOULAY, G.H. & ROGERS, A.T. (1977). Effects of intracranial dopamine perfusion: behavioural arousal and reversal of cerebral arterial spasm following surgery for clipping of ruptured cerebral aneurysms. *Proc. Roy. Soc. Med.*, **70**, Suppl. 2, 55–70.

BOULLIN, D.J. & GLENTON, P.A.M. (1978). Characterisation of receptors mediating 5-hydroxytryptamine and catecholamine induced platelet aggregation, assessed by the actions of α- and β-blockers, butyrophenones, 5-HT antagonists and chlorpromazine. *Br. J. Pharmac.*, **62**, 537–542.

BOULLIN, D.J., GREEN, A.R. & PRICE, K.S. (1972). The mechanism of adenosine diphosphate induced platelet aggregation: Binding to platelet receptors and inhibition of binding and aggregation by prostaglandin E^1. *J. Physiol.*, **221**, 415–426.

BOULLIN, D.J. & GRIMES, R.P.J. (1976). Increased platelet aggregation in patients receiving chlorpromazine: responses to 5-hydroxytryptamine, dopamine and *n*-dimethyldopamine. *Br. J. clin. Pharmac.*, **3**, 649–653.

BOULLIN, D.J., MOHAN, J. & GRAHAME-SMITH, D.G. (1976). Evidence for the presence of a vasoactive substance (possibly involved in the aetiology of cerebral arterial spasm) in cerebrospinal fluid from patients with subarachnoid haemorrhage. *J. Neurol. Neurosurg. Psychiat.*, **39**, 756–766.

BOULLIN, D.J. & MOHAN, J. (1977). Effects of (+) and (–) propranolol on the responses of the human isolated basilar artery to cerebrospinal fluid obtained from patients with subarachnoid haemorrhage and cerebral arterial spasm. *Br. J. clin. Pharmac.*, **4**, 27–31.

BRAWLEY, B.W., STRANDNESS, B.E. & KELLY, W.A. (1968). The biphasic response of cerebral vasospasm in experimental subarachnoid haemorrhage. *J. Neurosurg.*, **28**, 1–8.

BUNTING, S., MONCADA, S. & VANE, J.R. (1976). The effects of prostaglandin endoperoxides and thromboxane A_2 on strips of rabbit coeliac artery and certain other smooth muscle preparations. *Br. J. Pharmac.*, **57**, 462–463P.

DU BOULAY, G., KENDALL, B.E., SYMON, L., PASZTOR, E., CROCKARD, A., BELLONI, G. & SAGE, M. (1975). The vasodilator action of angiography with urografin 60% on the basal vessels of the brain of the baboon. *Neuroradiology*, **9**, 133–137.

DU BOULAY, G. & SYMON, L. (1971). The anaesthetist's effect upon the cerebral arteries. *Proc. Roy. Soc. Med.*, **64**, 77–80.

DUSTING, G.J., LATTIMER, N., MONCADA, S. & VANE, J.R. (1977). Prostaglandin X, the vascular metabolite of arachidonic acid responsible for relaxation of bovine coronary artery strips. *Br. J. Pharmac.*, **59**, 443P.

ELLIS, E.F., OELZ, O., ROBERTS, L.J., PAYNE, N.A., SWEETMAN, B.J., NIES, A.S. & OATES, J.A. (1976). Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A_2. *Science*, **193**, 1135–1137.

ERSPAMER, V. (1966). Occurrence of indolealkylamines in nature. In *Handbook of Experimental Pharmacology*, eds Eichler, O. & Farah, A., pp. 132–166. New York: Springer-Verlag.

FRASER, P.J. (1970). Antagonists of the embryocidal effect of 5-hydroxytryptamine in the rat. *Br. J. Pharmac.*, **39**, 224.

FURCHGOTT, R.S. & BHADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

LA TORRE, E., PATRONO, C., FORTUNA, A. & GROSSI-BELLONI, D. (1974). Role of prostaglandin $F_{2\alpha}$ in human cerebral vasospasm. *J. Neurosurg.*, **41**, 293–299.

MAWSON, C. & WHITTINGTON, H. (1970). Evaluation of the peripheral and central antagonistic activities against 5-hydroxytryptamine of some new agents. *Br. J. Pharmac.*, **39**, 223.

NEEDLEMAN, P., MONCADA, S., BUNTING, S. & VANE, J.R. (1976). Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides. *Nature*, **261**, 558–560.

PELOFSKY, S., JACOBSON, E.D. & FISHER, R.G. (1972). Effects of prostaglandin E^2 on experimental cerebral vasospasm. *J. Neurosurg.*, **36**, 634–639,

SEVERINGHAUS, J.W. & BRADLEY, A.F. (1958). Electrodes for blood PO_2 and PCO_2 determination. *J. appl. Physiol.*, **13**, 515–520.

SIGGAARD, O., ANDERSEN, A.E. & ENGEL, K. (1960). A new acid base nomogram. An improved method for the calculation of the relevant blood acid-base data. *Scand. J. clin. lab. Invest.*, **12**, 177–186.

STACEY, R.S. (1966). Clinical aspects of cerebral and extracerebral 5-hydroxytryptamine. In *Handbook of Experimental Pharmacology*, eds Eichler, O. & Farah, A., pp. 744–774. New York: Springer-Verlag.

STARLING, L.M., BOULLIN, D.J., GRAHAME-SMITH, D.G., ADAMS, C.B.T. & GYE, R.S. (1975). Responses of isolated human basilar arteries to 5-HT, NA, serum, platelets and erythrocytes. *J. Neurol. Neurosurg. Psychiat.*, **7**, 650–656.

SYMON, L., DU BOULAY, G., ACKERMAN, R.H., DORSCH, N.W.C. & SHAH, S.H. (1973). The time course of blood induced spasm of cerebral arteries in baboons. *Neuroradiology*, **5**, 40–42.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac.*, **12**, 344–349.

VANE, J.R. (1969). The release and fate of vasoactive hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

WHITE, R.P., HEATON, J.A. & DENTON, I.C. (1971). Pharmacological comparison of prostaglandin $F_{2\alpha}$, serotonin and norepinephrine *Eur. J. Pharmac.*, **15**, 300–309.

WILKINS, R.H. (1976). Aneurysm rupture during angiography: does acute vasospasm occur? *Surg. Neurol.*, **5**, 299–303.

WILKINS, R.H., WILKINS, G.K., GUNNELLS, J.C. & ODOM, G.L. (1967). Experimental studies of intracranial arterial spasm using aortic strip assays. *J. Neurosurg.*, **27**, 490–500.

WURTMAN, R.J., LAVYNE, M.H. & ZERVAS, N.T. (1974).

Brain catecholamines in relation to cerebral blood vessels. In *Cerebral Vascular Diseases,* eds Whisnant, J.P. & Sandok, B.A., pp. 13–26. New York: Grune & Stratton.

YAMAMOTO, L.Y., FEINDEL, W., WOLFE, L.S., KATOH, H. & HODGE, C.P. (1972). Experimental vasoconstriction of cerebral arteries by prostaglandins. *J. Neurosurg.,* **37,** 385–397.

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