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MODELS FOR INVESTIGATING THE AETIOLOGY OF CEREBRAL ARTERIAL SPASM: COMPARATIVE RESPONSES OF THE HUMAN BASILAR ARTERY WITH RAT COLON, ANOCOCCYGEUS, STOMACH FUNDUS, AND AORTA AND GUINEA-PIG ILEUM AND COLON

D.J. BOULLIN, TERESA M. HUNT & A.T. ROGERS*

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford
*Nuffield Institute of Comparative Medicine, Zoological Society of London, Regents Park, London NW1

1 The pharmacological similarity between human basilar artery and a number of isolated tissues (rat colon, anococcygeus, stomach fundus and aorta and guinea-pig ileum and colon) has been assessed during investigations of the aetiology of cerebral arterial spasm.

2 The responses of each of the six tissues to human normal and abnormal cerebrospinal fluid (CSF) and to human serum were compared with those of the human isolated basilar artery.

3 These studies revealed the presence of a vasodilator factor in CSF from subarachnoid haemorrhage patients and several tissues may be of use for further work in identifying the vasoactive substances in CSF.

4 No tissue displayed exactly the same spectrum of biological reactivity as the human basilar artery. The rat stomach fundus showed the closest similarity and was further studied for similarities in drug-induced responses.

5 The rat stomach fundus, like the human basilar artery, was contracted by 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$ and histamine and relaxed by dopamine. However, noradrenaline relaxed the fundus but contracted the basilar artery.

Introduction

There are many methods for investigating the cause of cerebral arterial spasm (CAS) following rupture of cerebral arterial berryaneurysms. These include investigations of cerebral blood flow (CBF), the simulation of human subarachnoid haemorrhage (SAH) in various mammalian species by damage of the cerebral vasculature (Echlin, 1965; 1968; 1971; Simeone & Finall, 1975); injection of drugs into the region of the Circle of Willis (Kapp, Mahaley & Odom, 1968; Kuwayama, Zervas, Belson, Shintani & Pickren, 1972; Peterson, Searle, Mandy & Leblanc, 1973; Nagai, Suzuki, Sugiura, Noda & Mabe, 1974; for other references see Boullin, Mohan & Grahame-Smith, 1976) and studies of the responses of isolated organs to whole blood, blood cellular extracts and CSF from SAH patients with and without pre- or post-operative CAS (for references see Boullin *et al.*, 1976).

In the last 3 years we have studied extensively the responses of the human isolated basilar artery to the various tissue fractions described above. We have detected in the CSF of SAH patients with pre-operative CAS a vasoactive substance which contracts the isolated basilar artery (Boullin *et al.*, 1976; Allen, Gross, French & Chou, 1976). Moreover dopamine reverses this spasm *in vitro* and also reverses post-operative CAS in some subjects following intracranial perfusion of the region of the aneurysm with dopamine (Boullin, Adams, Mohan, Green, Hunt, Du Boulay & Rogers, 1977).

Although the human basilar artery obtained 1 to 5 days after death responds to drugs in about 50% of cases (Starling, Boullin, Grahame-Smith, Adams & Gye, 1975) and has yielded considerable information, we were anxious to see if any other mammalian tissue would serve as a model system giving qualitatively similar responses.

Accordingly we have examined the responses of other isolated tissues to various substances including the potentially vasoactive materials mentioned above, in the hope that we might be able to find a more viable and easily obtainable substitute for the human basilar artery for investigating the aetiology of CAS.

The criteria we have used for suitability for a model system are based upon certain responses of the human isolated basilar artery described here and in

previous papers (Starling *et al.*, 1975; Boullin *et al.*, 1976). These are as follows: (1) The isolated tissue should fail to contract to normal human CSF but should contract to CSF from patients with SAH; this contraction should preferably be prolonged. (2) Dopamine should produce relaxation of the tissue. It should reverse contractions caused by prostaglandins and/or other drugs and contractions produced by CSF from SAH patients with CAS. (3) Non-specific spasmolytic drugs such as papaverine should cause prolonged relaxation.

Methods

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 aortic strip and other smooth muscles. Sprague-Dawley male and female rats (250 to 300 g) were killed by decapitation and one or more of the following tissues removed: the aortic strip (Furchgott & Bhadrakom, 1953); the anococcygeus muscle dissected as described by Gillespie (1972) except that the dissection of the extrinsic nerve was omitted; the stomach fundus strip prepared according to Vane (1957); and the proximal colon according to conventional pharmacological methods (Edinburgh Staff, 1970).

Guinea-pig intestine. The proximal ileum and proximal colon were set up in the same way as the rat colon. The movements of all tissues were recorded isotonically as described by Boullin *et al.* (1976). The observations described for each tissue are based on results obtained from 3 to 6 experiments.

Fluid samples

Cerebrospinal fluid (CSF). This was obtained from SAH patients prior to surgery for clipping or cyanoacrylate wrapping of ruptured berryaneurysms. The CSF was divided into 2 groups on the basis of evidence for pre-operative spasm according to angiograms made within 4 to 10 h of CSF collection: the fluid from patients with pre-operative spasm was termed VS CSF and that from similar patients with no angiographic evidence of spasm was termed NVS CSF. We also obtained 'normal' CSF from other subjects undergoing myelography for suspected prolapsed intravertebral disc. The effects of VS CSF and NVS CSF 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 sequence 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 either sex (aged 18 to 46 years) and allowed to clot. The serum supernatant fluid was removed and kept at −20°C until required.

Saline solutions

The preparations were set up in a 10 or 13 ml isolated organ bath containing physiological saline medium at 37°C. 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 and glucose 11.1) was used for all preparations except guinea-pig ileum and colon and the rat colon. The latter were immersed in Tyrode solution (composition: mmol/l: NaCl 137, KCl 2.7, $CaCl_26H_2O$ 1.8, $MgSO_4.7H_2O$ 1.06, $NaH_2PO_42H_2O$ 5.6, $NaHCO_3$ 11.9 and glucose 5.6.) The Krebs solution was gassed with 5% CO_2 in O_2 and the Tyrode solution with pure O_2; the pH of both solutions was 7.4.

Drugs

5-Hydroxytryptamine (5-HT), (±)-noradrenaline (NA), (±)-adrenaline, histamine, dopamine and papaverine were obtained from Sigma Chemical Company. The following compounds were kindly donated as indicated below: prostaglandin $F_{2\alpha}$, tromethamine salt ($PGF_{2\alpha}$) by Dr J. E. Pike, The Upjohn Company, Kalamazoo, MI 49001, U.S.A.; phentolamine by Ciba Laboratories; haloperidol by Janssen Pharmaceutical Ltd.

All drugs except the prostaglandin were dissolved in ascorbic acid (1 mg/ml); prostaglandin $F_{2\alpha}$ was dissolved in (2%) ethanol to give a stock solution of 1 to 10 mmol/l and then diluted with the appropriate saline medium. 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 to 250 µl into a 10 ml organ bath. The final concentration of ethanol in the diluted stock solution (prostaglandin) was 0.002%; this concentration did not affect the responses of the various isolated tissues.

Results

Table 1 shows that apart from the human basilar artery only 2 tissues specifically contracted to VS CSF but not to NVS or normal CSF. The rat stomach

fundus strip and the guinea-pig colon responded with a prolonged contraction as shown previously with the human basilar artery (Boullin *et al.*, 1976).

The effects of the 3 types of CSF on the rat stomach fundus are illustrated in Figure 1. Only VS CSF produced a slowly developing contraction while NVS CSF and normal CSF caused transient biphasic relaxations and contractions which were quite different. Similarly on the guinea-pig colon, only VS CSF caused a slowly developing sustained contraction. NVS CSF relaxed and normal CSF caused a slight contraction, which in any event was much less than the response to VS CSF.

As described previously, VS CSF does sometimes produce an initial transient relaxation of the human basilar artery (Boullin *et al.*, 1976) and it will be noted from Figure 1 that relaxation also occurred with VS CSF on the rat fundus. Although these responses were of small magnitude and short duration, they did suggest that a relaxing factor is present in VS CSF in addition to the contractile substances.

This phenomenon was investigated in detail on the rat stomach fundus. Although VS CSF samples invariably produced a contraction on the first occasion of testing, the responses changed when the tests were repeated at a later date. Thus Figure 2 (a and b) shows the differing responses obtained with 2 samples of VS CSF tested on rat stomach fundus at 2-weekly intervals with thawing and refreezing at 20°C before and after each test. In the first sample (Figure 2a) the initial slowly developing contraction was diminished at the second test; on the third test there was only a very small transient contraction, which was followed by a prolonged relaxation.

In the second sample (Figure 2b) the changes in response were even more dramatic; the response to test 1 was a rapidly developing sustained contraction, but to test 2 made two weeks later the response was exactly the opposite: a rapidly developing sustained relaxation. A smooth muscle relaxing substance was detected in 6 different samples subjected to 18 tests.

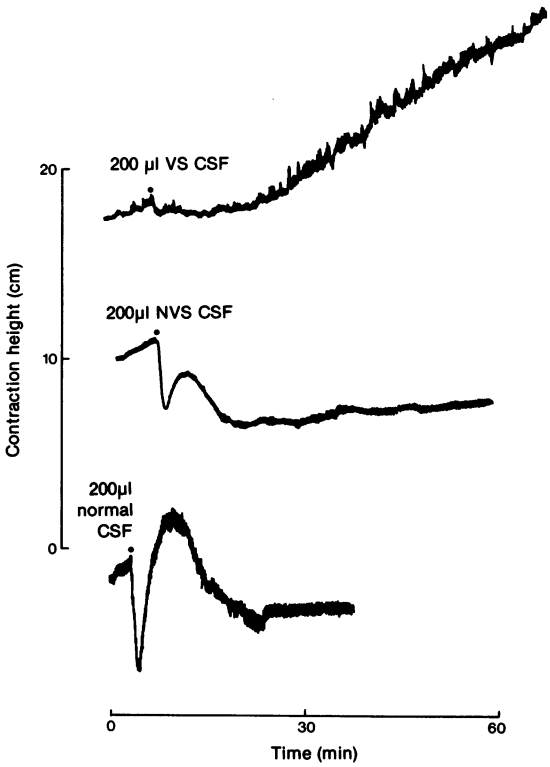

Figure 1 Responses of the rat stomach fundus to vasospastic (VS), non-vasospastic (NVS) and normal cerebrospinal fluid (CSF). Movements were recorded isotonically by a transducer and displayed on a single channel potentiometric recorder. Abscissa scale: time; ordinate scale: amplified change in tissue length.

This alteration in response to VS CSF was most clearly demonstrated by the fundus, although changes

Table 1 Effects of human cerebrospinal fluid (CSF) and serum samples on isolated tissues

Tissue	*VS CSF*	*NVS CSF*	*Normal CSF*	*Serum*
Human basilar artery	C	NE	NE	C
Rat stomach fundus	C	R	TR	C
Guinea-pig ileum	C	C	NE	NE
Guinea-pig colon	C	R	NE	NE
Rat colon	R	C	R	C
Rat aorta	TC	TC	TC	C
Rat anococcygeus muscle	NE	NE	R	C

C = contraction; R = relaxation; NE = no effect; TC = transient contraction; TR = transient relaxation; VS CSF = cerebrospinal fluid from patients with pre-operative spasm prior to surgery on ruptured berry-aneurysms; NVS CSF = fluid from similar patients with no angiographic evidence of spasm; Normal CSF = fluid from subjects undergoing myelography for suspected prolapsed intravertebral disc.

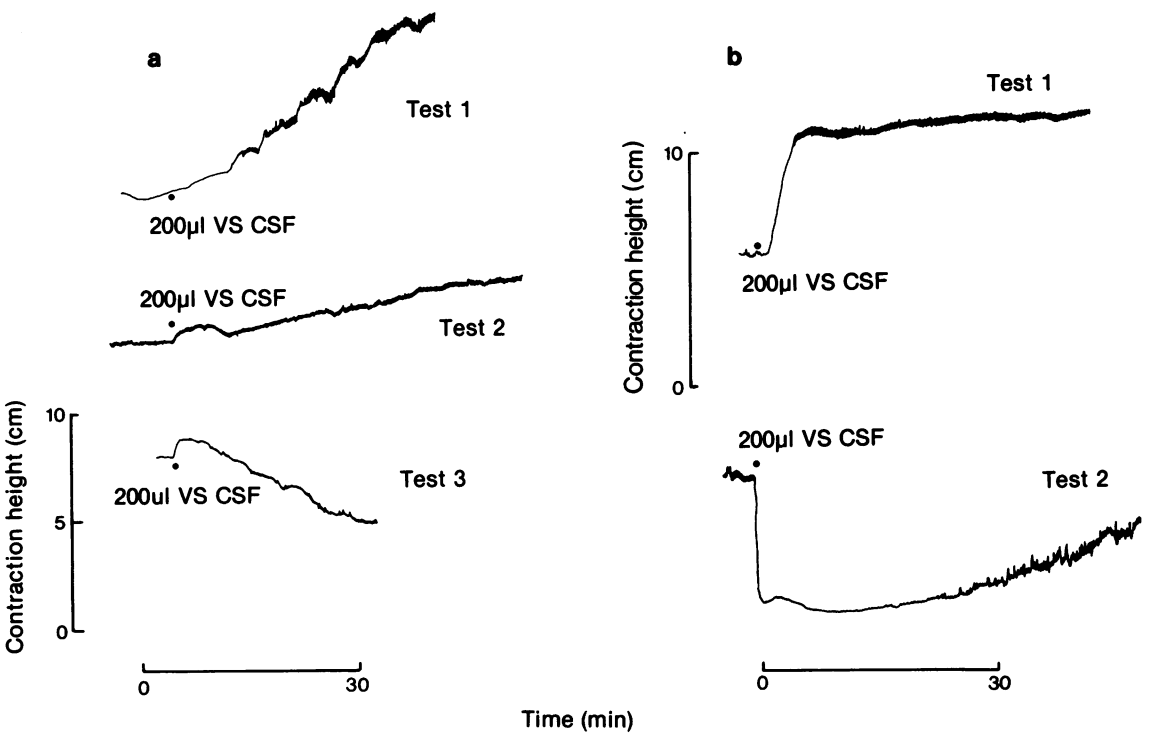

Figure 2 Changes in the response of the rat stomach fundus to vasospastic (VS) CSF. Movements were recorded isotonically by a transducer and displayed on a single channel potentiometric recorder. (a) Response of the rat stomach fundus to the same sample of VS CSF tested at 2 weekly intervals (tests 1 to 3). (b) Response of the fundus to another sample of VS CSF tested at a 2 weekly interval. Note: The response of the fundus changes from contraction to relaxation. Abscissae, time; ordinates, amplified change in tissue length.

also occurred on the human basilar artery. In contrast with this, the responses of the fundus or basilar artery to human serum did not change during several tests.

Effects of human serum

Because the presence of blood in the CSF is an essential factor for CAS to develop, we also compared the effects of normal human serum on the human isolated basilar artery and the animal tissues. Figure 3 illustrates the effects of VS CSF and human serum on the basilar artery: both caused a gradually developing sustained contraction. Table 1 compares the effects of VS CSF and serum and shows not only that these fluids display different spectra of contractile activity on different tissues, but also that there is a similarity between the rat stomach fundus and human basilar artery.

Responses to drugs

The responses of the various tissues to drugs were then studied, with emphasis on the rat stomach fundus. Table 2 shows the EC_{50} values of some spasmogenic drugs. $PGF_{2\alpha}$ was generally of high potency; and all tissues were contracted by 5-HT, which was much more potent than noradrenaline (NA) on the basilar artery. In contrast, noradrenaline was much more potent than 5-HT on the extracerebral tissues. From Table 2 calculations reveal that the potency ratio 5-HT:NA on the basilar artery was 20:1 whereas the ratio on extracerebral rat tissues was only 0.02 (calculated from the examples in which noradrenaline caused a contraction). These data support the view that cerebral arteries show much greater sensitivity to 5-HT than to the catecholamines (Nielson & Owman, 1971; Allen *et al.*, 1976).

Table 3 shows the EC_{50} values of some drugs which relax the various preparations. The effects of dopamine were of particular interest: dopamine relaxed the human isolated basilar artery *in vitro* and successive applications of increasing concentrations of dopamine (0.1 to 10 µmol/l) caused dose-dependent relaxations. Biphasic effects (relaxation followed by contraction) were observed with concentrations above 50 µmol/l which were not dose-dependent. These con-

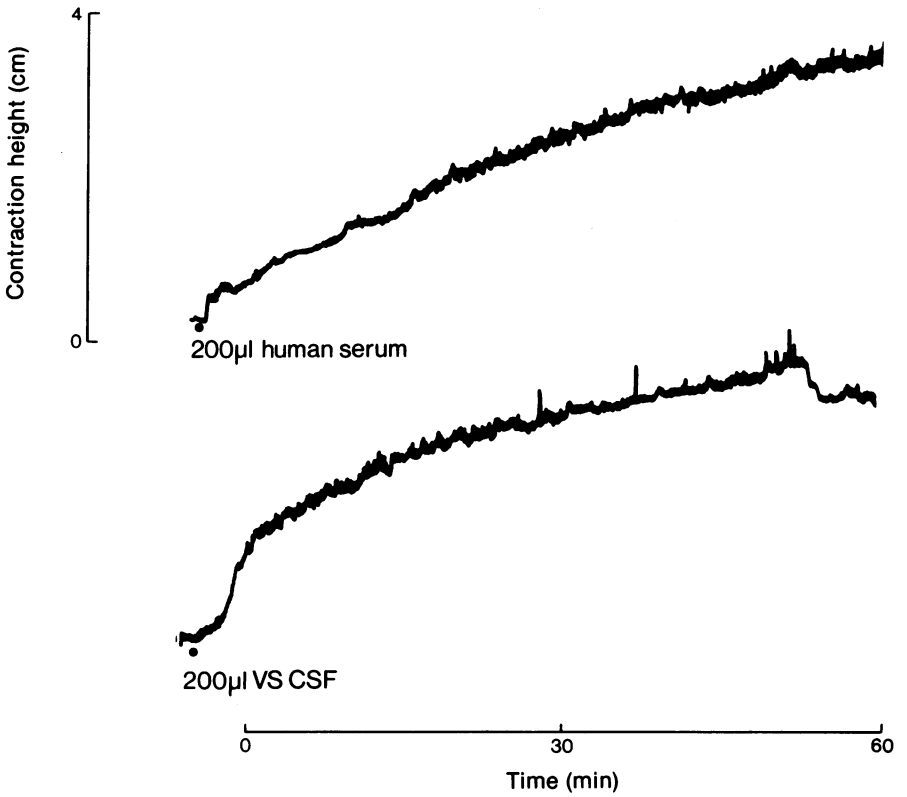

Figure 3 Responses of the human basilar artery to human serum and vasoactive (VS) cerebrospinal fluid (CSF). Movements were recorded isotonically by a transducer and displayed on a single channel potentiometric recorder. Abscissa scale: time; ordinate scale; amplified change in length of tissue.

tractions were blocked by phentolamine (1 µmol/litre).

Dopamine not only caused a spontaneous relaxation of human basilar arteries, but also reversed the contractions produced either by VS CSF or by drugs.

The effects of dopamine and other drugs were then investigated on the rat stomach fundus. 5-HT contracted while dopamine and noradrenaline relaxed the rat stomach fundus. The responses to 5-HT and dopamine were qualitatively similar to those of the human basilar artery but in contrast noradrenaline con-

Table 2 Potency of some drugs which contract cerebral and extracerebral tissues

	EC_{50} values (µm/l)				
Tissue	*5-HT*	*Noradrenaline*	*Dopamine*	*$PGF_{2\alpha}$*	*Histamine*
Arteries					
Human basilar	10 ± 0.9	200 ± 18	NT	10 ± 1.6	10 ± 0.7
Rat aortic strip	0.5 ± 0.06	0.01 ± 0.001	0.6 ± 0.09	9.1 ± 1.2	0
Rat stomach fundus	0.13 ± 0.01	*	*	0.2 ± 0.01	0.1 ± 0.01
Rat colon	2 ± 0.18	*	*	0.17 ± 0.02	16
Rat anococcygeus muscle	0.5 ± 0.03	0.01 ± 0.001	2	0.4 ± 0.06	0
Guinea-pig ileum	1.8 ± 0.2	0.47 ± 0.07	36.4 ± 5.8	0	0.27 ± 0.04
Guinea-pig colon	0	0	0	0	1.06 ± 0.27

All values are mean ± s.e. mean of data from 3 to 6 experiments; NT = not tested; * = tissue relaxed by this drug (see Table 3); EC_{50} is the concentration needed to produce 50% maximum contraction.

tracted the basilar artery. Haloperidol (10–100 μmol/l) selectively blocked the dopamine-induced relaxations of the rat stomach fundus but higher concentrations of the antagonist were required than were previously used on the basilar artery (Boullin *et al.*, 1977). Phentolamine (0.1 to 100 μmol/l) did not affect the dopamine-induced relaxation of the fundus.

High concentrations of dopamine (50 to 100 μmol/l) did not contract the rat stomach fundus which contrasts with the responses obtained with this drug on the human basilar artery.

Finally, dopamine produced other qualitatively similar effects on the fundus to those seen on the human isolated basilar artery, such as inhibiting the contractions of the fundus produced by 5-HT, prostaglandin $F_{2\alpha}$, and VS CSF.

Discussion

Many experimental studies concerned with the aetiology of CAS have assayed the biological activity of vasoactive substances on various isolated tissues (see Introduction). In spite of this, few investigators have used human isolated cerebral arteries, and no investigation to date has included a comprehensive study of the effects of vasoconstrictor and vasodilator agents on the cerebral vessels which show angiographic evidence of spasm. These vessels include the vertebro-basilar system together with the Circle of Willis and its branches.

Of the 6 animal tissues studied here, there was no single preparation which could be selected as an ideal model precisely mimicking the responses of the human basilar artery to contracting and relaxing agents. However, several preparations resembled it in some degree and the rat stomach fundus showed the closest similarity. Thus the fundus contracted specifically to VS CSF but not to NVS or normal CSF. Moreover, on the first test, VS CSF-induced contractions were similar in time of onset and duration to those produced on the human basilar artery.

Dopamine also antagonized VS CSF-induced contractions of the fundus as it did of the basilar artery (see also Boullin *et al.*, 1976). Of particular pharmacological significance was the fact that dopamine-induced relaxations of the fundus were antagonized by high doses of the specific dopamine antagonist haloperidol; again this was similar to the haloperidol-sensitive relaxation of the basilar artery. The final resemblance between the fundus and the basilar artery concerned the extreme sensitivity of both tissues to 5-HT and prostaglandins (Vane, 1957; Boullin *et al.*, 1976). One dissimilarity concerned responses to noradrenaline; whereas the basilar artery contracts to noradrenaline the fundus relaxes.

Apart from the fundus, the guinea-pig colon is worthy of mention since it contracted specifically to VS CSF while remaining unresponsive to normal CSF. However, it was not particularly sensitive to 5-HT or noradrenaline and did not respond to the prostaglandins tested here. Furthermore, dopamine produced α-adrenoceptor-mediated contractions.

It was surprising to find that, from our data, aortic tissue was not a suitable model since this artery has been used in earlier investigations relating to CAS (Wilkins, Wilkins, Gunnells & Odom, 1967). First, its contractile responses to CSF do not enable a distinction to be made between the 3 types of CSF. Second, dopamine causes α-adrenoceptor-mediated contractions (inhibited by phentolamine); and third, the artery is preferentially sensitive to noradrenaline rather than 5-HT.

Another interesting point concerns the differential responses of the one human and 6 animal tissues to normal CSF, NVS CSF, VS CSF and normal serum. It is clear from Table 1 that not only do no two tissues display an identical spectrum of biological responses to the fractions tested but also that the tissue responses to serum and CSF differ. Thus, it appears that the vasoactive principles in serum and CSF do not have the same chemical identities. However, it seems very likely that blood borne substances are involved in the generation of the vasoconstrictor prin-

Table 3 Potency of drugs relaxing cerebral and extracerebral tissues

	EC_{50} values (μm/l)			
Tissue	*Noradrenaline*	*Dopamine*	*Isoprenaline*	*Papaverine*
Human basilar artery		10.0 ± 1.7		1.0 ± 0.09
Rat stomach fundus	1.2 ± 0.23	3.0 ± 0.47	0.03 ± 0.007	1.0 ± 0.12
Rat colon	3.0 ± 0.47	2.8 ± 0.54	0.002 ± 0.0003	
Guinea-pig colon	21.3 ± 3.1			

All values are the mean ± s.e. mean of data from 3 to 6 experiments; EC_{50} is the concentration needed to produce 50% maximum relaxation.

ciple in CSF (Zervas, Kuwayama, Rosoff & Salzman, 1973), and one possible explanation of the different effects of CSF and serum is that a substance synthesized during blood clotting is converted to some other chemical by serum, tissues, blood cells or by the CSF itself.

Experiments with several tissues reveal the presence of relaxing as well as contracting factors in all 3 types of CSF and also human serum. The relaxing factor was most evident in the experiments with the rat stomach fundus, but it was also seen with the guinea-pig and rat colon, the guinea-pig ileum and the rat anococcygeus muscle.

At this stage we do not know whether the relaxing or vasodilator factors present in the various groups of CSF are specifically related to CAS. The experiments on rat stomach fundus and human basilar artery, in which samples of VS CSF were tested on several occasions after storage, suggest that the spasmogenic material is less stable than the relaxing factor. We can suggest two possible candidates for the relaxing factor: the first is dopamine and the second is the recently discovered prostaglandin, named prostacyclin, which has artery dilating properties (Johnson, Morton, Kinner, Gorman, McGuire & Sun, 1976; Moncada, Vane & Higgs, 1977).

The development of CAS in patients with SAH may involve a reduction in the quantity of relaxing factor present in the region of spasm, or a change in the sensitivity of arteries to the relaxing and contracting factors.

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