The 5-hydroxytryptamine antagonist ketanserin inhibits the vasoconstrictor activity of per-operative CSF, from subarachnoid haemorrhage patients, on isolated tissues

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SUMMARY Peri-aneurysmal CSF was obtained at operation from 13 patients with subarachnoid haemorrhage from ruptured intracranial aneurysms. The 5-hydroxytryptamine antagonist ketanserin inhibited contractions of isolated human intracranial arteries, elicited by this CSF. The presence of 5-HT in CSF was confirmed by high performance liquid chromatography. The use of ketanserin in the therapy of postoperative cerebral vasospasm is discussed.

Delayed cerebral vasospasm, leading to cerebral ischaemia and neurological deficits, may follow subarachnoid haemorrhage (SAH) from ruptured intracranial aneurysms.¹ Accordingly, neurosurgeons time aneurysm clipping to occur before the onset of cerebral arterial spasm or until the likelihood of its occurrence has receded. The cause of delayed cerebral vasospasm remains obscure; vasoconstrictor substances in the cerebrospinal fluid (CSF) have been implicated in the development of prolonged cerebral arterial narrowing in the immediate post-operative phase leading to morbidity and mortality.²⁻⁵ This implies that post-operative cerebral vasospasm has a pharmacological basis. Recent investigations have focused on the identification and assay of vasoconstrictor substances appearing in the CSF after subarachnoid haemorrhage, with the intention of developing suitable pharmacological antagonists for clinical use.

A number of vasoconstrictor agents have been detected in the CSF after aneurysm rupture, including oxyhaemoglobin,⁶ thrombin,⁷ prostaglandins⁷ and 5-hydroxytryptamine (5-HT).⁸ The last of these has been overlooked in the investigation of cerebral arterial spasm for two reasons. Firstly, 5-HT does not appear to be the prime cause of prolonged vasospasm in experimental animal models.⁹ Secondly, 5-HT

antagonists in clinical use, such as methysergide, have direct vasoconstrictor actions on cerebral arteries.¹⁰

We now report experiments with two isolated tissues which demonstrate that there are substantial quantities of 5-HT-like vasoconstrictors in the cisternal CSF around the aneurysm at the time of surgery for clipping of ruptured aneurysms. We also show that their vasoconstrictor effects on human intracranial arteries can be antagonised by a novel 5-HT antagonist, the quinazoline derivative ketanserin.^{11,12} We discuss the relevance of this drug (which lacks intrinsic vasoconstrictor activity) to the treatment of pathological cerebral arterial narrowing after aneurysm rupture and clipping.

Methods

Isolated tissues

Two *in vitro* systems for the detection of vasoactive substances were used, the isolated human cerebral artery obtained *post mortem*⁴ and the isolated rat stomach fundus strip.¹³ 5-HT is a potent constrictor agent on both preparations although its actions may be mediated via different sub-types of 5-HT receptor.¹⁴⁻¹⁶ The cerebral vessels used were the basilar, both branches of the vertebral artery, and the arteries making up the anterior portion of the circle of Willis. The isolated tissues were mounted in 5 ml organ baths in Krebs bicarbonate pH 7.4 at 37°C continuously gassed with a 95%/5% mixture of O₂ and CO₂. Isotonic contractions were recorded on a potentiometric chart recorder.⁴ Preliminary experiments were performed to test for vasoconstrictor actions of ketanserin (ketanserin tartrate; Janssen Pharmaceuticals, Marlow, Bucks, UK) on isolated human intracranial arteries, and establish effective

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concentrations for antagonism studies. Dose response curves for 5-HT (serotonin creatinine sulphate; Sigma Biochemicals, Poole, Dorset, UK) in the presence and absence of ketanserin (2×10^{-5} M) were obtained for all types of cerebral vessel used to ensure homogeneity of antagonist response. Similar experiments were performed with the rat stomach fundus preparation using methysergide (methysergide hydrogen maleinate; Sandoz Pharmaceuticals, Basel, Switzerland) as the 5-HT antagonist.

The CSF was centrifuged for 5 minutes at 6500 g in an Eppendorf bench centrifuge, to remove whole cells and debris. All samples were frozen to -20°C within 1 hour of collection. Samples were thawed, kept on ice, and 500 μl aliquots assayed for vasoconstrictor activity on isolated tissues. Vasoactivity was expressed in terms of the equivalent bath concentration of 5-HT required to elicit an equivalent contraction (5-HT equivalents) using a log-dose response curve for 5-HT obtained for each separate preparation. CSF samples were tested on human cerebral vessels in the presence and absence of ketanserin tartrate at a bath concentration of 2×10^{-5} M, and on the rat stomach strip in the presence and absence of 2×10^{-5} M methysergide hydrogen maleinate. These concentrations gave complete inhibition of 5-HT induced contractions of the magnitude elicited by post-SAH cerebrospinal fluid. Bioassays were performed within 0–14 days of sample collection.

High-performance liquid chromatography

Samples from four patients in the study were also analysed within 10 days of collection by high-performance liquid chromatography with electrochemical detection (HPLC-ED).¹⁸ Aliquots (1 ml) of each were deproteinated by the addition of 0.25 ml of a solution of 50% trichloroacetic acid in 50 mM sodium metabisulphite with 0.5% EDTA and centrifuged as above. 50 μl of the supernatant was applied to a C18 reversephase chromatography column. This was eluted with 100 mM sodium acetate in 15% methanol (pH 4.2) at a flow rate of 1.2 ml/minute. 5-HT and its major metabolite 5-hydroxyindole acetic acid (5-HIAA) were detected by an electrochemical cell with an oxidation potential of 0.6V and quantitated against external standards. The lower limit of detection was 1 pmol in 50 μl (20 pmols/ml). The relationship between peak height and 5-HT/5-HIAA concentration was linear in the range 20 pmols/ml to 20 nmols/ml, within which fell all the concentrations detected in the CSF.

Collection of CSF

Samples of CSF were obtained per-operatively from 13 patients after subarachnoid haemorrhage following aneurysm rupture (table 1). All patients were grades I or II¹⁷ at the time of operation for aneurysm occlusion, which was performed using microneurosurgical techniques 7–18 days after the initial SAH. CSF was obtained from the immediate vicinity of the ruptured aneurysm by direct suction and great care was taken to avoid contaminating these samples with blood released into the subarachnoid space by operative procedures.

Assessment of vasospasm

The patients in the study were assessed for pre-operative vasospasm by cerebral angiography.¹⁹ Angiograms were

Table 1 Details of the patients involved in the study

Patient	Age (yr)	Sex	Location of aneurysm	Time of CSF collection	Pre-op spasm	Outcome
4	57	f	ICA	17	none	excellent
5	46	m	ACoA	17	moderate	good
6	50	f	ICA	18	none	excellent
7	49	f	ICA	17	none	excellent
8	54	m	MCA	13	none	excellent
9	61	f	ICA	13	none	excellent
10	39	f	MCA	13	none	excellent
12	21	m	MCA	14	none	excellent
13	44	f	ICA	14	none	excellent
14	61	f	ICA	32	moderate	good
15	42	m	ICA	20	none	good
16	52	f	MCA	8	mild	good
17	50	f	MCA	7	moderate	good

Aneurysms were located on the anterior communicating artery (ACoA), internal carotid artery (ICA) and middle cerebral artery (MCA). Pre-operative arterial vasospasm was determined by angiography (see METHODS for explanation of rating). The time of collection of CSF was determined as the number of days elapsed since the subarachnoid haemorrhage occurred. Outcome was determined on follow-up (see Methods).

measured at the following positions and the sum of the values obtained (after correction for magnification) compared with the normal values described by Gabrielsen *et al*²⁰: the internal carotid (IC) 5 mm proximal to its termination (normal value 4.57 ± 0.46 mm), the middle cerebral (normal value 3.82 ± 0.43 mm) and anterior cerebral (normal value 3.02 ± 0.5 mm) arteries 5 mm beyond their origins. Spasm was described as "mild" if there was 25–50% narrowing. If the reduction in arterial was more than 50%, spasm was "moderate". Vasospasm was thought to be "severe" if, in addition to arterial narrowing of greater than 50%, there was a segmental reduction in

Fig 1 Dose-response curves for the effect of 5-HT on the isolated human intracranial artery and the isolated rat stomach strip. Isotonic concentrations (volts, ordinate) are plotted against bath concentration of 5-HT (M, abscissa). Values are the mean $\pm$ standard error for the human intracranial artery (closed circles) and the rat stomach strip (closed triangles) as indicated.

Fig 2 The effect of ketanserin on 5-HT-induced contractions of the isolated human intracranial artery. Isotonic contractions (volts, ordinate) are plotted against bath concentration of 5-HT (M, abscissa). Values are the mean $\pm$ standard error for arteries in the presence of 5-HT alone (unbroken line), and in the presence of 5-HT and ketanserin at 2×10^{-5} M bath concentration (broken line) as indicated.

diameter to less than 2 mm in IC or 1 mm in the A1 of M1 segment.

The outcome of aneurysm surgery was assessed after 3 months. If the patient had no neurological deficit and had returned to the same occupation, then the outcome was termed "excellent". Mild deficit but a return to the same occupation was described as a "good" outcome. A significant neurological deficit and a return to a different occupation was regarded as "fair". Severe deficit with the patient not independent was termed "bad".

Results

5-hydroxytryptamine produced dose-related contractions of all human intracranial artery and rat fundus preparations used in this study (fig 1). Ketanserin was shown to be a potent competitive antagonist of 5-HT by a parallel shift to the right of the log-dose response curve for 5-HT induced contractions of isolated human intracranial arteries (fig 2). Potency was similar for all vessels. Ketanserin (bath concentrations up to 2×10^{-4} M) had no vasoconstrictor activity on these vessels (agonist activity) and completely inhibited 5-HT (2×10^{-8} M) contractile activity as shown in fig 3 (right record). Partial antagonism of contractions induced by post-SAH CSF is also demonstrated. The similar activity of methysergide on the rat stomach strip is illustrated in fig 4.

Fig 3 The effects of ketanserin, a specific 5-HT antagonist, on contractions of the isolated human basilar artery elicited by CSF collected during the occlusion of a ruptured cerebral aneurysm. Contractions of a single basilar artery (volts, ordinate; minutes, abscissa) were obtained to 5-HT and CSF collected during occlusion of a ruptured cerebral aneurysm (SUCTION TRAP CSF). Addition of ketanserin (2×10^{-5} M) to the bath partially inhibited responses to CSF and wholly inhibited responses to 5-HT. The tissue remained responsive to prostaglandin E_2 (PGE_2).

Fig 4 Effects of methysergide, a specific 5-HT antagonist, on contractions of the isolated rat stomach strip elicited by CSF collected during the occlusion of a ruptured cerebral aneurysm. Responses are plotted in the format of fig 3. 5-HT and CSF contracted the rat stomach strip. Methysergide ($2 \times 10^{-5} M$) partially inhibited CSF induced contractions, and completely inhibited 5-HT responses. The tissue remained sensitive to prostaglandin E_2 (PGE_2).

Table 2 Constrictor activity of post-SAH CSF on isolated human intracranial arteries and rat stomach fundus: values for 5-HT concentration determined by inhibition of constrictor responses with ketanserin and methysergide

Patient	Vasoconstrictor activity of CSF (5-HT equivalents nmol/l)		% Inhibition of response by 5-HT antagonists		5-HT Concentration in CSF (nmol/l)		Outcome
	A	B	C	D	E	F	
	ARTERY	FUNDUS	ARTERY	FUNDUS	ARTERY	FUNDUS	
4	5.4	20.0	70	79	38	158	excellent
5	11.0	9.9	58	72	64	71	good
6	41.0	12.0	95	83	390	100	excellent
7	170.0	4.0	100	63	1700	25	excellent
8	12.0	26.0	81	95	97	246	excellent
9	9.5	3.8	82	47	78	3	excellent
10	75	3.8	49	47	370	18	excellent
12	0.4	23.0	60	26	2	60	excellent
13	37.0	44.0	85	91	314	401	excellent
Mean $\pm$ SEM	40.1 $\pm$ 18.0	16.3 $\pm$ 4.5	75.6 $\pm$ 5.8	67.0 $\pm$ 7.7	339.2 $\pm$ 177.3	120.2 $\pm$ 43.4	

Vasoconstrictor response is expressed in terms of 5-HT equivalents (nmol/l bath concentration). The bath concentration of ketanserin was $2 \times 10^{-5} M$ added to isolated human intracranial arteries (ARTERY) 5 minutes before challenge with CSF. The bath concentration of methysergide was $2 \times 10^{-6} M$ added to isolate rat stomach strips (FUNDUS) as above. 500 μ l aliquots of CSF were added to a 5 ml bath. The values for the concentration of 5-HT-like material are given as nmol 5-HT/l CSF. Each value is a single determination.

Brief details of the patients in the study are given in table 1. The contractile responses of isolated human intracranial arteries to CSF from these patients is given in table 2, together with the percentage inhibition of contraction caused by incubation of the

artery with ketanserin. Mean values are given $\pm$ standard error (SE) where appropriate. The vasoconstrictor activity of each CSF sample was measured in terms of the equivalent amount of 5-HT required to induce a contraction of the same magnitude as that of

Table 3 Comparative estimations of 5-HT and derivatives in CSF by rat fundus bioassay and HPLC-ED methods

Patient	Fundus bioassay (5-HT nmol/l)	HPLC-ED determination (total indoles nmol/l)
14	640	433.0
15	555	422.6
16	38	240.0
17	220	183.8
Mean $\pm$ SEM	363.3 $\pm$ 141.3	319.9 $\pm$ 63.4
Correlation coefficient $r = 0.9$		

Fundus bioassay results are presented in the format of table 2. Total indoles (the sum of 5-HT and 5-HIAA concentrations) were measured by high performance liquid chromatography with electrochemical detection (HPLC-ED) against external standards. Each value is a single determination. The mean values for each assay were not significantly different ($p > 0.5$ Student's t test).

CSF itself (5-HT equivalents nmol/l) on the two tissues (columns A and B). The mean inhibition, by ketanserin, of CSF produced contraction was $75.6 \pm 5.8\%$ (column C). Table 2 also shows the data obtained using the isolated rat stomach fundus preparation. The mean inhibition of contractile response by incubation with methysergide was $67.0 \pm 7.7\%$ (column D). These values were not found to be significantly different (Student's t test $p > 0.5$).

As the component of the CSF-induced contraction attributable to 5-HT was completely inhibited by ketanserin (intracranial artery preparation) or methysergide (rat stomach strip), then values for the 5-HT-like activity of each CSF sample were obtained by multiplying the total vasoconstrictor activity by the percentage inhibition due to the antagonist, and a dilution factor. These values are shown in columns E and F, table 2.

The results of the HPLC-ED assay for total indoles (the sum of 5-HT and 5-HIAA concentrations) in the CSF are shown in table 3 with the equivalent values for 5-HT-like vasoconstrictors obtained by rat fundus bioassay. There was a high correlation ($r = 0.9$, $n = 4$) between bioassay for 5-HT-like material, and the concentration of indoles in the CSF detected by HPLC-ED although the actual quantities differed slightly (but not significantly, $p > 0.5$ Student's t test). There was no obvious correlation between either the total vasoconstrictor activity in the CSF sample, the concentration of 5-HT-like material or total indoles, and the clinical outcome of the patient.

Discussion

Of many substances thought to be involved in the aetiology of the cerebral vasospasm syndrome after SAH, the role of 5-HT is least clear. Buckell⁸ first detected 5-HT in haematoma fluid from subarachnoid haemorrhage patients in 1964, but since that time, its importance in the development of spasm

has not been established. Experimental evidence is largely contradictory. In animals where the blood-brain barrier is intact, intra-arterial 5-HT has been demonstrated to cause increased perfusion,²¹ implying vasodilatation. Zervas *et al*²² showed that blood from reserpinised animals (depleted of monoamines including 5-HT) was incapable of producing prolonged arterial narrowing when released into the cisternal space of dogs and monkeys. However, Boullin *et al* failed to abort autologous blood induced prolonged spasm in baboons by intracisternal administration of BW 501C67, an experimental 5-HT antagonist without agonist activity on cerebral arteries.²³ Furthermore, injection of 5-HT into the subarachnoid space of naïve animals did *not* produce prolonged (2 or more days) vasospasm, but did intensify chronic spasm in animals pretreated with blood intracisternally.²²

This data, generated from work in a number of animal species, and confined to the immediate (experimental) post-SAH phase, is in marked contrast to clinical investigations, which have concentrated on the post-operative occurrence of cerebral vasospasm. Two longitudinal studies of the vasoactivity of cerebrospinal fluid in patients after surgery for aneurysm clipping have been performed in this centre.^{2,3} In both series, 5-HT-like activity was detected in the CSF, together with unidentified vasoconstrictor substances, but was not quantified.

The data we have presented demonstrate that 70% of the vasoactivity in the CSF sampled per-operatively can be accounted for by 5-HT-like substances active at the cerebral artery receptor (antagonised by ketanserin) and rat fundus receptor (antagonised by methysergide). There was some variability between the values for the concentration of 5-HT-like material in the CSF obtained by rat fundus, and by human artery bioassay, the latter giving higher values. This might be explained by variations in the sensitivity of individual human arteries to 5-HT vasoconcentration, as there was a good correlation between fundus bioassay and HPLC-ED measurement of 5-HT and metabolite concentrations in the CSF of four patients (table 3). Such variations of response to 5-HT and CSF have been reported in the responses of isolated human pial arteries.²⁴ Furthermore, there was very good agreement between the percentile inhibition of CSF induced contractions by ketanserin (isolated human intracranial arteries: table 2, column C) and inhibition by methysergide (rat stomach strip: table 2, column D).

In the present study, CSF samples were obtained from around the region of the ruptured aneurysm, where vascular spasm is most likely to occur. The data presented show no obvious correlations between the concentration of 5-HT-like material in the cisternal

CSF, and either pre-operative spasm or the outcome of aneurysm surgery. However, Voldby *et al*²⁵ have recently shown that high levels of 5-HT (26 and 60 nmol/l) measured by radioimmunoassay in per-operative cisternal CSF samples were associated with severe post-operative cerebral vasospasm in patients with ruptured aneurysms. These values are lower than those reported here (intracranial artery bioassay, mean $\pm$ SE 339.2 ± 177.3 nmols/l $n = 9$; rat fundus bioassay, 195.0 ± 59.2 $n = 13$; HPLC-ED 319.9 ± 63.4 $n = 4$). This disparity may represent differences in assay technique or diffusion gradients of 5-HT and metabolite across different sites of CSF collection (26).

Our results, and the findings of Voldby *et al*²⁵ suggest that 5-HT-like substances may impose a considerable vasoconstrictor stress on the cerebral vasculature, post-operatively. We have shown that ketanserin is an inhibitor of 5-HT and CSF-induced vasoconstriction in isolated human intracranial arteries at 2×10^{-5} M (inhibition may be more potent with *in vivo* use). Furthermore, ketanserin has no intrinsic contractile activity on isolated human intracranial arteries. Therefore, the pharmacological consequences of high concentrations of 5-HT, in the CSF, on the cerebral vasculature might be prevented or reversed by ketanserin. It may thus be regarded as a candidate for prophylaxis or therapy of post-operative spasm, where 5-HT is implicated, by systemic or intracisternal administration.

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