Nature of the vasoactive substance in CSF from patients with subarachnoid hemorrhage

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The purpose of this experiment was to study the nature of vasoactive substance in cerebrospinal fluid (CSF) from patients with aneurysmal subarachnoid hemorrhage. The authors have examined the effects of disulfide bond-reducing agents, a sulfhydryl group oxidizing agent, and specific antagonists of so-called "classical" neurotransmitters on the in vitro isometric contraction of canine basilar arterial strips induced by bloody human CSF. The disulfide bond-reducing agents dithiothreitol (10⁻⁴ M) and dithioerythritol (10⁻⁴ M) suppressed the contraction induced by bloody CSF by an average of 40.3% and 61.2%, respectively. This suppression was achieved under conditions that did not alter KCl-induced contraction. The sulfhydryl group oxidizing agent, 5,5'-dithiobis-(2-nitrobenzoic acid), 10⁻⁴ M, reversed the inhibitory effect of dithioerythritol on the contractile response to bloody CSF. No significant suppression of any response in any preparation was observed with methysergide (10⁻⁷ M), mepyramine (10⁻⁷ M), phenoxybenzamine (10⁻⁵ M), propranolol (10⁻⁶ M), or atropine (10⁻⁶ M).

These results indicate that disulfide bonds in the arterial smooth-muscle cells are involved in the contractile responses of canine basilar artery to bloody CSF. Prostaglandins, hemoglobin, and lipid hydroperoxides may all be spasmogens in bloody CSF, while serotonin, histamine, norepinephrine, and acetylcholine are probably not involved.

KEY WORDS • cerebral artery • cerebrospinal fluid • subarachnoid hemorrhage • vasospasm • disulfide bond

Cerebral vasospasm is a well known cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH) following rupture of a cerebral aneurysm.²⁰,²⁵ In spite of extensive efforts, the precise mechanism of cerebral vasospasm has not yet been clarified. It has recently been reported that cerebrospinal fluid (CSF) obtained from patients with SAH produced sustained constriction of cerebral arteries in vitro¹⁶-⁸,¹⁶ and in vivo,⁹ and that bloody CSF samples obtained from patients with angiographic evidence of vasospasm were significantly more active than those obtained from patients without vasospasm.⁷,¹⁶ Therefore, it seems important to examine the nature of the vasoactive substance in bloody CSF.

Boullin, et al.,⁵ have shown that the sustained constriction induced by bloody CSF was not affected by the selective antagonists of norepinephrine, serotonin, isoproterenol, histamine, acetylcholine, or angiotensin II. Only nonspecific vasodilators, such as calcium-blocking agents,⁹,¹⁶ prostacyclin,⁴ or diatrizoate meglumine,²⁴ have been reported to inhibit the vasoconstriction induced by bloody CSF. In addition to the so-called "classical" transmitters, several prostaglandins, hemoglobin, and lipid hydroperoxides have also been suggested as possible spasmogens in bloody CSF. Recently it was reported that disulfide bond-reducing agents selectively inhibited the contractile responses of vascular smooth muscle to prostaglandin (PG) F₂α, A₂, B₂, E₁, D₂,¹¹ hemoglobin,¹⁰ and lipid hydroperoxides.³ If these prostaglandins, hemoglobin, or lipid hydroperoxides are involved in the contractile responses of cerebral arteries to bloody CSF, the vasoconstriction by bloody CSF should be inhibited by disulfide bond-reducing agents.

In an attempt to study the nature of vasoactive substances in bloody CSF, we examined the effects of disulfide bond-reducing agents, a sulfhydryl group oxidizing agent, and specific antagonists of so-called "classical" transmitters on the contraction of canine basilar arterial strips induced by bloody CSF.
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FIG. 1. Effects of specific antagonists of so-called “classical” neurotransmitters on the contraction of canine basilar arterial strips induced by human bloody cerebrospinal fluid (CSF). Upper: A: Reference contraction induced by 40 mM KCl. B: Contraction induced by 1 ml of bloody CSF, obtained on Day 14 from a patient without vasospasm. C, D, and E: Effects of pretreatment of the arterial strips with atropine (10^-6 M), propranolol (10^-6 M), phenoxymethylamine (10^-5 M), mepyramine (10^-7 M), or methysergide (10^-7 M) on the contraction induced by the same bloody CSF as in B. The contraction induced by the “nonvasospasm” bloody CSF (B) was not inhibited by pretreatment with atropine, propranolol, phenoxybenzamine, mepyramine, or methysergide. Mepyramine and methysergide slightly increased the basal tension of the arterial strips. Lower: A: Reference contraction induced by 40 mM KCl. B: Effects of phenoxybenzamine (10^-5 M), propranolol (10^-5 M), atropine (10^-6 M), methysergide (10^-6 M), and mepyramine (10^-6 M) on the contraction induced by bloody CSF obtained on Day 5 from a patient with vasospasm. The contraction induced by the “vasospasm” bloody CSF was not reversed by the specific antagonists of “classical” neurotransmitters. Methysergide slightly increased the tension of the contracted strips.

Materials and Methods

Samples of CSF were drawn from patients with SAH following rupture of a cerebral aneurysm either by lumbar puncture, or through cisternal or ventricular drainage catheters. The purpose of this experiment was to study the nature of vasoactive substances in bloody CSF, not to relate the vasoconstrictile activity of the bloody CSF to changes in the neurological state of each patient, to the onset of angiographic spasm, or to the periods after SAH. Accordingly, we were not concerned with the methods, or the timing of the collection of CSF, or with the presence of angiographic spasm. The
CSF was centrifuged for 15 minutes at 3500 rpm and the supernatant was collected, frozen, and stored at -20°C until needed.

Adult mongrel dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg) and sacrificed by exsanguination from the femoral artery. The brain was rapidly removed, and the basilar artery was dissected from the brain stem under magnification. The artery was placed immediately in oxygenated modified Krebs' bicarbonate solution at 37°C and maintained in an atmosphere of 95% O₂ and 5% CO₂.

The arteries were cut into 4-mm long ring segments, which were suspended between L-formed stainless steel holders in organ baths with a 10-ml working volume under a resting tension of 2 gm. The preparations were allowed to equilibrate at 37°C for 90 minutes before use. The composition of the nutrient-modified Krebs' solution was as follows (mM): NaCl 120, KCl 4.5, MgSO₄ 1.0, CaCl₂ 2.5, NaHCO₃ 27.0, KH₂PO₄ 1.0, and dextrose 10.0. The pH of the solution ranged from 7.40 to 7.45.

Bloody CSF, 1 ml, was added to 9 ml of the modified Krebs' solution in the organ bath and the contractions were recorded isometrically using a Nihon-Kohden force-displacement transducer, an amplifier, and an ink-writing recorder.* The contractions induced by bloody CSF are expressed as the percentage of the contraction elicited by a standard dose of 40 mM KCl.

Preparations were exposed for 5 minutes to selective antagonists of the so-called "classical" transmitters: phenoxymethylamine hydrochloride, methysergide, propranolol, mepyramine, and atropine. For investigating the effects of disulfide bond-reducing agents and a sulfhydryl group oxidizing agent on the vasocontractions induced by bloody CSF, dithiothreitol (DTT), dithioerythritol (DTE), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), alone or in combination, were added to the organ bath and allowed to remain in contact with the tissues for 20 minutes. The DTT, DTE, and DTNB were dissolved using 50 mM phosphate buffer solution (pH 7.4). Exposure of any vessel to DTT or DTE necessitated the preparation of a new specimen for continued investigation of reactivity to bloody CSF, since those agents had long-acting effects on cerebral vessels, causing the contractile response recovery time to be very slow.

The vasocontractile activity of each CSF sample was tested, and those with vasocontractile activity too weak to evaluate the inhibitory effects of the drugs used in this experiment were discarded. The drugs used in this study were DTT, DTE, DTNB, atropine, propranolol, phenoxybenzamine hydrochloride, mepyramine maleate, and methysergide.
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FIG. 5. Inhibition by DTNB of the effect of dithioerythritol (DTE) on bloody cerebrospinal fluid (CSF)-induced contractile response of canine basilar artery. The DTE diminishes the contractile response of the artery to bloody CSF. The DTNB reduces the inhibition caused by DTE when administered following DTE, and eliminates the inhibitory effect of DTE when administered simultaneously. W = wash.

FIG. 4. Influence of dithioerythritol (DTE) on bloody cerebrospinal fluid (CSF)-induced contractions. The abscissa shows the contractile activity of each CSF sample, expressed as a percentage of the contraction elicited by a standard dose of 40 mM KCl. The ordinate shows the percent inhibition of contraction caused by DTE (10^-4 M) for each sample. Incubation of basilar arterial strips in DTE (10^-4 M) suppressed by an average of 61.2% the contractions induced by bloody CSF samples obtained from 18 subarachnoid hemorrhage patients.

Results

Exposure of canine basilar artery strips to phenoxycbenzamine (10^-5 M), propranolol (10^-6 M), methysergide (10^-7 M), mepyramine (10^-7 M), and atropine (10^-6 M) did not inhibit the contractile responses of the arterial strips to bloody CSF tested (in four tests each). Additions of these antagonists to arterial strips already contracted by bloody CSF did not reverse the CSF-induced contractions (in four tests each) (Fig. 1).

Constrictions induced by KCl were not affected by the exposure of arterial strips to either DTT (10^-4 M) or DTE (10^-4 M) for 20 minutes (Fig. 2). The effects of DTT on contractions induced by bloody CSF were evaluated in 17 CSF samples collected from 13 patients with SAH. Exposure of arterial strips to DTT (10^-4 M) inhibited the contractile responses of the arterial strips to bloody CSF (Fig. 3). Bloody CSF-induced contractions that were under 50% of the standard contraction by 40 mM KCl were inhibited with DTT (10^-4 M) by 47.6% (in seven tests). Contractions induced by bloody CSF that were over 50% of the standard contraction by KCl were inhibited by 35.2% (in 10 tests). However, no statistical difference of the inhibition rate by DTT was observed between those two groups. The overall inhibition rate by DTT (10^-4 M) was 40.3% (in 17 tests).

The effects of DTE on vasoconstriction induced by bloody CSF were evaluated in 19 samples drawn from 18 patients with SAH. All the CSF-induced contractions of the arterial strips tested were inhibited by DTE (10^-4 M) (Fig. 4). The contractions that were under 50% of the standard contraction by KCl showed an average 67.7% inhibition by 10^-4 M DTE (in 11 tests). On the other hand, DTE (10^-4 M) caused 52.3% inhibition of the bloody CSF-induced contractions that were over 50% of the standard contraction by KCl (in eight tests). No statistical difference of the inhibition rate by DTE, however, was observed between the two contraction groups. The overall inhibition rate by DTE (10^-4 M) was 61.2% (in 19 tests).

In five of six CSF samples tested, DTNB (10^-4 M) reversed the inhibitory effects of DTE (10^-4 M) on the contractile responses to bloody CSF. In addition, bloody CSF-induced contractions were not inhibited when arterial strips were exposed to DTE in combination with DTNB (Fig. 5). Exposure of arterial strips to DTNB (10^-4 M) alone did not have any effect on the bloody CSF-induced contractions.

Discussion

Bloody CSF collected from patients with SAH has been reported to produce sustained contractions of canine cerebral artery, rat stomach fundus, cat pial arterioles, and human cerebral artery. Despite extensive efforts, the vasoactive substances responsible for this contraction have not been identified.

In this experiment, the specific antagonists of so-called "classical" transmitters methysergide, mepyramine, phenoxycbenzamine, propranolol, and atropine did not inhibit or reverse the contractions of canine basilar arterial strips produced by bloody CSF. The same results have previously been reported by Boullin, et al., and Okwuasaba, et al. Boullin, et al. also reported that sarcosine-alanine-angiotensin II did not inhibit bloody CSF-induced contractions of canine cerebral arteries. These results indicate that serotonin, histamine, norepinephrine, acetylcholine, and angiotensin II are probably not involved in bloody CSF-induced contractions.

The results of this experiment also revealed that the disulfide bond-reducing agents, DTT and DTE, inhibited contractile responses of canine arterial strips to bloody CSF, and that a sulfhydryl group oxidizing agent DTNB reversed the DTE-induced inhibition of the...
vasoconstrictions caused by bloody CSF. These findings indicate that disulfide bonds in the arterial smooth-muscle cells are involved in the contractile responses of canine basilar artery strips to bloody CSF. Exposure of preparations to DTT or DTE may inhibit the contractile responses of the arterial strips to bloody CSF by reducing the disulfide bonds in vascular smooth-muscle cell membrane to sulfhydryl bases. Addition of DTNB to muscle strips exposed to DTT or DTE will oxidize the sulfhydryl bases to disulfide bonds, and may reverse the DTT- or DTE-induced inhibition. For clarifying this hypothesis, further studies, including measurement of tissue sulfhydryl groups, are warranted.

In contrast to the inhibitory effect that DTT or DTE exerts on the contractile responses induced by bloody CSF, these disulfide bond-reducing agents had no effect on contractions produced by KCl. Although DTT does not alter the contractile response of rabbit renal arteries to norepinephrine or KCl, it does inhibit the contractions produced by PGF2α, A2, B2, E1, D2, and angiotensin II. In addition, DTNB reverses the DTT-induced inhibition of contractile responses to those prostaglandins. It has also been reported that DTT inhibited the contractile responses of rabbit aortic strips to lipid hydroperoxides, and those of canine basilar arteries to hemoglobin. Therefore, it appears that DTT or DTE acts through specific inhibitory mechanisms rather than exerting nonspecific inhibitory effects such as local anesthetic or calcium-blocking agents. These findings are consistent with the concept that prostaglandins, oxyhemoglobin or lipid hydroperoxides may be possible spasmogens in bloody CSF. All these substances exert vasoconstrictive activities on the cerebral vessels. With the exception of PGF2α, the concentrations of these vasoactive substances in CSF after SAH have not been evaluated in detail. Such studies should be performed in an attempt to identify the spasmogens in bloody CSF.

References

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