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Contractile Effects of Hemoglobin-Free Human Cerebrospinal Fluid on Isolated Porcine Cerebral Arteries

Yung Hong Baik, Seon Young Kang, Hyun Kook, Sang Keun Chyung, Young Johng Kook, and Sam Suk Kang[1]

Department of Pharmacology and [1]Neurosurgery, Research Institute of Medical Sciences, Chonnam University Medical School, Kwangju 501-190, Korea

To elucidate the mechanism involved in the cerebral vascular spasm following subarachnoid hemorrhage (SAH), the effects of the cerebrospinal fluid (CSF) obtained from the SAH patients on the resting tension and its influence on the contractile responses to various vasoactive agents and to hypoxia were investigated in isolated porcine cerebral arteries. All the CSFs containing hemoglobin (Hb) produced contraction and some Hb-free CSFs also elicited contraction. When the Hb-free CSF was separated by microfilter, the filtrate of $<30,000$ MW did not produce contraction, while the fraction above 30,000 MW elicited more marked contractile responses than the unfractionated CSF. The CSF contraction was significantly attenuated in the presence of indomethacin or nimodipine, whereas the contractions induced by KCl, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), or endothelin-1 (ET-1) were not affected by the CSF pretreatment. However, the contractile responses induced by 5-hydroxytryptamine (5-HT) and phenylephrine (PE) were markedly potentiated by the pretreatment. Hypoxia-induced vasoconstriction was significantly potentiated by the pretreatment with either unfractionated CSF or the CSF fraction of above 30,000 MW. These results suggest that unknown vasocontractile substance(s) exists in the Hb-free CSF and that the substance, with its MW above 30,000, is activated by hypoxia and acts synergistically with 5-HT and PE, and that extracellular calcium influx and cyclooxygenase are also involved in the cerebral vasoconstrictory effect of Hb-free CSF.

Key Words: Subarachnoid hemorrhage, Cerebrospinal fluids, Isolated porcine cerebral artery, Hb-containing CSF, Hb-free CSF

INTRODUCTION

Cerebral arterial spasm that invariably ensue subarachnoid hemorrhage (SAH) leads to cerebral ischemia, posing a grave threat to the afflicted. However, the nature and the pathophysiology of the spasm remain obscure in spite of extensive research efforts, which have been centered mostly in two categories: the influence of hypoxia and the mediation of endogenous bioactive substances.

Following SAH, the cerebral arteries are exposed to lysed blood and ischemic condition. In vascular smooth muscles, hypoxia is one of the components of ischemia. In elucidating the influence of hypoxia on the cerebral vessels, Kim (1993) observed that in the isolated porcine cerebral arteries, hypoxia elicits contraction followed by relaxation. But, whether the hypoxia responses are mediated by some vasoactive substances, and if then, from where they are derived, the vessel or adjacent tissue, still remains unsettled (Busse et al, 1983; Holden & McCall, 1984; Madden et al, 1992).

Recently, the subarachnoid clot attracted attention as a possible source of vasoconstrictive substance causing cerebral arterial spasm after SAH. It has been reported that 5-hydroxytryptamine (5-HT) and thromboxane A_2 play important roles in cerebral vasocon-

Corresponding to: Yung Hong Baik, Department of Pharmacology, Chonnam University Medical School 5 Hak-dong, Dong-ku, Kwangju 501-190, Korea (Tel) 062-220-4232, (Fax) 062-232-6974, E-mail: yhbaik@orion.chonnam.ac.kr

striction (Kassell et al, 1985; Von Holst et al, 1982; Zervas et al, 1975). Otherwise, Some reports have suggested that extravasated erythrocytes or their breakdown products, such as hemoglobin (Hb), methemoglobin (MetHb), oxyhemoglobin (OxyHb) and ferrous heme protein cause prolonged cerebral vasoconstriction (Osaka, 1977; Wellum et al, 1982).

When blood enters the subarachnoid space, blood pigments are released into the cerebrospinal fluid (CSF). Thus, research efforts have been focused on identifying vasoconstrictor materials in CSF from the SAH patients. CSF samples collected from the patients were tested on human and canine cerebral arterial segments for contractile activity (Allen et al, 1976; Boullin et al, 1976). In Hb-containing CSF, there is little doubt that blood pigments released are important in the pathogenesis of arterial spasm. However, the effects of Hb-free CSF on the cerebral arteries have not been well studied so far. This prompted us to examine the effects of human CSF on the cerebral vessels, on the responses induced by various vasoactive agents, and on the hypoxia-induced responses.

METHODS

Preparations and tension experiments

Heads of pigs (male Yorkshire, weighing 40~45 kg), obtained from a local slaughterhouse, were cooled immediately after decapitation by infusing cold saline into foramen magnum. The entire brain was rapidly excised from the skull, and basilar and circle of Willis arteries were carefully dissected out. The isolated arteries were placed in cold (4°C) physiological salt solution (PSS) and cleaned of connective and adipose tissues under stereoscope. Then, the arteries were cut into rings of 3~4 mm in length. The ring segments were mounted in an organ bath by sliding them over two parellel stainless-steel hooks. The lower hook was fixed on the bottom of the bath and the upper was connected to an isometric transducer (Grass FT03) with thread, and the tension was recorded on polygraph (Grass 7D). The double jacketed organ bath was connected to a circulator and filled with 4 ml PSS saturated with 95% O_2 + 5% CO_2 at 37°C (pH 7.4). All arterial rings were equilibrated for 2 hrs and maintained under the resting tension of 1 g. The rings which did not respond to 50 mM KCl were discarded. Hypoxia was produced by substituting 95% N_2 + 5% CO_2 (0% O_2) for aeration. After the introduction of the hypoxic gas, the basal tone was readjusted. pO_2 of bath fluid was measured with gas analyzer (Ciba-Corning 228 Blood Gas System). The pO_2 was 486±23.9 mmHg (n=4) during normal oxygenation and it decreased to 175±9.3, 157±5.4, 74±3.0 and 49±2.1 mmHg, at 1, 2, 5 and 10 min after changing to 0% O_2.

CSF studies

The samples of CSF were obtained from the patients with intracranial diseases, who had been admitted to the Department of Neurosurgery, Chonnam University Hospital from March through September 1994, and they were classified into two groups: Hb-containing CSF and Hb-free CSF. The presence of Hb was detected by spectrophotometry (Fig. 1). The Hb-free CSFs were frozen and stored at −20°C, and warmed immediately prior to the experiment. Some of the Hb-free specimens did not produce contractions, and for the present study the CSF specimens that elicited prominent vasocontraction stronger than 0.1 g were employed. In some experiments, the Hb-free CSF was fractionated by passing them through molecular sieve of M.W.

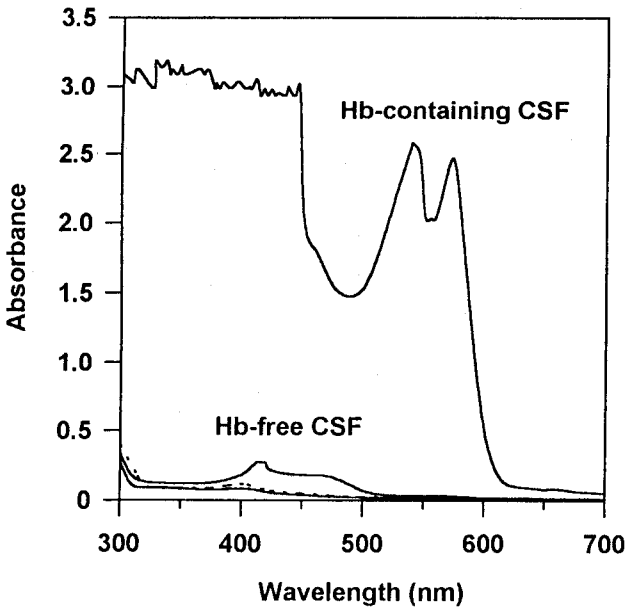

Fig. 1. Absorbance spectrum of the CSF samples obtained from the SAH patients. Hemolyzed sample shows the typical two-peak patterns of the OxyHb.

30,000 (Amicon, MWCO: 30,000) and was tested for the contractile activities.

Drugs

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 5-hydroxytryptamine (5-HT) and endothelin-1 (ET) were obtained from Sigma (St. Louis, MO). All drugs were dissolved and diluted with distilled water.

The composition of PSS was NaCl 115, $NaHCO_3$ 35, KH_2PO_4 1.2, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 2.5, EDTA 0.03, glucose 11.1 mM.

Statistical analysis

Statistical significances were examined by Student's unpaired t-test and two-way ANOVA with repeated measure. $p<0.05$ was considered significant.

RESULTS

Effects of CSF samples on the tension of porcine cerebral artery

All Hb-containing CSFs from the SAH patients produced contraction in a dose-related fashion. On the other hand, some of the Hb-free CSFs elicited marked contraction, whereas others produced no vasoconstriction (data not shown). Fig. 2 shows contractile responses to Hb-free CSF in relation to the amount of the sample cumulatively added up to 1 ml. Fig. 2 also shows further that when the CSF was filtered with the molcular sieve of M.W. 30,000 no contraction was elicited by the filtrate, while the remaining CSFs that retain substances above M.W. 30,000, did produce markedly augmented contractions.

Influence of various blockers on the CSF-induced contraction

The contraction elicited by the Hb-free CSF was

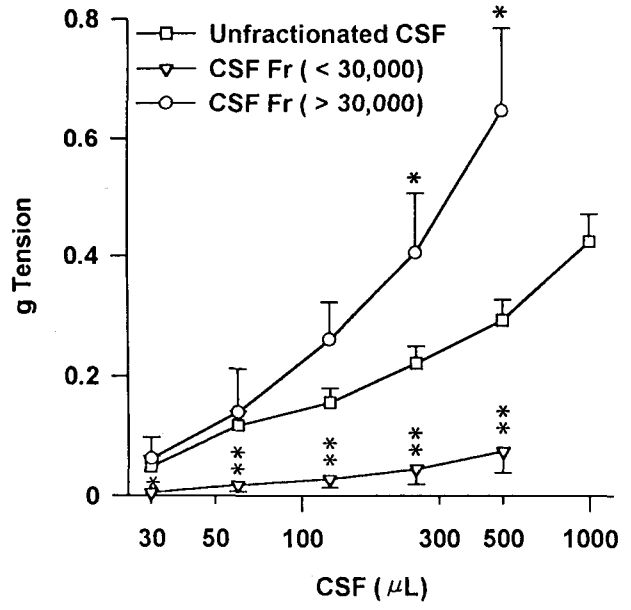

Fig. 2. Responses of the isolated porcine cerebral arterial rings to cumulative addition of the Hb-free CSF. The quadrangles represent the responses to the unfractionated CSF (Control); the triangles denote the CSF Fr < 30,000 that contains molecules below 30,000; and the circles indicate the fraction above 30,000 MW (CSF Fr > 30,000). Each point represents mean ± SEM from 30 preparations. Asterisks indicate significant diffrences from Control (*$p<0.05$; **$p<0.01$)

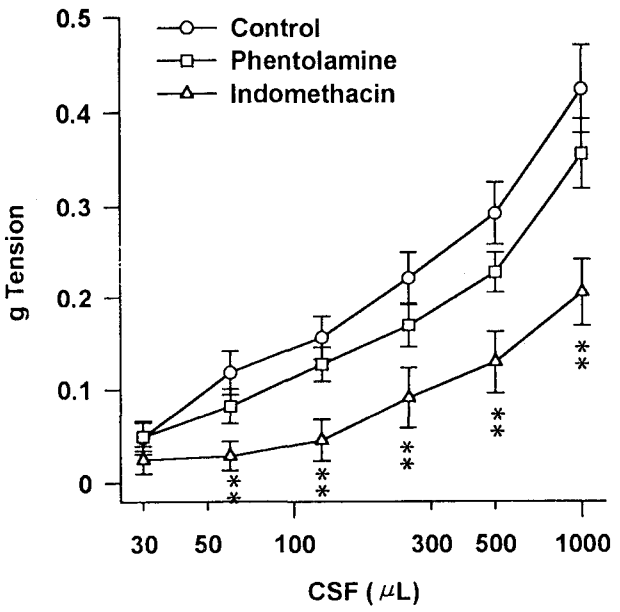

Fig. 3. Influence of pretreatment with phentolamine or indomethacin on the contractile response of the arterial rings to unfractionated CSF. The circles represent the control group with no pretreatment; the quadrangles the phentolamine (10^{-6} M) pretreated; and the triangles indomethacin (10^{-6} M) pretreated. Mean ± SEM from 11 ring preparations. Other legends as in Fig 2.

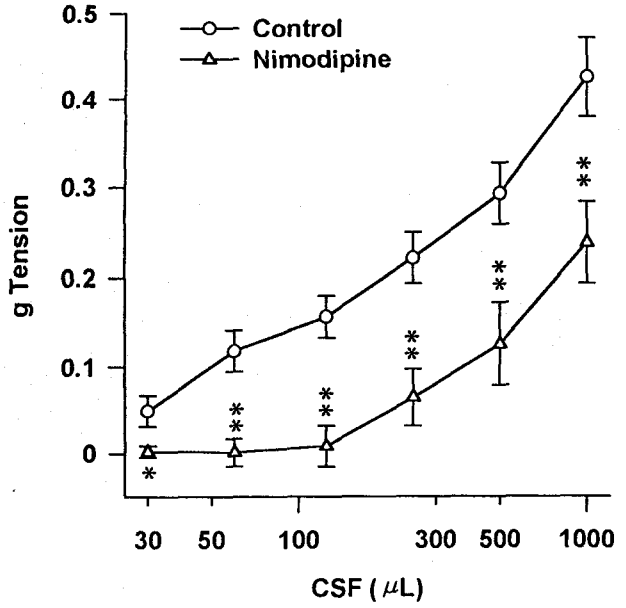

Fig. 4. Influence of nimodipine (10^{-6} M) pretreatment on the contractile response of the arterial rings to unfractionated CSF. Mean±SEM from 9 ring preparations. Other legends as in Fig. 2.

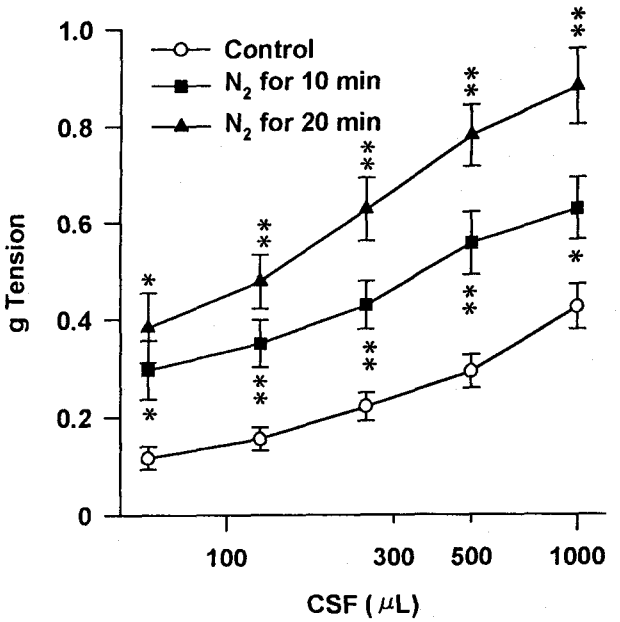

Fig. 5. Influence of hypoxia on the contractile response of the arterial rings to unfractionated CSF. Ten or 20 min after switching the aeration to N_2, CSF was added. Mean ±SEM from 17 ring preparations. Other legends as in Fig. 2.

not affected by the pretreatment with phentolamine (10^{-6} M), but it was significantly attenuated in the presence of indomethacin (10^{-6} M) as shown in Fig. 3. Fig. 4 shows that the CSF-induced vasoconstriction was markedly reduced by the pretreatment with nimodipine (10^{-6} M), a Ca^{2+} channel antagonist.

Influence of hypoxia on the CSF-induced contraction

In isolated porcine cerebral arteries, hypoxia produced an abrupt and transient contraction 3~5 min later, followed by relaxation reaching below the basal level within 7~8 min (data not shown). When the Hb-free CSF was added to the ring preperations under hypoxia, marked potentiation of vasoconstriction was observed. As shown in Fig. 5, the contraction was further strengthened at 20 min as the hypoxia progresses.

Influence of CSF on the contractions induced by various vasoconstrictors and hypoxia

The contractions induced by KCl, $PGF_{2\alpha}$, or ET were not changed by the pretreatment with CSF (Fig. 6). On the other hand, the contractile responses induced by 5-HT or PE were markedly potentiated by the CSF pretreatment (Fig. 7A). The hypoxia-induced

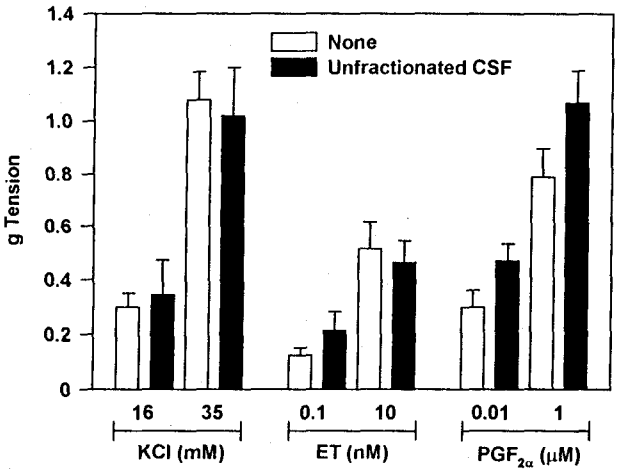

Fig. 6. Influence of 500$\mu\ell$ unfractionated CSF pretreatment on the contractile responses of the arterial rings to potassium chloride (KCl), endothelin-1 (ET), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Each column represents mean value from 26 preparations, with the bars indicating one SEM.

contraction was not affected by pretreatment with CSF-filtrate (<30,000), but it was significantly potentiated by pretreatment with either unfractionated CSF or the CSF fraction above 30,000 MW (Fig. 7B).

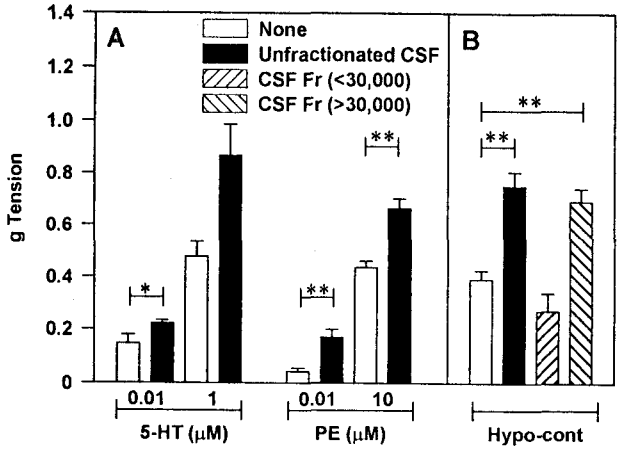

Fig. 7. A. Influence of pretreatment with unfractionated CSF on the contractile responses to 5-hydroxytryptamine (5-HT) and phenylephrine (PE). B. Influence of pretreatment with CSF fractions on the contractile responses to hypoxia (Hypo-cont). Hypoxic gas was introduced to the bath 5 min after pretreatment with 250$\mu\ell$ of each CSF fractions. CSF Fr $<$30,000 represent the fraction below 30,000 M.W; CSF Fr $>$30,000 the fraction above 30,000 M.W. Each column represents data from 28 rings. Significant differences were marked with asterisks (*$p<0.05$; **$p<0.01$).

DISCUSSION

Several lines of evidence support the concept that the development of cerebral arterial spasm after SAH is closely associated with Hb present in CSF (Duff et al, 1988; Mayberg et al, 1990). The Hb-containing CSFs of the patients suffering from SAH have been shown to cause cerebral arterial spasm (Allen et al, 1976; Brandt et al, 1981; Hunt et al, 1979), and it has also been demonstrated that injection of autologous arterial blood into the cisterna magna in experimental animals produced cerebral arterial spasm (Peerless & Kendall, 1993; Shigeno et al, 1991). In the present study we confirmed that all Hb-containing CSF from SAH patients evoked contractions of arterial preparations. It was further found, however, that Hb-free CSF from some patients can produce contractions. We also observed that the Hb-free CSF fraction above 30,000 MW elicited more marked contractile responses, while no contraction was observed with the filtrate containing substances of less than 30,000 MW.

Concerning the mechanisms of Hb or OxyHb in producing cerebral arterial spasm, several hypotheses have been presented. MacDonald et al (1991) speculated that through the oxidative conversion of the released Hb or OxyHb to MetHb, free radical such as superoxide is produced, which then may be responsible for the arterial spasm. They postulated that inhibition of endothelium-dependent relaxing factor (EDRF) and augmented release of ET caused by Hb or OxyHb lead to arterial spasm. Kassell et al (1985) and von Holst et al (1982) suggested that 5-HT or thromboxane A_2 released from blood component such as platelets is involved in producing vasoconstriction and spasm. However, our present finding that the CSF entirely free of Hb can also induce cerebral vasoconstrictions in some cases renders it difficult to interpret in light of the Hb- or OxyHb-implicated hypotheses. Rather, it is more likely that the cerebral vasospasm is not associated with blood products having low molecular weight such as monoamines, prostaglandins, angiotensin and peptides, and that certain vasoconstrictive substances not yet defined, such as polypeptides structurally related to Hb, is ultimately responsible for the spasm. The Hb-free CSF-induced contraction was not affected by phentolamine, but it was significantly attenuated by either indomethacin or nimodipine. These findings seem to indicate that the involvement of the cyclooxygenase product may play a role in the cerebral vasospasm. And, the extracellular Ca^{2+} may also be involved in some degree.

When the cerebral artery was pretreated with CSF, the vasocontractile effects of 5-HT and PE were markedly potentiated, whereas those induced by KCl, $PGF_{2\alpha}$ and ET were not changed. Presently, 5-HT is generally accepted as a potent spasmogen of brain arteries (Connor & Feniuk, 1989; Müller-Schweinitzer & Engel, 1983; Peroutka et al, 1986). Furthermore, the findings that 5-HT (Connor & Feniuk, 1989) and PE (Demirel et al, 1989) cause the release of EDRF led us to speculate that the abilities of 5-HT and PE to release EDRF may have been attenuated by the CSF. To substantiate the point, however, decrease in the contents of nitric oxide in the CSF-pretreated cerebral artery should be established. Hypoxia has been implicated in the vasospasm long since. The effects of hypoxia upon the isolated cerebral vessels may be mediated by the inhibition of EDRF released from the endothelial cells, augmentation of the release of ET (Katusic & Vanhoutte, 1986; Klaas & Wadsworth, 1989), vasoconstrictive activities of prostaglandins (Kim, 1993; Rubany &

Paul, 1984) or leukotrienes (Gu et al, 1991) and our previous experiments showing that the hypoxia-induced vasoconstriction in isolated porcine cerebral artery is endothelium-dependent (Kim, 1993). In the present study, we have shown that the CSF pretreatment potentiated the hypoxia-induced vasoconstriction. The similarity of the CSF potentiation of hypoxia vasoconstriction to those treated with 5-HT and PE may be indicative of a EDRF-involved mechanism. In other words, this may be due to potentiated inhibition of the EDRF release in addition to blocking the effect of released EDRF by synergistic effect of CSF to hypoxia. However, the filtered CSF containing subtances of less than 30,000 MW did not significantly affect the hypoxia response as contrasted with the marked potentiation of hypoxia-induced vasoconstriction by the CSF fraction of above 30,000 MW. This indicate that the potentiation may be due to the unidentified cerebral vasoconstrictor substance above 30,000 MW present in CSF.

In conclusion, our present findings may suggest that CSF from the SAH patients and also from patients not suffering from SAH contain certain unidentified cerebral vasoconstrictive substance(s) of $>30,000$ MW and that these substances may be activated by hypoxia and act synergistically with 5-HT and PE. It is further suggested that inhibition of EDRF, extracellular Ca^{2+} influx, and cyclooxygenase may participate in the CSF-induced vasoconstriction.

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