Pharmacological studies on relaxation of spastic primate cerebral arteries in subarachnoid hemorrhage

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Chronic cerebral vasospasm was induced in 16 monkeys by direct placement of a clot of autologous blood over the arteries of the circle of Willis on the right side. The middle cerebral arteries (MCA's) on the clot side all showed angiographic vasospasm, which was maximal 7 days after subarachnoid hemorrhage. Animals were sacrificed at this time and vascular responses to acetylcholine (ACh), histamine, and the calcium ionophore A23187 were studied in MCA rings from the clot (spastic) side and the non-clot (control) side. In control preparations with an intact endothelium, which had been precontracted by prostaglandin F2α (PGF2α), histamine and A23187 produced significant relaxation. The same concentrations of histamine and A23187 did not relax vascular tissues in which the endothelium had been mechanically removed. Acetylcholine did not produce a significant endothelium-dependent relaxation of primate MCA rings, but did relax rings of primate common carotid artery. Pretreatment with chlorpheniramine (an H1-receptor antagonist) prevented histamine-induced relaxation; however, cimetidine (an H2-receptor antagonist) had no inhibitory action. It thus seems that histamine mediates relaxation of intact MCA's mostly by an H1-receptor-mediated release of endothelium-derived relaxing factor (EDRF). Relaxations induced by histamine and A23187 in MCA's from the clot side were substantially reduced. Moreover, the small component of ACh-induced relaxation was also abolished. Endothelium-independent relaxation induced by glyceryl trinitrate (GTN) occurred in arteries from both the control and the clot sides. Constrictions induced by KCl and PGF2α were reduced on the clot side of the MCA's. These results suggest that subarachnoid hemorrhage influences both the generation of EDRF and the constriction of affected arteries. The small contraction which was elicited in spastic arteries was fully relaxed by GTN.

KEY WORDS • endothelium-derived relaxing factor • histamine • calcium ionophore • acetylcholine • subarachnoid hemorrhage • vasospasm • cynomolgus monkey

It has been demonstrated that experimental subarachnoid hemorrhage (SAH) and the administration of hemoglobin can impair endothelium-dependent relaxation. However, it remains controversial if the inhibition of endothelium-derived relaxing factor (EDRF) by hemoglobin is a critical cause of cerebral vasospasm or a result of prolonged vasospasm. Despite the fact that most arterial ring segment studies have shown the inhibitory effects on EDRF by hemoglobin and SAH, some investigators reported the ineffectiveness on EDRF of hemoglobin and SAH in a tissue-mounted perfusion study. The inconsistent results of EDRF studies may be due to the differences in the experimental conditions, animal species, and arteries tested. Since no data are available regarding EDRF in human cerebral arteries, the role of EDRF inhibition in the pathogenesis of vasospasm is still inconclusive. It was suggested that the monkey cerebral arteries have more pharmacological similarities to human cerebral arteries than do dog cerebral arteries.

The purpose of the present investigation was to compare relaxations of the clot side of middle cerebral arteries (MCA's) with those of the non-clot side of MCA's obtained from a primate model of SAH, which approximates very closely the situation in man. The emphasis of the study was on the comparison of endothelium-dependent relaxations.

Materials and Methods

Animal Model

Animal Groups and Operative Procedures. Sixteen female cynomolgus monkeys (Macaca fascicularis),
were conducted with strict adherence to the standards. Temperature was maintained at 37°C by a heating pad. Procaine penicillin (100,000 IU/kg) was given intra-vessels four times with a calibrated optical micrometer, and a mean value was determined. Arteries were measured bilaterally at the stem of the MCA's. Series measurements were recorded as a percentage of the baseline value in individual monkeys.

**In Vitro Studies**

**Arterial Preparation and Tension Recording.** The MCA's were isolated from the brains, and the MCA's and CCA's were quickly immersed in Krebs bicarbonate solution (KBS) equilibrated with 95% O₂ and 5% CO₂ at room temperature. The composition of the KBS was (mM): 132 Na⁺, 5.9 K⁺, 2.5 Ca²⁺, 1.2 Mg²⁺, 122.7 Cl⁻, 25.0 HCO₃⁻, 1.2 SO₄⁻, 1.2 H₂PO₄⁻, and 11.0 dextrose. The arteries were then cut into rings 2 mm long, according to the method previously described, and mounted vertically between small hooks in a water-jacketed tissue bath containing 10 ml KBS maintained at 37°C ± 0.5°C and bubbled with 95% O₂ and 5% CO₂. The optimal resting tensions applied to the rings were 0.5 gm for MCA rings and 1.0 gm for CCA rings. Contractions were recorded isometrically using strain gauges connected to a polygraph.

**Arterial Relaxation Response to ACh, A23187, Histamine, and GTN.** The basic experimental protocol employed a control period during which the intact arterial rings were contracted with concentration of prostaglandin F₂α (PGF₂α) close to the median effective dose and relaxed by the cumulative addition of acetylcholine chloride (ACh), A23187, histamine, and glyceryl trinitrate (GTN). At the end of each series of experiments, 10⁻⁴ M papaverine was added to attain maximum relaxation according to the report by Toda. Values relative to the papaverine-induced relaxation are presented in the text and figures. Preparations had been treated for 20 minutes with the histamine receptor antagonists chlorpheniramine (H₁-antagonist) and cimetidine (H₂-antagonist) before the response to histamine was obtained.

**Removal of Endothelial Cells.** The endothelial cells of the arterial rings were mechanically removed by a standard brief, gentle rubbing of the intimal surface with a No. 25 to 30 stainless steel rod with a diameter equivalent to the lumen of the arteries, as described elsewhere. Changes in endothelium function were

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† Tele-thermometer manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio.
Relaxation of spastic primate cerebral arteries confirmed by testing the A23187-induced dilatation at the end of each experiment.

Drugs. The drugs used were as follows: ACh, calcium ionophore A23187, histamine dihydrochloride, papaverine hydrochloride, PGF$_{2\alpha}$, chlorpheniramine maleate, cimetidine, and GTN. The A23187 was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO had no effect on the contractile response to agonists.

Statistical Analysis. The results were expressed as the mean ± standard deviation, and comparisons were made using Student’s t-test. A probability of 0.05 or less was considered significant.

Results

Angiography

On angiograms taken on Day 7 after SAH, severe vasospasm (mean vessel caliber 30% of baseline value) was observed in the MCA’s on the clot side in all animals studied. The contralateral MCA’s showed no vasospasm.

Relaxation Response to ACh, A23187, and Histamine

Rings of the MCA’s with endothelium from the non-clot side were contracted with PGF$_{2\alpha}$, and the addition of 10^-9 to 10^-7 M A23187 caused a dose-dependent relaxation (Fig. 1B). However, even at high concentrations, ACh did not produce significant relaxation in the MCA rings with endothelium from the non-clot side (Fig. 1A). Both ACh and A23187 induced significant relaxation in the intact CCA rings (Fig. 1). While ACh produced significantly greater relaxation in the CCA than in the MCA rings from the non-clot side (Fig. 1A), dose-response curves for A23187 were not different between MCA and CCA rings from the non-clot side (Fig. 1B). These results confirmed the previous finding that responses of intracranial arteries to muscarinic stimulation were appreciably different from that of extracranial arteries. Histamine, on the other hand, has a dilator effect on monkey, cat, and rabbit cerebral arteries. In view of the potential importance of histamine in cerebrovascular regulation, we determined the effects of histamine on MCA rings on the non-clot side in both intact and endothelium-denuded preparations. Figure 2 depicts the cumulative dose-response relationship, in both the presence and absence of endothelium, to relaxation stimulated by ACh, A23187, and histamine exhibited by MCA rings from the non-clot side that had been precontracted with PGF$_{2\alpha}$. Figure 2B and C clearly demonstrate that relaxation of the primate MCA’s by the calcium ionophore A23187 and by histamine is completely dependent on the presence of endothelial cells. However, ACh could not induce significant endothelium-dependent relaxation in primate MCA’s (Fig. 2A).

Type of Receptor Involved in Histamine-Induced Release of EDRF

The type of receptor (H$_1$ or H$_2$) in the MCA rings from the non-clot side was determined from the dose-response curves to histamine after pretreatment with the H$_1$-antagonist chlorpheniramine (10^-6 M) or the H$_2$-antagonist cimetidine (10^-5 M). The results show the inhibitory effect of chlorpheniramine but not of cimetidine, suggesting the involvement of H$_1$-receptors (Fig. 3).

Effect of SAH on Relaxation Induced by ACh, A23187, Histamine, and GTN

In the MCA rings from the SAH side, endothelium-dependent relaxation to A23187 and histamine was significantly reduced compared with the contralateral side (Fig. 4B and C). It is remarkable that even a small

Fig. 1. Dose-response curves showing relaxation of middle cerebral artery (MCA) on the non-clot side (open circles) and common carotid artery (CCA) rings (closed circles), both with endothelium. A: Acetylcholine (ACh)-induced relaxation during contractions induced by 3 x 10^-6 M prostaglandin (PG)F$_{2\alpha}$ for MCA and 10^-6 M PGF$_{2\alpha}$ for CCA rings. B: A23187-induced relaxation, same protocol as in A. Vertical lines are standard deviations; numbers in parentheses are number of preparations. * = p < 0.05.

Fig. 2. A: Dose-response curves showing relaxation to acetylcholine (ACh) with (closed circles) and without (open circles) endothelium on middle cerebral artery rings from the non-clot side. The difference is not significant. B: Dose-response curves showing relaxation to A23187, same protocol as in A. * = p < 0.05; † = p < 0.01. C: Dose-response curves showing relaxation to histamine (His), same protocol as in A. Vertical lines are standard deviations; numbers in parentheses are number of preparations. * = p < 0.05; † = p < 0.01.
relaxation induced by ACh was also abolished in the MCA rings on the clot side (Fig 4A). Although A23187-induced relaxation was abolished, the MCA rings from the SAH side could be relaxed by a higher concentration of histamine (Fig. 4C). Glyceril trinitrate, a well-known endothelium-independent relaxant, induced a significant relaxation in MCA rings from both the non-clot and clot sides (Fig. 4D).

**Effect of SAH on Contraction Induced by KCl and PGF$_{2\alpha}$**

In each case there was a highly significant reduction in the response of the MCA's on the clot side relative to those on the non-clot side (Fig. 5). The maximal responses to 60 mM KCl or 10$^{-5}$ M PGF$_{2\alpha}$ did not differ significantly in the rings from either the clot or the non-clot side.

**Discussion**

The principal findings of this work are that: 1) histamine and A23187 produced endothelium-dependent relaxation in MCA rings from the non-clot side and histamine caused a relaxation by an H$_1$-receptor-mediated release of EDRF; 2) although ACh did not sufficiently produce an endothelium-dependent relaxation of MCA rings from the non-clot side, ACh did produce significant relaxation in CCA rings; 3) relaxation induced by ACh, A23187, and histamine was substantially reduced in the MCA rings from the clot side, but GTN-induced relaxation was preserved; and 4) contractions induced by KCl and PGF$_{2\alpha}$ were reduced in the MCA rings from the clot side.

We are unaware of a previous demonstration that histamine and A23187 induce endothelium-dependent relaxation in MCA's of primates. Histamine has several potential sources of synthesis, storage, and release near cerebral blood vessels. Since it is a putative cerebral neurotransmitter, neuronal discharge may liberate histamine in the vicinity of cerebral resistance vessels. Mast cells located within perivascular spaces contain histamine, which may be released during pathological conditions. Blood basophils are also a potential source of histamine. Histamine may be contained in non-mast cell stores within smooth-muscle cell layers of cerebral vessels. Collectively, this suggests that certain
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physiological and/or pathological circumstances might involve histamine release and its subsequent action on the cerebral vasculature. It has been demonstrated that there was an increase in the mast cell population of cerebral arterial walls taken from patients after SAH.11 This was mainly in the muscular layer and the number of mast cells was higher in arteries closer to the aneurysm.14 Thus, histamine released from mast cells in the vicinity of the aneurysm may affect the local cerebral circulation. Reportedly, histamine-induced endothelium-dependent relaxation is mediated by H1-receptors in noncerebral arteries,41 by H1- and H2-receptors in cat cerebral arteries,7 and by H2-receptors in rabbit cerebral arteries.33 These findings suggest that the histamine-induced relaxation may be mediated by receptors varying with the site of arteries and the species. On the other hand, A23187 has been known to induce relaxation in arteries by a receptor-independent mechanism (Ca ++ influx via the plasma membrane). It has been shown that A23187 is approximately 10 to 30 times more potent than ACh in producing endothelium-dependent relaxation of rabbit aorta.16-17 In the presence of full relaxing activity of A23187 (10 -6 M), no relaxation by any of the other agents can be demonstrated.16,17 It was therefore considered possible to estimate the release of EDRF in the normal vascular preparations by using A23187. It appeared that there were no differences in the release of EDRF between MCA’s on the non-clot side and CCA’s, since the dose-response curves were almost the same in both preparations.

Acetylcholine is a powerful inducer of EDRF release in almost all extracranial arteries13 and in cerebral arteries of rat,32 cat,27 and rabbit.15,20,31 However, ACh does not produce a significant endothelium-dependent relaxation of canine basilar artery.21 Significant differences may be due to individual characteristics of muscarinic cholinergic receptors present in cerebral and peripheral arteries.29 The current results show that the small relaxation response to ACh was abolished in the MCA’s on the clot side. Several causes for this impairment may be postulated: 1) since ACh directly relaxes smooth-muscle cells,4 SAH may cause a decrease in or loss of responsiveness of the smooth-muscle cells to ACh; 2) ACh may produce endothelium-dependent constricting factor in the MCA’s on the clot side and may potentiate the initial contractile tone;25 or 3) since ACh has been reported to stimulate the release of prostacyclin from endothelium,2 the small relaxation induced by ACh may be due to the release of prostacyclin from the MCA’s on the non-clot side. In addition, SAH may diminish the synthesis of prostacyclin.28,34 Endothelium-derived relaxing factor release induced by histamine and A23187 was reduced in the MCA’s on the side of SAH compared with those on the non-clot side. Since about 75% of red blood cells released into the subarachnoid space became enmeshed and fixed in the subarachnoid space,1 the MCA’s on the clot side may have been exposed to a substantial concentra-

tion of hemoglobin at least until Day 7 after SAH. In long-term exposure to hemoglobin, it is possible that EDRF may be inactivated by the generation of superoxide anion (O2)19 produced by the autooxidation of hemoglobin to methemoglobin. In short-term in vitro studies it was demonstrated that hemoglobin-induced vasoconstriction was unaffected by the removal of endothelium despite the inhibition of EDRF.5,14,30 A tissue-mounted perfusion study also demonstrated that extraluminally administered oxymethemoglobin failed to affect the relaxation induced by intraluminal ACh.38 Kim, et al.,24 suggested that the luminal release of EDRF was not impaired during chronic vasospasm in dogs and that the loss of endothelium-dependent relaxation is due to a decreased transfer of the EDRF or a reduced responsiveness of the smooth muscle to the factor. In view of several studies that strongly support free radical generation and lipid peroxidation as a key factor in vasospasm after SAH,35 it is plausible that continuously generated free radicals may eliminate abluminal EDRF in the period of chronic vasospasm.

Glyceryl trinitrate, an endothelium-independent relaxant,15 did relax the MCA rings on the clot side that were precontracted with PGF2α. Reportedly, GTN generates nitric oxide in vascular tissues and causes vasodilation through the pathway mediated by cyclic guanosine monophosphate (GMP).45 Accumulation of cyclic GMP activates cyclic GMP-dependent protein kinase which phosphorylates membrane proteins, whereby Ca ++ efflux takes place.43 As a result, vascular smooth muscle is relaxed. Our results are consistent with the observations that SAH did not influence the activity of protein kinases in canine basilar arteries.39 It can be inferred that GTN may have a therapeutic potential in vasospasm after SAH. Frazee, et al.,12 demonstrated the efficacy of intravenous GTN on chronic cerebral vasospastic in the primate model.

Subarachnoid hemorrhage also reduced the vascular smooth muscle contractility induced by the maximum force developed in response to KCl and PGF2α. Bevan, et al.,2 also demonstrated the reduction of contractility to norepinephrine and serotonin in MCA’s on the clot side in primate. For this reason, Krueger, et al.,16 hypothesized that, since there is a maximal force that can be exerted by the artery, contractions in response to pharmacological agents are measured from a baseline that is elevated by the existence of vasospasm.

In conclusion, although both EDRF and contractility were significantly impaired in chronic vasospasm, an endothelium-independent relaxation mechanism remained to act as an antagonist system to established smooth muscle contraction. These findings provide encouragement for efforts to discover an effective pharmacological agent for the reversal of cerebral vasospasm after SAH.

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References

38. Tanaka Y, Chiba S: Relationship between extraluminal oxyhemoglobin and endothelium-dependent vasodilata-
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