Effect of Subarachnoid Hemorrhage on Calcitonin Gene-Related Peptide–Induced Relaxation in Rabbit Basilar Artery

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An isometric tension measurement of ring segments was performed in the rabbit basilar and common carotid arteries in vitro to investigate the regional differences in the calcitonin gene-related peptide (CGRP)–induced vasodilation and the effect of subarachnoid hemorrhage on CGRP-induced vasodilation. CGRP elicited vasodilation of the rabbit basilar artery in a dose-dependent fashion when the artery was precontracted by $10^{-5}$ M 5-hydroxytryptamine, whereas almost no relaxation occurred in the rabbit common carotid artery. The relaxation of the basilar artery was $64.03 \pm 1.85\%$ at $3 \times 10^{-8}$ M CGRP, with an $EC_{50}$ of $8.46 \pm 0.08$. Two days after experimental subarachnoid hemorrhage, CGRP-induced relaxation of the rabbit basilar artery was $53.96 \pm 8.08\%$ of the $10^{-5}$ M 5-hydroxytryptamine–induced contraction, not significantly different from that of the basilar artery of the control rabbit. Our findings suggest that CGRP induces potent vasodilation in the rabbit basilar artery and that no impairment of vasodilation occurred after experimental subarachnoid hemorrhage. We speculate that CGRP may have therapeutic potential in cerebrovascular disease such as vasospasm after subarachnoid hemorrhage. (Stroke 1989;20:100–104)

Cerebral blood vessels have been shown to have innervation with a vasodilatory effect.\textsuperscript{1–3} However, the nature of the vasodilatory transmitters has not yet been fully characterized. Recent studies have revealed that some vasoactive peptides may regulate the systemic circulation, and calcitonin gene–related peptide (CGRP) may be one of these regulators.\textsuperscript{6–8} CGRP, a newly discovered peptide, has been localized in many regions of the central nervous system (most notably the spinal cord, medullary and pontine nuclei, amygdala, and hypothalamus) and in elements of the peripheral nervous system.\textsuperscript{9} Thus, CGRP has been shown to have strong vasodilatory actions.\textsuperscript{6–8,10,11} Although many studies have been performed regarding vascular responsiveness to CGRP, there have been few studies of the effect of CGRP on the cerebral arteries.\textsuperscript{10,12–14}

The pathogenesis of vasospasm after subarachnoid hemorrhage (SAH) is not fully understood. However, impairment of the vasodilatory activity of cerebral arteries following SAH may play a role in the development of arterial narrowing. We have demonstrated that vasoactive intestinal peptide (VIP)-induced vasodilation is decreased after SAH in a rabbit model (T. Tsukahara, K. Hongo, N.F. Kassell, O. van Beek, H. Ogawa, S.B. Hudson, G.I. Asban, unpublished data). Greenberg et al\textsuperscript{15} reported use of an immunohistochemical technique that demonstrated that CGRP fibers were drastically reduced after SAH in rats.

No reports have been published regarding alterations in CGRP-induced vasodilation after SAH. Our experiments were conducted to investigate the regional differences in CGRP-induced vasodilation, and the effect of SAH or CGRP-induced vasodilation of the cerebral arteries of rabbits.

Materials and Methods

Adult male New Zealand White rabbits weighing 2.9–3.4 kg were anesthetized with an intramuscular injection of a mixture of 20 mg/kg ketamine, 5 mg/kg xylazine, and 0.25 mg/kg acepromazine and killed by exsanguination from the femoral artery. The brain, with the basilar artery in situ, and the common carotid arteries were removed and placed in dissecting chambers filled with a modified Krebs’ bicarbonate solution of the following millimolar composition: NaCl 120, KCl 4.5, MgSO\textsubscript{4} 1.0, NaHCO\textsubscript{3} 27.0, KH\textsubscript{2}PO\textsubscript{4} 1.0, CaCl\textsubscript{2} 2.5, and dextrose 10.0. The basilar and the common carotid arteries

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were dissected free under magnification. Ring segments (3 mm in length) of basilar and common carotid arteries were then prepared and suspended between two L-shaped stainless steel rods in an organ bath with a 10-ml working volume of Krebs' solution, which was aerated with 95% O₂-5% CO₂. The pH of the solution ranged from 7.40 to 7.50. The preparations were allowed to equilibrate at 37°C for 60 minutes before use. Resting tension was adjusted to 400 mg and 3 g for the basilar and common carotid arteries, respectively. Contraction force was recorded isometrically using a force-displacement transducer (FT.03, Grass Instrument Co., Quincy, Massachusetts) connected to one rod and displayed on a Soltec 3418 polygraph (San Fernando, California). To affirm appropriate activity in each specimen, the contractile response to 40 mM KCl was first obtained in each ring segment. Only those specimens that showed a good response to 40 mM KCl were used for the experiments. For relaxation studies, submaximal tone was induced with 10⁻⁵ M 5-hydroxytryptamine (5-HT); CGRP was then added in a cumulative fashion. The relaxation induced by CGRP was expressed as a percentage of the tonic phase of the contraction induced by 10⁻⁵ M 5-HT.

We used some arteries to test the endothelium dependency of CGRP. Endothelium was removed by infusing 0.1 mg/ml saponin, and absence of the endothelium was confirmed pharmacologically by applying 10⁻⁸ to 10⁻⁵ M acetylcholine. Arteries that did not show any vasodilation induced by acetylcholine were used as endothelium-denuded arteries. An equal number of rabbit basilar arteries with endothelium were used for a comparison of endothelium dependency.

We also tested the effect of SAH on CGRP-induced relaxation. SAH was induced in rabbits as reported. Briefly, 5 ml of fresh, autologous, non-heparinized arterial blood withdrawn from the central ear artery was injected percutaneously into the cisterna magna over 10 seconds. The rabbits were killed 2 days after SAH, the basilar artery was removed, and the isometric tension study was performed. The control rabbit received no injection of blood.

KCl, 5-HT, and saponin were obtained from Sigma Chemical Co. (St. Louis, Missouri); rat CGRP was kindly provided by Peptide Institute, Inc. (Osaka, Japan). KCl and CGRP were dissolved in distilled water and 5-HT was dissolved in 0.1N HCl with 0.1% ascorbic acid to make stock solutions. Each agent was then dissolved in Krebs' solution before use, and volumes of <0.1 ml were added to the organ bath.

The data are expressed as mean ± standard error of the mean (SEM). Statistical comparison was done using Student's t test for unpaired observations. Values were considered to be significantly different when p<0.01.

Results
When the rabbit basilar arteries were precontracted by 40 mM KCl, 10⁻¹¹ to 3×10⁻⁸ M CGRP induced dose-dependent vasodilation. However, CGRP did not induce any notable relaxation of rabbit common carotid arteries (Figure 1). There was a significant difference in relaxation between these two arteries (p<0.005). The maximal relaxation of rabbit basilar artery was elicited by 3×10⁻⁸ M CGRP, with an EC₅₀ of 8.40±0.08, and the degree of relaxation was 20%. When the arteries were precontracted by 10⁻⁵ M 5-HT, the basilar artery showed marked relaxation (64% of 5-HT-induced contraction at 3×10⁻⁸ M CGRP, with an EC₅₀ of 8.46±0.08); however, almost no relaxation occurred in the rabbit common carotid artery (only 3% of 5-HT-induced contraction, Figures 2 and 3).

CGRP (10⁻¹¹ to 10⁻⁸ M) induced a dose-dependent vasodilation of rabbit basilar arteries both with and without endothelium (Figure 4). There was no significant difference in the degree of vasodilation between the groups.
Following SAH, 10^{-11} to 10^{-8} M CGRP elicited relaxation in rabbit basilar artery precontracted by either KCl or 5-HT as well as in the artery of the control rabbit in a dose-dependent manner (Figures 5, 6, and 7). There was no significant difference in relaxation between the arteries of the control and the SAH rabbits. EC50 in the artery of the control and SAH rabbits precontracted by 40 mM KCl was 8.40±0.08 and 8.28±0.06, respectively. When the arteries were precontracted by 10^{-5} M 5-HT, EC50 was 8.46±0.08 in the control and 8.54±0.06 in the SAH rabbits.

**Discussion**

Our study demonstrates that 1) CGRP induced a pronounced relaxation of rabbit basilar artery precontracted by 5-HT, but no relaxation occurred when the artery was precontracted by KCl; 2) almost no relaxation occurred in the rabbit common carotid artery precontracted by either 5-HT or KCl; 3) there was no significant difference in vasodilation between the arteries with and without endothelium; and 4) there was no significant difference in the CGRP-induced relaxation between the arteries of the control and the SAH rabbits.

CGRP is a potent vasodilator of feline middle cerebral and basilar arteries,10,112 rabbit basilar14 and mesenteric18 arteries, and rat aortas.6,19 Edvinsson et al14 reported a pD2 of 8.5±0.2 and a maximal relaxation of 55±8.8% induced by CGRP in rabbit basilar arteries precontracted by 10^{-5} M 5-HT; these data are quite similar to ours. Uddman et al7 compared the CGRP-induced vasodilation among various arteries (basilar, femoral, gastroepiploic, and mesenteric arteries, aorta) of guinea pigs. From their finding that large blood vessels such as the femoral artery and the aorta responded poorly to CGRP, they suggested that CGRP was important for the regulation of blood flow only in certain vascular beds and not in others. A significant difference was noted in the vasodilatory effect of

**Figure 3.** Graph. Effect of different concentrations of calcitonin gene-related peptide (CGRP) on 9 basilar arteries (BA) and 8 common carotid arteries (CCA) from rabbits. Data are percent relaxation after precontraction by 10^{-5} M 5-hydroxytryptamine. *p<0.01, **p<0.005.

**Figure 4.** Graph. Effect of endothelium removal on calcitonin gene-related peptide (CGRP)-induced vasodilation in rabbit basilar artery precontracted by 10^{-5} M 5-HT. Data are percent relaxation after precontraction by 10^{-5} M 5-hydroxytryptamine. E+, endothelium intact (n=4); E−, endothelium removed (n=4).

**Figure 5.** Typical pattern of effect of different concentrations of calcitonin gene-related protein (CGRP) on 5-hydroxytryptamine (5-HT)-induced contraction of normal control rabbit and rabbit after subarachnoid hemorrhage (SAH). Numbers with arrows indicate negative log-molar concentrations.

**Figure 6.** Graph. Effect of subarachnoid hemorrhage (SAH) on calcitonin gene-related peptide (CGRP)-induced vasodilation in rabbit basilar artery precontracted by 40 mM KCl. Data are percent relaxation after precontraction by 40 mM KCl. Fourteen rabbits in each group.
CGRP depending on the vessels tested; the rabbit basilar artery showed more potent vasodilation by CGRP than the rabbit common carotid artery. Although the precise mechanism for the difference in the degree of relaxation between arteries precontracted by 5-HT and KCl remains unclear, CGRP-induced vasodilation occurs when the contraction is induced by a receptor-oriented agonist. It has been reported that another peptide, VIP, does not modify the membrane potential in the rabbit mesenteric artery.20

Recently, Greenberg et al.,15 using an immunohistochemical technique in rats, reported that CGRP-containing fibers showed a marked decrease in the intensity of staining after experimental SAH. Our results, however, showed that vasodilation was not impaired by exogenously applied CGRP after SAH. One possible explanation is that the vascular damage produced by SAH may not be enough to promote the impairment of CGRP-induced vasodilation. Nakagomi et al.21 previously demonstrated that endothelium-dependent relaxation by acetylcholine was not impaired in the basilar artery of a rabbit sacrificed after a single injection of blood but that it was impaired in a rabbit sacrificed after two injections of blood. In spite of the possibility of conflicting results due to different models, it is likely that the presynaptic mechanism may be disturbed after SAH but that the postsynaptic mechanism may not be disturbed during the course of SAH in our model.

Regarding the dependency of CGRP-induced vasodilation on the vascular endothelium, no consistent results have been obtained. Hanko et al.10 reported that CGRP elicited endothelium-independent vasodilation in feline middle cerebral and basilar artery and in rabbit basilar artery; Edvinsson et al.22 also demonstrated it in feline middle cerebral artery. On the other hand, Kubota et al.19 and Brain et al.6 reported that CGRP-induced relaxation requires intact endothelium in rat aorta. In our study, rabbit basilar artery was relaxed by CGRP independent of the endothelium; this may be due to species and regional differences.

There have been several studies performed in human vessels in vitro.10,11,13 and clinical studies were carried out by several investigators.8,23–26 Such studies have shown that CGRP circulates in human plasma, is released mainly from perivascular nerves,3,24 and that nerve fibers containing CGRP-like immunoreactivity are found in the walls of human cerebral arteries.11 Considering these findings, it is consistent that CGRP is a potent vasodilator in human vessels also. Moreover, it is likely that CGRP has an important role in the regulation of the cerebral circulation and that CGRP may have some therapeutic potential in vascular disease, such as vasospasm after SAH.

Acknowledgments

The authors are grateful to Dr. T. Watanabe, Peptide Institute, Inc., Osaka, Japan, for the generous donation of the CGRP. We also thank Sarah Hudson and Grace I. Asban for technical assistance and Lucille Staiger for manuscript preparation.

References


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**FIGURE 7.** Graph. Effect of subarachnoid hemorrhage (SAH) on calcitonin gene–related peptide (CGRP)-induced vasodilation in rabbit basilar artery precontracted by 10−3 M 5-hydroxytryptamine. Data are percent relaxation. Control, n=9; SAH, n=12.


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Stroke. 1989;20:100-104
doi: 10.1161/01.STR.20.1.100

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