Relaxant effect of calcitonin gene-related peptide on cerebral arterial spasm induced by experimental subarachnoid hemorrhage in dogs

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This study examines the relaxant effect of calcitonin gene-related peptide (CGRP), a 37-amino acid peptide with a potent vasodilator action, on cerebral arterial spasm after subarachnoid hemorrhage (SAH). The spasm was induced by injecting autologous arterial blood percutaneously into the cisterna magna in adult mongrel dogs. The single-injection model of SAH was produced by injection of 1.0 ml/kg body weight of blood (on Day 0), and the double-injection model involved two successive injections of 0.5 ml/kg body weight of blood made 48 hours apart (on Day 0 and Day 2). On vertebral angiograms, arterial narrowing of the major cerebral arteries was most prominent on Day 3 after SAH in the single-injection model and on Day 7 in the double-injection model. When 10^-11 mol/kg of CGRP was administered intracisternally in the single-injection model on Day 3, the diameter of the spastic cerebral arteries, as determined by angiography, recovered to normal. After intracisternal administration of 10^-11 to 2 x 10^-10 mol/kg of CGRP on Day 7 in double-injection models, spastic cerebral arteries dilated in a dose-dependent manner. The dilatory effect of CGRP continued for a few hours after administration. The results suggest that CGRP injected intracisternally may reverse cerebral arterial spasm after SAH.

KEY WORDS • cerebral vasospasm • calcitonin gene-related peptide • subarachnoid hemorrhage • dog

Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide, is widely distributed in the central and peripheral nervous system, particularly in nerves supplying blood vessels, and may regulate the blood flow of organs as a neurotransmitter or a neuromodulator. It has a potent vasodilating action in both cerebral and peripheral arteries, and appears to be the most potent dilator of all known neuroactive substances in the pial vessels.

Cerebral vasospasm is one of the major complications that influences the morbidity and mortality of patients suffering from subarachnoid hemorrhage (SAH), but its pathogenesis and therapeutic management are still matters of debate. The present study examines the vasodilating effect of CGRP on cerebral vasospasm after SAH produced experimentally in dogs.

Materials and Methods

In Vitro Experiments

Adult mongrel dogs of either sex, each weighing 7 to 13 kg, were used for this study. Fifteen dogs, anesthetized with intramuscular ketamine hydrochloride (5 mg/kg) and intravenous sodium pentobarbital (20 to 30 mg/kg), were sacrificed by rapid exsanguination from the common carotid arteries. The basilar artery was dissected free and cut into rings of 3 mm length under an operating microscope. The arterial rings were suspended from two fine stainless steel wires inserted through the lumen, and isometric contraction was recorded. The rings were mounted in organ chambers filled with 20 ml of modified Ringer-Locke solution, composed of (mM): 139.7 Na, 5.4 K, 2.2 Ca, 1.0 Mg, 131.5 Cl, 20.0 HCO3, and 5.6 glucose. The solution was maintained at 37°C and aerated continuously with a
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TABLE 1
Timing and dose of CGRP administered in vivo to dogs with SAH

<table>
<thead>
<tr>
<th>Group</th>
<th>CGRP (mol/kg)</th>
<th>No. of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal group</td>
<td>10^-10</td>
<td>5</td>
</tr>
<tr>
<td>single-injection SAH model, Day 3</td>
<td>10^-10</td>
<td>6</td>
</tr>
<tr>
<td>double-injection SAH model, Day 7</td>
<td>10^-11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10^-10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2 x 10^-10</td>
<td>6</td>
</tr>
</tbody>
</table>

* CGRP = calcitonin gene-related peptide; SAH = subarachnoid hemorrhage. In three additional normal dogs, only vehicle (100 μl of 0.1% bovine serum albumin) was injected intracisternally.

95% O₂/5% CO₂ gas mixture. Tension changes were recorded by a strain gauge transducer coupled with an ink-writing oscillograph. The resting tension was adjusted to 1.5 g, which had previously been determined as the optimal length. The rings were allowed to equilibrate for 60 minutes before the experiments were performed.

The contractile response to 30 mM K was first obtained in each ring. The relaxation in response to human CGRP was recorded in a cumulative manner in a ring which was precontracted with 2.5 x 10^-6 M prostaglandin (PG) F₂α. The maximum relaxation was obtained by exposure to 10^-4 M papaverine. In some rings, the endothelium was removed mechanically: a needle with an appropriate diameter was inserted into the ring, and the ring was rolled back and forth gently on a saline-wetted paper. In separate experiments, before the administration of CGRP, one of the following was added to the organ bath: 10^-6 M phentolamine mesylate (an alpha-adrenergic blocker), 2 x 10^-6 M propranolol hydrochloride (a beta-adrenergic blocker), 10^-6 M atropine sulfate (an anti-cholinergic agent), 10^-5 M chlorpheniramine maleate (a histamine H₁ blocker), 10^-4 M cimetidine (a histamine H₂ blocker), 10^-5 M methysergide (a serotonin blocker), or 10^-7 M indomethacin (a PG-synthesis inhibitor).

In Vivo Experiments

Thirty dogs were anesthetized as described above. Spontaneous respiration was permitted via an endotracheal tube. Blood pressure, heart rate, respiratory rate, and arterial pO₂, pCO₂, and pH were monitored during the experiments.

A single-injection model and a double-injection SAH model were produced by percutaneous injection of fresh autologous nonheparinized arterial blood into the cisterna magna: 1.0 ml/kg of blood was injected in the single-injection model (on Day 0), while 0.5 ml/kg of blood was injected successively 48 hours apart in the double-injection model (on Day 0 and Day 2). After the injection, the dogs were maintained in a head-down position for 30 minutes to facilitate settling of the blood around the basilar artery by gravity. Vertebral angiography was performed by transfemoral catheterization under fluoroscopy. Angiography (3 ml) was injected rapidly by hand, and the angiography was performed in the ventrodorsal projection at a fixed distance. Exposure factors were set at 60 to 80 kV and 10 to 15 mA. The diameters of the basilar artery at three determined positions were measured on the angiograms under optical magnification. The averages of the three diameters were compared before and after the intracisternal injection of blood, and expressed as a percentage of the values before the injection (% diameter).

The effect of CGRP on cerebral vasospasm was examined on the day when cerebral arterial narrowing was most marked after SAH (Table 1). The CGRP, at doses determined from the in vitro studies (1 x 10^-11 mol/kg to 2 x 10^-10 mol/kg) and dissolved in 100 μl of 0.1% bovine serum albumin (BSA) or 100 μl of 0.1% BSA without CGRP, was administered percutaneously into the cisterna magna via a No. 22 needle. Angiography was performed at 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 1 day after the administration of CGRP. Changes in the diameter of the basilar artery were evaluated on the angiograms as described above.

Statistical Analysis

The two-tailed Student t-test for paired samples was used to compare the percent diameters of the basilar artery before and after the intracisternal administration of CGRP. Differences with a p value < 0.01 were considered to be statistically significant. Mean data are given ± standard error of the mean.

Results

In Vitro Experiments

The amount of CGRP administered was determined from in vitro data so that maximum relaxation could be obtained in vivo. The contractile response to 30 mM K was first determined in each of the rings obtained from the basilar artery. Prostaglandin F₂α (2.5 x 10^-6 M) produced a moderate contraction (1373 ± 548 mg), which was 64% ± 14% of the contraction produced with 30 mM K (Fig. 1). Administration of CGRP caused relaxation of the rings precontracted with 2.5 x 10^-6 M PGF₂α. The maximum relaxation produced with CGRP was 63.5% ± 1.8% of the relaxation with 10^-4 M papaverine (Figs. 1 and 2). This relaxation started 10 to 30 seconds after the addition of CGRP and lasted for at least 10 minutes. It was not influenced by pretreatment with phenolamine (10^-6 M), propranolol (2 x 10^-6 M), atropine (10^-6 M), chlorpheniramine mesylate (10^-4 M), or a PG-synthesis inhibitor.

* Oscillograph manufactured San-Ei Instrument Co., Tokyo, Japan.
† Human CGRP obtained from Peptide Laboratory, Osaka, Japan.
‡ Prostaglandin F₂α obtained from Ono Pharmaceutical Co., Osaka, Japan.
§ Drugs obtained from Sandoz Ltd., Basel, Switzerland.
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FIG. 1. Effect of calcitonin gene-related peptide (CGRP) on the contraction induced by $2.5 \times 10^{-6}$ M prostaglandin (PG) F$_{2\alpha}$ in the basilar artery. The contraction induced by 30 mM K is also shown. The numbers above the filled circles indicate the $-\log$ molar concentration of CGRP.

(10^{-6} M), cimetidine (10^{-4} M), methysergide (10^{-5} M), or indomethacin (10^{-5} M). It was found that CGRP showed more relaxant effects upon the rings of the basilar arteries that were devoid of endothelium than upon the rings obtained from normal basilar arteries (Fig. 2).

In Vivo Experiments

The most marked constriction of the basilar artery was seen on Day 3 after SAH in the single-injection model (50.0% \pm 4.7% diameter), and on Day 7 in the

FIG. 2. Dose-response curves for relaxation induced in the basilar artery by calcitonin gene-related peptide (CGRP). The basilar arteries were precontracted by $2.5 \times 10^{-6}$ M prostaglandin F$_{2\alpha}$. Data are mean values expressed as a percentage of the maximum relaxation induced by $10^{-4}$ M papaverine. Vertical bars indicate two standard errors of the mean for eight samples. Open triangles = effects of CGRP upon the basilar arteries with intact endothelium (endothelium +); filled triangles = effects of CGRP upon the basilar arteries which were devoid of endothelium (endothelium -).

FIG. 3. Angiograms in a single-injection model showing the effects produced by intracisternal administration of $10^{-10}$ mol/kg of calcitonin gene-related peptide (CGRP) upon the major cerebral arteries. a: Before subarachnoid hemorrhage (SAH). b: On Day 3 after SAH. c: At 30 minutes after CGRP administration on Day 3 after SAH.

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When \(10^{-10}\) mol/kg of CGRP was administered intracisternally on Day 3 in the single-injection model, cerebral vasospasm reversed completely (Fig. 3). The effect began to appear 5 minutes after CGRP administration, continued for 4 hours, and disappeared by 24 hours after the administration (Fig. 4). When CGRP was administered at doses of \(10^{-11}\) to \(2 \times 10^{-10}\) mol/kg on Day 7 after SAH in the double-injection model, the cerebral vasospasm was reversed in a dose-dependent manner; \(2 \times 10^{-10}\) mol/kg of CGRP reversed the vasospasm completely (Fig. 5). The effect began to appear 5 minutes after the CGRP administration, continued for 4 hours, and disappeared by 24 hours (Fig. 6).
When CGRP was administered at a dose of 10^{-10} mol/kg into the cisterna magna in normal dogs, the basilar artery was markedly relaxed 5 minutes after the administration. This vasodilation lasted for 4 hours, and the diameter of the basilar artery returned to the normal level by 24 hours after CGRP administration. The maximum dilation was observed 30 to 60 minutes after administration of the peptide (150% ± 10.2% diameter). In additional normal dogs which were intracisternally injected with vehicle only (100 μl of 0.1% BSA), no changes were observed in the diameter of the major cerebral arteries.

When CGRP, 10^{-10} mol/kg, was administered intravenously, mean arterial blood pressure (MABP) decreased and the heart rate increased markedly a few minutes after the injection. These changes lasted for 30 to 40 minutes (Fig. 7). On the other hand, when the same amount of CGRP was administered intracisternally, both MABP and heart rate were only slightly increased and returned to the previous levels in several minutes (Fig. 8). No significant changes were observed in the other parameters that were monitored in the present study.

Discussion

Results of the present in vitro experiments showed that CGRP markedly dilated the precontracted basilar arteries, and that the relaxant effect of CGRP was not influenced by phentolamine, propranolol, atropine, chlorpheniramine, cimetidine, methysergide, or indo- methacin. These results indicate that the sites of CGRP action were different from those of adrenergic, cholinergic, histaminergic, or serotoninergic actions, and that the arachidonic cascade was not involved in the vasodilatory action of CGRP. Similar effects of CGRP on the middle and anterior cerebral arteries have also been reported in an in vitro study in humans, cats, and pigs.

It has been suggested that endothelial cells are damaged to some degree after SAH. 7,14,22,25,31,32 If this is the case, the agents acting on the arteries via endothelial cells fail to reverse cerebral vasospasm after SAH. In the present experiments, CGRP showed its relaxant effects not only upon the normal basilar arteries, but also upon those without endothelium. The in vivo experiments showed that CGRP markedly dilated both normal and spastic basilar arteries when injected into the cisterna magna, and that this action lasted for at least 4 hours and disappeared by 24 hours after CGRP administration. A slight increase in MABP and heart rate after the intracisternal administration of CGRP, and a profound decrease in MABP and a marked increase in heart rate after intravenous administration of CGRP have also been observed in the present study, as reported previously in the rat and the dog.

The results of previous in vitro experiments in the rat reported by Kline and Pang suggest that the degree of CGRP-induced relaxation of the tail arteries is dependent upon intracellular concentration of calcium in the perivascular smooth-muscle cells. If CGRP acts on the cerebral arteries of the dog in the same way as on the tail arteries of the rat, the relaxant CGRP action on cerebral vasospasm after SAH can be considered to be produced by inhibiting the mobilization of intracellular calcium in perivascular smooth-muscle cells of the cerebral arteries.

The presence of CGRP in plasma and cerebrospinal fluid has already been proved in normal subjects. Although the metabolism or mechanism of CGRP...
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clearance in plasma and cerebrospinal fluid has not yet been elucidated,\textsuperscript{4,38} the present study appears to imply possible therapeutic use of CGRP in the treatment of cerebral vasospasm in clinical cases.

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