Increased Neuropeptide Y Concentrations in Cerebrospinal Fluid From Patients With Aneurysmal Subarachnoid Hemorrhage

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We investigated the possible relation between neuropeptides and cerebral vasoconstriction in samples of ventricular or cisternal cerebrospinal fluid from 14 patients with subarachnoid hemorrhage. Neuropeptide Y, calcitonin gene-related peptide, atrial natriuretic peptide, and pituitary polypeptide 7B2 were present in the cerebrospinal fluid of these patients. Concentrations of calcitonin gene-related peptide and 7B2 were not significantly different from those in control subjects, but that of atrial natriuretic peptide was significantly lower. Although the mean concentration of neuropeptide Y was not significantly higher than control, consecutive determinations showed an increase 6–11 days after the onset of subarachnoid hemorrhage. An initially high 7B2 concentration decreased gradually, although half the patients showed a second increase >10 days after the onset. Considering the well-recognized vasoconstrictive effect of neuropeptide Y, it is possible that this increase in its concentration in the cerebrospinal fluid plays a role in the pathogenesis of the cerebral vasospasm that is often seen after subarachnoid hemorrhage. (Stroke 1989;20:1680–1684)

Neurologic deterioration due to cerebral vasospasm complicates a significant number of cases of subarachnoid hemorrhage (SAH) although the pathogenesis of this vasospasm is still a matter of discussion. Many investigations have suggested that the catecholaminergic perivascular nerve fibers of the arteries are important. However, in a recent reclassification of the autonomic nervous system, it has been proposed that, in addition to the classical neurotransmitters, there exist a number of neuropeptides that play roles as neurotransmitters or neuromodulators in the central nervous system. A number of vasoactive neuropeptides have been isolated in recent years. Neuropeptide Y (NPY)-immunoreactive nerve terminals form a dense plexus around the cerebral and coronary vessels, and pharmacologic experiments indicate a role for NPY in vasocostriction. Calcinon gene-related peptide (CGRP), which is thought to be generated as a result of the calcitonin gene, has been shown to possess a very potent vasodilator action. Atrial natriuretic peptide (ANP) shows potent natriuretic and diuretic actions. In addition to these vasoactive peptides, a pituitary polypeptide, 7B2, which was first extracted from porcine and human pituitary glands, has been shown to be abundant in the central nervous system and to be present in the cerebrospinal fluid (CSF), with an unknown function. We examine possible changes in the concentrations of these vasoactive peptides and 7B2 in consecutive samples of CSF from patients with SAH.

Subjects and Methods

The ventricular and cisternal CSF samples were collected from three men and 11 women (mean ± SEM age 64 ± 3 years) with SAH 3–19 days after the onset. The clinical courses of the 14 SAH patients were assessed according to the Hunt and Kosnic grading, and the degrees of SAH were determined by computed tomography (CT) (Toshiba 60-A, Toshiba Co. Ltd., Tokyo, Japan). Vasospasm was diagnosed when transient or persistent neurologic deficits occurred with an impaired level of consciousness and without CT evidence of rebleeding. The SAH patients were divided into two subgroups, those presenting with (n=11) and those presenting without (n=3) clinical and radiologic evidence of vasospasm. The operation had been performed at 3.4 ± 2.7 days after
onset. CSF was obtained from the ventricles or cisterns by intraoperative puncture or by postoperative drainage for intracranial decompression; the samples were stored at -20°C until extraction and further processing. Twenty control spinal CSF samples were also obtained from seven men and 13 women (mean±SEM age 62±3 years) suspected of having brain or spinal tumors. Control CSF samples were taken by lumbar puncture with the subjects in the lying position after an overnight fast.

The extracted CSF samples were analyzed by high-performance liquid chromatography (HPLC, Waters Associates, Ltd., Milford, Massachusetts). Usually 20 ml CSF was passed through the cartridge and eluted with 2 ml 60% acetonitrile (ACN, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 0.1% trifluoroacetic acid (TFA, Nakarai Chemicals Ltd., Kyoto, Japan); the dry residue was reconstituted in 1 ml assay buffer. Because of the small amount of control CSF, the mean concentrations of NPY, CGRP, and ANP were calculated from 10 extracted CSF samples. Immunoreactive NPY, CGRP, and ANP concentrations were determined with commercially available antibody (Peninsula Laboratories, Inc., Belmont, California) and corresponding iodinated peptides (Amersham Japan Co., Tokyo, Japan). 

For the determination of immunoreactivity 7B2 concentration, 50-100 μl CSF was measured directly. The radioimmunoassay for 7B2 has been described elsewhere.24 Inhibition doses for 50% binding of these assays were 136 fmol for NPY, 12.5 fmol for CGRP, 8.1 fmol for ANP, and 12 fmol for 7B2.22-24

The extracted CSF samples were analyzed by high-performance liquid chromatography (HPLC, Shimazu, Inc., Kyoto, Japan) using a 10-60% gradient of ACN in water with 0.1% TFA for 50 minutes. To characterize immunoreactive 7B2 in the CSF, randomly selected samples were applied to a 1.4×90 cm column of Sephadex G-100 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) that was precalibrated with several molecular markers.

Concentrations of immunoreactive NPY, CGRP, and ANP are expressed as picomoles per liter of CSF; concentrations of immunoreactive 7B2 are expressed in molar equivalents of synthetic 7B2 fragments as picomoles per liter of CSF. All concentrations are quoted as mean±SEM. Statistical analysis was performed using one-way analysis of variance or unpaired t test. Because NPY concentration seemed to change according to the time course, statistical analysis was done using paired t test. The level of significance was accepted as being p<0.05.

### Results

As shown in Table 1, ventricular and cisternal ANP concentrations in the SAH patients were significantly lower than that in the controls (p<0.05); CGRP concentrations in the SAH patients also seemed to be lower. There were, however, no significant differences in CGRP or 7B2 concentrations between groups. No significant differences were observed in mean NPY concentrations between the patients and controls (Table 1) or in maximum NPY concentrations between the spasm and no-spasm patient subgroups (87.5±24.6 vs. 35.4±11.0 pmol/l, p>0.05). No significant differences between subgroups were observed for CGRP, ANP, or 7B2 concentrations (Table 1).

The time courses for NPY (n=14), CGRP (n=10), ANP (n=11), and 7B2 (n=12) concentrations in CSF from the SAH patients are depicted in Figure 1. CGRP and ANP concentrations seemed to be stable after surgery. NPY concentrations, however, were low during the initial stage (the first 4–5 days after the onset of SAH), sharply increased during the next 3–6 days, then decreased gradually during the late stage (>11 days after onset). NPY concentrations during these three stages in paired data were 24.6±6.8 (during the initial stage) and 62.7±14.0 pmol/l (7–11 days after onset) or 86.1±34.4 (7–11 days after onset) and 23.9±8.8 pmol/l (during the late stage). NPY concentration 6–11 days after onset was significantly higher than

### Table 1. Concentrations of Neuropeptides in Cerebrospinal Fluid of Patients With Subarachnoid Hemorrhage and Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Neuropeptide</th>
<th>Calcitonin gene-related peptide</th>
<th>Atrial natriuretic peptide</th>
<th>Pituitary polypeptide 7B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients sampling site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricles</td>
<td>14</td>
<td>NPY</td>
<td>4.5±1.0</td>
<td>0.5±0.2*</td>
<td>1123.7±360.5</td>
</tr>
<tr>
<td>Cistern</td>
<td>14</td>
<td>NPY</td>
<td>3.0±0.4</td>
<td>1.1±0.3*</td>
<td>938.1±121.9</td>
</tr>
<tr>
<td>Delayed vasospasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>11</td>
<td>NPY</td>
<td>3.4±0.5</td>
<td>0.7±0.2</td>
<td>1058.6±222.7</td>
</tr>
<tr>
<td>Without</td>
<td>3</td>
<td>NPY</td>
<td>2.8±0.1</td>
<td>0.9±0.4</td>
<td>712.1±39.7</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>NPY</td>
<td>19.1±6.1†</td>
<td>19.0±7.7†</td>
<td>712.1±39.7‡</td>
</tr>
</tbody>
</table>

Data are mean±SEM pmol/l.

* p<0.05 different from corresponding control value by unpaired t test.

† Calculated from 10 samples.

‡ Calculated from 20 samples.
FIGURE 1. Time course for concentrations of immunoreactive neuropeptide Y (IR-NPY) (n=14), calcitonin gene-related peptide (CGRP) (n=10), atrial natriuretic peptide (IR-ANP) (n=11), and pituitary polypeptide 7B2 (IR-7B2) (n=12) in cerebrospinal fluid from patients with subarachnoid hemorrhage (SAH). The operation had been performed at 3.4±2.7 days after onset.

that during the initial or late stages (both p<0.05). 7B2 concentration seemed to decrease gradually. Six of 12 patients showed a second increase 1-2 weeks after the onset of SAH (from 626.4±118.4 to 1,538.5±126.1 pmol/l).

Immunoreactive NPY, CGRP, and ANP extracted from CSF samples were fractionated by reverse-phase HPLC. The main immunoreactive peaks eluted at positions identical to those of corresponding synthetic standards (profiles not shown). Minor components eluted at either more hydrophobic or more hydrophilic positions. The gel chromatographic profile of immunoreactive 7B2 from CSF was similar to that of the porcine pituitary gland extract (profile not shown).25

Discussion

Significant concentrations of neuropeptides were present in CSF from SAH patients, and the main immunoreactivities of these substances corresponded to those of synthetic standards or to the main peak found in porcine pituitary gland. NPY, one of the most abundant neuropeptides in the brain,26 was also found in the CSF at concentrations comparable to those in previous works.27,28 ANP was also found in the CSF, and its HPLC profile revealed that the main immunoreactivity corresponded to that of α-hANP, suggesting a biologic activity in the CSF. Recent studies by Marumo et al29 and Levin30 also showed the immunoreactivity of ANP to be compatible with that of α-hANP. In our series, the concentration of ANP in the CSF was comparable to that in plasma,23 although we could not measure corresponding plasma samples. Levin30 observed that plasma ANP levels were ≤50% of those in CSF from patients with radiculopathy. Doczi et al31 demonstrated that ANP levels in the CSF of SAH patients with raised intracranial pressure were significantly higher than concentrations in the CSF of SAH patients with normal intracranial pressure. The reason for this discrepancy seemed to be the differences in assay methods. Doczi et al31 employed a radioreceptor assay, in which indistinguishable displacement of [125I]ANP was observed by the addition of brain natriuretic peptide.32 CGRP has also been found in the CSF.33 Wimalawansa and MacIntyre33 reported multiple immunoreactive forms of CGRP in CSF, and the level in CSF was lower than that in plasma. Contrary to levels of these peptides, the level of 7B2 in the CSF was much higher than its level in normal plasma. Gel chromatographic profiles indicated that authentic 7B2 was responsible for the observed immunoreactivity; 7B2 has also been demonstrated
to be abundant in the central nervous system and to be stable in CSF by our previous study for 7B2 degradation. \(^4\) This high 7B2 level could be important for neuronal growth or transmission. The gradual decrease in 7B2 concentration found in our time course study also suggests its origin to be the central nervous system.

The innervation of cerebral blood vessels of many species has been studied, and the presence of not only adrenergic but also nonadrenergic nerves has been suggested. \(^6\) NPY has a potent vasoconstrictive activity, \(^8-10\) and NPY-immunoreactive nerve terminals are very common around cerebral vessels. \(^8,9\) Previous study has demonstrated the presence of NPY in the circle of Willis, and a prolonged vasoconstrictive effect of 50 pmol intracarotid NPY infusion was observed. \(^10\) Other studies have also demonstrated that 1.2 or 50 pmol NPY induced sustained vasoconstriction, which seemed to be compatible with the observed 40–50 pmol/L increase of NPY in the CSF. \(^8,9\) Furthermore, in our time course study, NPY levels rose 6–11 days after the onset of SAH and then decreased, differing from ANP and CGRP. This NPY change paralleled the clinical observation of cerebral vasospasm.

Considering its vasoconstrictive effect, NPY might have a pathologic significance in cerebral vasospasm. Uemura et al have demonstrated marked decreases in neuropeptides' immunoreactivities in perivascular nerve fibers during the initial stage after experimentally produced SAH. While vasoactive intestinal polypeptide and substance P immunoreactivities at perivascular axon terminals were gradually restored, NPY immunoreactive nerve fibers remained suppressed 63 days after SAH. The authors supposed that the marked decreases in the neuropeptides' immunoreactivities might be due to the excessive release of and/or the blocking of reuptake of the neuropeptides at perivascular axon terminals. The observed increase of NPY concentration in the CSF might be additional evidence for the immunohistochemical study and relevant for a role of NPY in the pathogenesis of cerebral vasospasm.

Acknowledgment

We would like to express our gratitude to Professor S.R. Bloom, Royal Postgraduate Medical School, London, UK, for his kind gift of the 7B2 antisera.

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KEY WORDS • cerebral vasospasm • neuropeptide Y • subarachnoid hemorrhage
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*Stroke*. 1989;20:1680-1684
doi: 10.1161/01.STR.20.12.1680

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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World Wide Web at:
http://stroke.ahajournals.org/content/20/12/1680

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