Effects of Atrial Natriuretic Peptide on Ischemic Brain Edema in Rats Evaluated by Proton Magnetic Resonance Method

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We examined the effect of atrial natriuretic peptide on cerebral edema in 96 rats. Forty-four rats were given 30 (n=11), 120 (n=26), or 150 (n=7) μg/kg of the peptide intravenously over 24 hours after occlusion of the left middle cerebral artery to induce cerebral ischemia. We then measured the brain water content, the brain sodium and potassium contents, the in vitro proton nuclear magnetic resonance longitudinal (T1) and transverse (T2) relaxation times, and the area of the edematous regions. Compared with saline treatment (n=39), peptide treatment decreased the brain water content in a dose-dependent manner and decreased the brain sodium content significantly (p<0.05). Peptide treatment also suppressed the lengthening of both T1 and T2 in edematous tissue (p<0.05 and p<0.01, respectively) and reduced the area of the edematous regions observed by magnetic resonance imaging (p<0.01). Atrial natriuretic peptide appears to have a pharmacological effect on ischemic brain edema, possibly by suppressing the elevation of water content through regulation of electrolyte transport in the brain. (Stroke 1991;22:61-65)

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Since its initial discovery as a substance with a diuretic effect,1 atrial natriuretic peptide (ANP) has been shown to be a unique hormone that regulates blood pressure and fluid homeostasis.2-5 Recent studies have shown that this hormone regulates sodium transport in vascular endothelial6-7 and other8-9 cells through activation of guanylate cyclase, suggesting that ANP may regulate water content in tissue.

Binding sites for ANP have been found on brain microvessel endothelial cells10 and astrocytes,11 and elevated levels of guanosine 3',5'-cyclic monophosphate (cGMP) have been noted in these cells following ANP binding. In view of the known role of these cells in the origin and development of brain edema,12-13 we inferred that ANP may have a pharmacological effect on brain edema. To investigate this hypothesis, we monitored changes in brain water, sodium, and potassium contents; changes in proton nuclear magnetic resonance (NMR) relaxation times in vitro; and changes in the area of edematous regions detected by in vivo magnetic resonance imaging (MRI) to examine the effects of ANP on ischemic brain edema induced in rats.

Materials and Methods

We used 96 male Wistar rats aged 8-11 weeks. Ninety-one rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital. In eight sham-operated rats, the dura mater and arachnoid membrane were opened without middle cerebral artery (MCA) occlusion. In the other 83 rats, the main trunk of the left MCA was occluded adjacent to the olfactory tract with a bipolar electrocoagulator, as described by Tamura et al.14 In 44 of these rats, purified rat ANP provided by Asahi Chemical Industry Co., Ltd. (Fuji, Japan) and dissolved in saline was administered continuously at 0.4 ml/hr through a tail vein over the 24 hours immediately after MCA occlusion; 11 rats received 30, 26 received 120, and seven received 150 μg/kg ANP. The remaining 39 rats received saline only. At 24 hours after sham operation or MCA occlusion, the rats were sacrificed by decapitation under ether anesthesia and the brains were excised. The MRI study was carried out before sacrifice.

Water content in each hemisphere of all 96 rats was measured by the dry-wet method. Each hemisphere of fresh brain was weighed in a humid chamber to obtain Wf, then dried at 100°C for 3 days and
weighed again to obtain $W_2$. Brain water content was calculated as $100\% \times (W_1 - W_2) / W_1$.

Brain sodium and potassium were extracted from the dried hemispheres of four sham-operated, nine saline-treated, and 10 ANP-treated (120 $\mu$g/kg) rats by soaking in 3 ml of 60% nitric acid solution. The sodium and potassium contents were measured with a Zeeman atomic absorption spectrophotometer (Model 180-16, Hitachi, Tokyo, Japan) and a flame photometer (Model 710, Hitachi), respectively, and expressed as milliequivalents per kilogram dry weight.

The NMR relaxation times in the five untreated, four saline-treated, and six ANP-treated (120 $\mu$g/kg) rats were measured in vitro at 40°C with a Minispec PC-20 (Bruker, Karlsruhe, F.R.G.) at a resonance frequency of 20 MHz. Cerebral cortex containing the ischemic area was placed in a 1.0 cm i.d. NMR tube containing at its bottom a small scroll of filter paper moistened with 1 ml of $D_2O$ to prevent tissue drying. The longitudinal relaxation time ($T_1$) was measured by the inversion–recovery method with eight different interpulse intervals between the 180° and 90° pulses. The transverse relaxation time ($T_2$) was measured by the Carr-Purcell-Meiboom-Gill method with 10 echoes at 10-msec intervals. Both $T_1$ and $T_2$ were calculated as single-component values. Following NMR, the water contents of these cerebral cortices were measured by the dry–wet method.

The MRI scans were obtained in vivo in four sham-operated, 13 saline-treated, and seven ANP-treated (150 $\mu$g/kg) rats with a 4.7-T SCM-200 device (JEOL, Tokyo, Japan). After anesthesia with 50 mg/kg i.p. sodium pentobarbital, each rat was placed in the magnet and MRI scans were obtained using a 5.0 cm diameter saddle-type coil. $T_2$-weighted spin-echo images were obtained with a 3,000 msec repetition time and a 96 msec echo time. Each slice was 2 mm thick and separated from other slices by 1.25-mm intervals. Over the 4.6-cm field of view, 256 frequency encodings and 128 phase encodings were carried out. The phase encoding steps were interpolated to 256 points after Fourier transformation to obtain an imaging matrix of 256x256 pixels. The theoretical imaging resolution was 0.2x0.2x2 mm.

To standardize the signal intensity, a 4 mm i.d. plastic tube of pure water was held next to the rat's head. In each image, the area having a signal intensity greater than a given proportion of the pure water standard was taken as the edematous region. For each rat, the area of the edematous region was the average of that in the three slices. Following MRI, the rats were sacrificed by decapitation under ether anesthesia and the brains were excised for the determination of water content by the dry–wet method.

Data are expressed as mean±SEM. Statistical comparisons were performed using Dunnett's test or Student's two-tailed $t$ test after analysis of variance. Differences were considered to be significant at $p<0.05$.

**Results**

Figure 1 shows the brain water content in each hemisphere of 13 saline-treated and 21 ANP-treated rats. In the saline-treated rats, water content in the occluded left hemisphere increased to 83.1% while that in the nonoccluded right hemisphere remained at 79.2%. In rats receiving ANP, however, water content in the left hemisphere was lower than that in rats receiving saline. This difference was slight when rats were given 30 $\mu$g/kg ANP but was significant ($p<0.01$) when the dose was 120 $\mu$g/kg. No significant differences in water content of the right hemisphere were noted among the groups.

Figure 2 shows the water, sodium, and potassium contents of each hemisphere of four sham-operated, nine saline-treated, and 10 ANP-treated rats. In the occluded left hemisphere of rats receiving saline, the water and sodium contents were significantly higher than those in sham-operated rats ($p<0.05$). In the occluded left hemisphere of rats receiving 120 $\mu$g/kg ANP, the water and sodium contents were significantly lower ($p<0.01$ and $p<0.05$, respectively) than those in rats receiving saline, but the potassium contents did not differ significantly. The water, sodium, and potassium contents in the right hemispheres did not differ significantly among the groups.
Table 1 shows T₁ and T₂ for the left hemispheres of the five untreated, four saline-treated, and six ANP-treated rats. Rats receiving saline had T₁ and T₂ values much greater than those in the untreated rats. Rats receiving 120 μg/kg ANP had T₁ and T₂ values significantly shorter than those of rats receiving saline (p<0.05 and p<0.01, respectively). These differences are consistent with the change in water content.

Figure 3 shows the calculated area of the edematous regions and the measured water contents for four sham-operated, 13 saline-treated, and seven ANP-treated rats. A good correlation (r=0.93) between water content and edematous area was found. Rats receiving ANP had significantly smaller areas of high intensity than saline-treated rats (p<0.01).

Discussion

The administration of ANP effectively suppresses the elevations in water and sodium contents in ischemic brain tissue without apparently affecting the water and sodium contents in nonoccluded brain tissue. Under the same conditions, ANP also has no apparent effect on the blood sodium level or the systemic blood pressure (unpublished data). These results suggest that ANP may be pharmacologically effective for the selective regulation of water and sodium contents in edematous brain tissue caused by ischemia. Furthermore, this effect does not seem to be caused by the systemic actions of ANP.

The ability of ANP to inhibit brain edema formation was confirmed by NMR. In vitro NMR showed that ANP suppressed the lengthening of proton relaxation times of edematous tissue. It has previously been reported that proton relaxation times are sensitive indicators of the state of water molecules in edematous tissue. Our findings therefore imply that increases in the fraction of freely mobile water molecules, the main component of edema fluid, are effectively suppressed by ANP.

In vivo MRI also showed the inhibition of brain edema formation. The known difficulty of quantifying and comparing regions of brain edema by MRI was effectively overcome by measuring areas in the MRI scans having signal intensities higher than a given
TABLE 1. Effects of ANP on Proton Relaxation Times of Ischemic Brain Tissue in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Water content (%)</th>
<th>Proton relaxation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5</td>
<td>78.66±0.37</td>
<td>T1 (msec): 660.1±27.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T2 (msec): 83.1±1.0</td>
</tr>
<tr>
<td>MCA occlusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>82.83±0.37</td>
<td>T1 (msec): 784.9±20.2</td>
</tr>
<tr>
<td>ANP (120 μg/kg)</td>
<td>6</td>
<td>81.95±0.32*</td>
<td>T1 (msec): 734.6±23.6f</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T2 (msec): 105.3±4.0*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. ANP, atrial natriuretic peptide; T1, longitudinal relaxation time; T2, transverse relaxation time; MCA, middle cerebral artery.

*p<0.01, 0.05, respectively, different from saline by Student's t test.

proportion of that for a pure water standard placed next to the rat's head. This allowed us to observe topographically the inhibition of edema formation after ANP administration. Compared with the brain water content determination and the in vitro NMR relaxation time measurement, which allow measurement only of the antiedema effect of ANP with respect to the entire brain tissue, the application of in vivo MRI permitted visual observation of the suppressive effect of ANP on the development of edema. Since MRI is widely used in the diagnosis of brain edema, our results suggest that the use of MRI in clinical investigations of the antiedema effects of ANP is possible.

All of our results indicate that ANP administration may diminish brain damage caused by edema. Although the mechanisms of this antiedema effect are not yet fully clear, our observation that the elevations of brain water and sodium contents are suppressed agrees with other reports on brain edema and the activities of ANP in vitro, suggesting that the effect may be attributed primarily to the regulation of electrolyte and fluid homeostasis in brain tissue.

At present, several drugs such as hyperosmolar agents, steroids, barbiturates, and loop diuretics are used in the treatment of brain edema. Some of these drugs are reported to influence cellular ion transport. It has been shown that ANP also has a regulatory effect on the intracellular electrolyte content in various cells. In vascular endothelial cells, renal inner collecting duct cells, and intestine, it has been reported that ANP regulates sodium transport through the activation of guanylate cyclase. The elevation of intracellular cGMP levels by guanylate cyclase is considered to mediate this. Brain tissue reportedly contains many ANP-specific receptors involved in the regulation of guanylate cyclase, and ANP has been shown to raise the intracellular cGMP level in cultured astroglia, suggesting that ANP prevents the swelling of astroglia in brain tissue through regulation of sodium transport.

It has been reported that ANP specifically inhibits amiloride-sensitive sodium uptake into isolated cerebral capillaries in vitro. Sodium influx from blood to brain across the blood–brain barrier may therefore be interrupted by ANP. Since sodium transport across the blood–brain barrier has been shown to be a rate-limiting step in edema formation, ANP may delay edema formation by inhibiting sodium transport in brain capillaries.

It is suggested, therefore, that ANP contributes to the regulation of sodium transport by causing an elevation in intracellular cGMP levels. It also seems likely that the effect of ANP on sodium transport is the primary reason for the antiedema effects of ANP in the brain. Our results suggest that ANP may be useful therapeutically or prophylactically in the treatment of ischemic brain edema. As such, ANP appears to be deserving of further investigation in the pathophysiology of brain edema.

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References


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