Arginine Vasopressin V1-Antagonist and Atrial Natriuretic Peptide Reduce Hemorrhagic Brain Edema in Rats

Gary A. Rosenberg, MD; Oscar Scremin, MD, PhD; Edward Estrada, BS; and Walter T. Kyner, PhD

Background and Purpose: Injection of arginine vasopressin into the cerebral ventricles in animals with brain injury increased brain water, whereas injection of atrial natriuretic peptide reduced water content. Therefore, to determine the role of endogenous arginine vasopressin in brain edema, we attempted to inhibit edema from a hemorrhagic lesion with an arginine vasopressin V1 receptor antagonist or atrial natriuretic peptide.

Methods: Adult Sprague-Dawley rats with hemorrhages induced by 0.4 IU bacterial collagenase were treated with 75 ng (n=9) or 8 μg (n=9) of the vasopressin V1 receptor antagonist d(CH2)5Tyr(Me)Arg, 3.2 μg (n=4) atrial natriuretic peptide injected intracerebrally, or 50 μg/kg per hour (n=7) atrial natriuretic peptide intraperitoneally. They were compared with control groups injected with 0.4 IU collagenase only. Brain water and electrolytes were measured 24 hours later. Brain uptake of [14C] sucrose was measured 30 minutes after lesions were induced by 0.4 IU collagenase alone (n=5) or after collagenase injection and 50 μg/kg per hour (n=5) atrial natriuretic peptide injected intravenously.

Results: The arginine vasopressin V1 receptor antagonist and atrial natriuretic peptide significantly reduced water and sodium contents in the posterior edematous regions. Brain uptake of [14C] sucrose was significantly reduced by intravenous atrial natriuretic peptide.

Conclusions: Antagonists to arginine vasopressin V1 receptors and atrial natriuretic peptide both significantly reduce hemorrhagic brain edema, and atrial natriuretic peptide appears to protect the blood–brain barrier. (Stroke 1992;23:1767–1774)

The neuropeptides arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) regulate water and electrolyte homeostasis by a series of complex actions at multiple sites in the body. There is increasing evidence that they play an important role in fluid balance in the central nervous system. Intravenous injection of AVP enhanced sodium transport into the cerebrospinal fluid (CSF) and lowered CSF production.1-2 Intraventricular injection of AVP increased transport of labeled water from blood to brain, enhanced water movement across the arachnoid villi, increased brain water content, aggravated cold-injury edema, and increased ependymal permeability but did not alter CSF production.3-7 In an earlier study we showed that the intracerebral injection of AVP caused an increase in brain water content that could be inhibited by an antagonist to the V1 receptor of vasopressin.8

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Intraventricular injection of ANP reduced CSF production and lowered brain water,9,10 while intraventricular or continuous intravenous infusion of ANP reduced cerebral edema secondary to ischemia.11,12 Recently, we developed a model of brain edema secondary to a hemorrhage produced by the intracerebral injection of bacterial collagenase.13 This model was used to study the action of intrinsic AVP in edema production and to test the effect on edema of either an antagonist to the AVP V1 receptor or ANP.

Materials and Methods

One hundred twenty-seven adult Sprague-Dawley rats (Harlan Farms, Altamont, N.Y.) weighing 250–350 g were used in the study. The method for induction of an intracerebral hemorrhage has been described.13 Briefly, rats were anesthetized with 50 mg/kg i.p. pentobarbital and placed in a stereotactic headholder (Kopf Instruments, Tujunga, Calif.). A burr hole was made 3 mm to the left of midline at the bregma. A 23-gauge infusion needle, connected by Teflon tubing to a 25-μl...
was determined by weighing the tissue wet (WW) and
with site 2 including the hippocampus and site 3 the
Animal Research Committees at the University of New
or without the agents to be tested was infused over 9
minutes. The experimental protocols, approved by the
Animal Research Committees at the University of New
Mexico School of Medicine and the Veterans Affairs
Medical Center, conformed to the guidelines established
for animal research by the National Institutes of Health.

Three groups of rats with collagenase lesions were
studied 24 hours after the intracerebral injection of the
enzyme with or without the agent to be tested. The
injection fluid contained both 0.4 IU bacterial collagen-
ase and either 75 ng (n=9) of the AVP V1 receptor
antagonist d(CH2)5Tyr(Me)Arg (Sigma), 8 µg (n=9) of
the antagonist, or 3.2 µg (n=4) ANP 5-28 (Bachem,
Inc., Torrance, Calif.). Each of the drug-treated groups
with collagenase lesions was compared with a separate
group with a similar number of untreated rats with
similar collagenase lesions.

A fourth group of seven rats without collagenase
lesions was injected intracerebrally with 8 µg of the
antagonist to determine the effect of the antagonist
alone; seven saline-injected rats were studied for com-
parison. A fifth group of eight rats with collagenase
lesions was treated with 3.2 µg ANP injected intracere-
brally and studied for water and electrolyte content at 4
hours; they were compared with eight untreated control
rats. A sixth group (n=7) of rats with 0.4 IU
enzymebased lesions were given 5 µg/kg per hour i.p. ANP by an implanted mini-infusion pump
(Alza Corp., Palo Alto, Calif.) and compared with six
untreated control rats.

At 4 or 24 hours after hematoma formation, the rats
were injected intraperitoneally with an overdose of
pentobarbital and their brains rapidly removed. Each
hemisphere was sectioned into four pieces. The anterior
section, which contained the olfactory nerve fibers, was
discarded. Water and electrolytes were measured in
three remaining sites: site 1, the left caudoputamen,
contained the hemorrhage; sites 2 and 3 were posterior,
with site 2 including the hippocampus and site 3 the
occipital region. The water content in each section
was determined by weighing the tissue wet (WW) and
after drying for 24 hours in a 100°C oven (DW). The
percentage of water was calculated using the formula
[(WW−DW)/WW]×100. Sodium and potassium con-
tent in the tissue was measured by flame photometry
(Corning Medical, Medfield, Mass.) after the dried
tissue was extracted with nitric acid. A sample of blood
obtained at the time of removal of the brain was used to
measure plasma sodium and potassium by flame pho-
tometry and osmolality by freezing point depression
(Osmette, Precision Systems, Inc., Natick, Mass.).

Physiological effects of ANP were determined in
another group of rats with collagenase lesions that were
either treated with 3.2 µg ANP (n=4) or had hemor-
rhages alone (n=4). Twenty-four hours after lesions
were made, these rats were anesthetized, and catheters
were inserted into the femoral artery to record blood
pressure with a transducer (Gould Corp., Oxnard, Cal-
if.) connected to a polygraph (Grass Instrument Co.,
Quincy, Mass.). Blood glucose was measured (Sigma).

Blood gases and pH were measured with a blood gas
analyzer (A Vision of Leadership Corp., Roswell, Ga.).
Rectal temperature was recorded. In all rats, including
those in which full physiological measurements were
made, plasma sodium, potassium, and osmolality were
measured at 24 hours after the lesions were produced.
In another group of rats, brain temperature was mea-
ured with an implanted probe using a telemetry system
(Mini-Mitter Corp., Sun River, Ore.). Temperature was
recorded for 24 hours in five rats with collagenase
lesions and compared with that in five rats with similar
lesions treated with 3.2 µg ANP.

Regional cerebral blood flow (rCBF) was measured in
12 rats by the autoradiographic [14C]iodoantipyrine
(IAP) technique 24 hours after hemorrhagic lesions
were induced by the infusion of 0.5 IU bacterial colla-
genase (n=6). CBF was then compared with blood flows
in control animals injected with 2 µl saline (n=6). The
rats were anesthetized with halothane (2.4% in air for
surgery and 1.7% for maintenance) for implantation of
arterial and venous catheters and allowed to recover
from anesthesia in a restraining device (Bollman cage)
for 2 hours. Blood flow was studied by the continuous
intravenous infusion over 30 seconds of 0.6 ml saline

![Figure 1](http://stroke.ahajournals.org/)

**FIGURE 1.** Line graph showing brain temperature (°C)
monitored by telemetry over 24 hours in rats injected with 0.4
IU bacterial collagenase (COLL) and those injected with the
same amount of collagenase and 3.2 µg atrial natriuretic
peptide (ANP).
Table 2. Effect of 8 μg Antagonist to Arginine Vasopressin Intracerebrally on Water and Electrolyte Contents 24 Hours After Injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H₂O (%)</th>
<th>Na⁺ (mEq/mg dry wt)</th>
<th>K⁺ (mEq/mg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
<td>R1</td>
</tr>
<tr>
<td>Saline (n=5)</td>
<td>79.57±0.21</td>
<td>78.71±0.15</td>
<td>79.33±0.18</td>
</tr>
<tr>
<td>8 μg antagonist to arginine vasopressin alone (n=5)</td>
<td>79.92±0.29</td>
<td>78.54±21</td>
<td>79.22±0.32</td>
</tr>
<tr>
<td>F</td>
<td>0.99</td>
<td>0.43</td>
<td>0.09</td>
</tr>
<tr>
<td>p&lt;</td>
<td>0.348</td>
<td>0.531</td>
<td>0.767</td>
</tr>
</tbody>
</table>

Values are mean±SEM. L1, left injected hemisphere, site 1 (anterior); L2, left injected hemisphere, site 2 (posterior); R1, right un.injected hemisphere, site 1 (anterior); R2, right un.injected hemisphere, site 2 (posterior).

Results

Plasma electrolytes and osmolality measured 24 hours after production of the hemorrhage were similar for the various groups. Physiological variables were similar for ANP-treated and untreated rats with collagenase lesions (Table 1). Brain temperature was similar in the rats treated with ANP compared to those with hemorrhages alone (Figure 1). The intracerebral injection of 0.4 IU bacterial collagenase as described above. Twenty minutes after hemorrhage, the rats were injected intravenously with 10 μCi [¹⁴C]sucrose (Dupont/New England Nuclear, Boston, Mass.). Ten minutes later they were injected with a lethal dose of potassium chloride, and their brains were removed and rapidly frozen. A sample of blood was removed and prepared for liquid scintillation counting (Beckman Instruments, Fullerton, Calif.). Brain tissue from around the injection site was weighed, dissolved in Protosol (Dupont/New England Nuclear, Boston, Mass.), and frozen in methylbutane at —70°C. They were cut in the brains were removed in less than 1 minute and frozen in methylbutane at —70°C. They were cut in cryostat into 20-μm-thick slices at —20°C, heat-dried on a glass slide, and exposed to x-ray film (Kodak AR-XOMAT, Rochester, N.Y.) along with eight radioactive standards. Optical density of films and standards was measured in a Philips CCD monochrome imaging module coupled to a Targa M-8 digitizing board and JAVA Software (Jandel Corp., Sausalito, Calif.). The data were analyzed by one-way analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons. Each treatment group was compared with a corresponding untreated collagenase-lesioned group. A value of p<0.05 was considered significant. All results were expressed as mean±SEM.

Figure 2. Bar graphs showing water and sodium contents in rat brains 24 hours after injection into the caudoputamen (site 1) of 0.4 IU bacterial collagenase alone (0.4U COL, filled bars) or with 75 ng AVP V₁ antagonist (75NG ANT, shaded bars). Water and sodium contents were measured in injection site and posterior regions (sites 2 and 3) of injected left hemisphere as described in text. *p<0.05 difference between drug-treated and untreated hemorrhage groups.
tion of the vasopressin antagonist alone had no effect on the brain water and electrolyte content (Table 2).

The 75-ng dose of the AVP V1 antagonist significantly reduced water and sodium contents in the posterior region (site 2) but not in the caudoputamen region (site 1) (Figure 2). Potassium values were also improved in the posterior regions by the 75-ng dose of the antagonist (507 ± 3 mEq/g dry wt at site 2 in untreated rats compared with 491 ± 3 mEq/g dry wt at site 2 in untreated rats; p < 0.001). The 8-μg dose also reduced water and sodium content in the posterior regions (Figure 3), but potassium content was not improved.

Atrial natriuretic peptide caused a more pronounced reduction in water and sodium in all sites of the injected hemisphere (Figure 4). The ANP increased the potassium content significantly only in the occipital region (site 3) (498 ± 5 mEq/mg dry wt in ANP-treated rats compared with 463 ± 9 mEq/mg dry wt in untreated rats at site 3; p < 0.01). The ANP also significantly reduced water and sodium in site 2 of the un.injected right hemisphere, whereas the V1 antagonists had little effect in the opposite hemisphere (data not shown).

Results of the 4-hour studies with ANP are shown in Table 3. A significant effect of treatment with 3.2 μg ANP was observed for water content in the posterior regions but not at the caudoputamen. Sodium and potassium content were significantly improved by ANP in several sites.

Table 4 shows the results of the intraperitoneal infusion of ANP by an implanted infusion pump. There was a significant reduction in water, sodium, and potassium content in the anterior (site 1) and posterior (site 2) regions of the injected left hemisphere. The un.injected right hemisphere also showed a significant reduction in water and sodium content but no change in potassium content.

Regional CBF was markedly reduced in the anterior region in the site of the hemorrhage but was not significantly reduced in the posterior regions, where brain water and electrolytes were increased (Figure 5).

Uptake of [14C]sucrose into brain as shown by the ratio of isotopes in the brain compared with blood was markedly increased in the collagenase-injected hemisphere compared with the opposite uninjected side (Table 5). Intravenous injection of ANP significantly reduced the brain uptake of [14C]sucrose.

Discussion

Collagenase-induced intracerebral hemorrhage resulted in edema at the injection site and in both posterior regions. The increase in water and sodium in the posterior regions was significantly reduced by either an antagonist to the V1 receptor of AVP or by ANP in animals with bacterial collagenase lesions. The ANP reduced the edema in the posterior regions at 4 hours and in all regions by 24 hours. The increased sodium content due to edema in the brains with collagenase injury was reduced by the V1 antagonist and ANP.

The ANP had no effects on the physiological measurements made 24 hours after the lesion was produced. Brain temperature remained normal in rats treated with ANP compared with that in untreated animals, eliminating hypothermia as a cause of the reduced edema. Although the temperature was monitored throughout the
24-hour period by the implanted sensor, the other measurements were made at the end of 24 hours, and there may have been an earlier effect that was not observed. Intracerebral injection of the vasopressin antagonist alone had no effect on brain water and electrolytes.

Bacterial collagenase produces a dose-dependent increase in brain edema. Collagenase causes a large lesion at the injection site that has the histological features of a hemorrhagic infarction, with edema in the white matter tracts around the hippocampus. There are several possible explanations for the edema in the posterior regions, including compromise of CBF to the edematous regions, spread of vasogenic edema from the site of hemorrhage, or activation of substances in the posterior edematous regions. The first possibility was eliminated by the normal rCBF with that of vasogenic edema. The reduction of substances in the posterior edematous regions. The factors controlling release and distribution of neuropeptides within the brain are poorly understood. Increased intracranial pressure caused an increase in CSF levels of vasopressin. Injection of AVP into CSF increased brain water in normal rats and cats with cold-injury edema. We found that intracerebral injection in cat caudate increased brain water in the posterior regions without significantly altering sodium. Vasopressin-deficient rats had an impaired regulation of sodium in the brain. This suggests that AVP acted to increase blood-brain barrier permeability to water.

Receptors to both AVP and ANP have been demonstrated in several extrahypothalamic regions. Vasopressin receptors have been found in the amygdala, the septum, hippocampus, and other brain regions. The vasopressin formed in nuclei of the hypothalamus is released under separate control mechanisms into the brain and the CSF, as well as into the blood. There are two primary types of AVP receptors, namely, a V1 receptor that modulates the pressor effect and a V2 receptor for the antidiuretic effect. From receptor binding studies, the V1 receptor is the primary receptor type in the brain.

Vasopressin V1 receptors have been linked to a phosphoinositide second-messenger system. Isolated cerebral microvessels have receptors for AVP that are of the V1 type. Phosphoinositide metabolism was altered by AVP infusion into brain. Astrocytes grown from cortex and cerebellum have receptors for AVP that lead to the release of inositol phospholipids. We have proposed that AVP caused the excessive stimulation of the AVP V1 receptor, increasing calcium and sodium levels.

Table 3. Effect of 3.2 μg Atrial Natriuretic Peptide on Water and Electrolyte Contents 4 Hours After Collagenase-Induced Hemorrhage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H2O (%) Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Na+ (mEq/mg dry wt) Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>K+ (mEq/mg dry wt) Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2 μg ANP+collagenase (n=8)</td>
<td>80.69±0.16</td>
<td>79.56±0.20</td>
<td>79.30±0.23</td>
<td>289±9</td>
<td>250±10</td>
<td>239±6</td>
<td>485±8</td>
<td>493±6</td>
<td>500±3</td>
</tr>
<tr>
<td>Collagenase (n=8)</td>
<td>81.04±0.07</td>
<td>80.32±0.16</td>
<td>80.01±0.12</td>
<td>324±5</td>
<td>285±5</td>
<td>258±6</td>
<td>449±9</td>
<td>476±4</td>
<td>496±8</td>
</tr>
<tr>
<td>F</td>
<td>4.2</td>
<td>8.7</td>
<td>7.5</td>
<td>12.5</td>
<td>9.5</td>
<td>4.4</td>
<td>9.6</td>
<td>5.4</td>
<td>0.17</td>
</tr>
<tr>
<td>p&lt;</td>
<td>NS</td>
<td>0.01</td>
<td>0.02</td>
<td>0.003</td>
<td>0.008</td>
<td>0.05</td>
<td>0.008</td>
<td>0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values for F and p determined by ANOVA with Bonferroni correction for multiple comparisons.

Table 4. Effect of 5 μg/kg per Hour ANP Intraperitoneally on Water and Electrolyte Contents 24 Hours After Collagenase-Induced Hemorrhage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H2O (%) L1</th>
<th>L2</th>
<th>L3</th>
<th>R1</th>
<th>R2</th>
<th>Na+ (mEq/mg dry wt) L1</th>
<th>L2</th>
<th>L3</th>
<th>R1</th>
<th>R2</th>
<th>K+ (mEq/mg dry wt) L1</th>
<th>L2</th>
<th>L3</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μg/kg per hour ANP+0.4 IU collagenase (n=7)</td>
<td>80.55±0.35</td>
<td>78.80±0.25</td>
<td>78.95±0.20</td>
<td>78.37±0.14</td>
<td>309±15</td>
<td>239±5</td>
<td>224±3</td>
<td>226±2</td>
<td>509±17</td>
<td>488±8</td>
<td>517±12</td>
<td>510±9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 IU collagenase (n=6)</td>
<td>82.39±0.24</td>
<td>80.88±0.3</td>
<td>80.01±0.31</td>
<td>80.04±0.29</td>
<td>402±17</td>
<td>302±10</td>
<td>253±5</td>
<td>273±8</td>
<td>442±15</td>
<td>488±8</td>
<td>517±12</td>
<td>510±9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>17.4</td>
<td>28.8</td>
<td>8.81</td>
<td>30.3</td>
<td>17.5</td>
<td>37.5</td>
<td>27.1</td>
<td>35.0</td>
<td>8.76</td>
<td>8.50</td>
<td>3.35</td>
<td>3.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td>0.0016</td>
<td>0.0002</td>
<td>0.0128</td>
<td>0.0002</td>
<td>0.0015</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0130</td>
<td>0.0142</td>
<td>0.0944</td>
<td>0.1017</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. L1, left injected hemisphere; L2, left uninjected hemisphere; R1, right injected hemisphere; R2, right uninjected hemisphere. Values for F and p determined by statistical comparison using ANOVA with Bonferroni correction for multiple comparisons.
in the cell,8 which is analogous to excitotoxic cell damage produced by excessive stimulation of the glutamate receptor.26 Receptors to ANP have also been shown to be present in multiple extrahypothalamic brain regions.30 ANP acts through cyclic guanosine monophosphate (cGMP) in cells, including isolated cerebral microvessels;31-35 it caused a marked increase in cGMP accumulation in rat glioma and pheochromocytoma cell cultures.36 In bovine aortic smooth muscle cells, cGMP inhibited the formation of inositol triphosphate and blocked the increase in intracellular calcium.37 The inhibition of edema may have occurred by a direct effect of the neuropeptides on brain cell volume or by an effect on the blood–brain barrier. The reduction in brain water content in the posterior regions observed at 4 hours after treatment of the collagenase lesion with ANP suggested that the hormone acts to reduce the damage to the blood–brain barrier. The opening of the blood–brain barrier by bacterial collagenase was blocked by ANP given intravenously. Although the mechanism is uncertain, ANP may prevent calcium-mediated injury to the cerebral capillaries. There may also be a direct action of ANP on cell volume, but this cannot be determined from these studies. A major effect of ANP on brain edema was noted early in the study and resulted in an emphasis on ANP in the subsequent studies.

The receptors for ANP on the cerebral capillary have been shown to be on the luminal surface, which may help explain the reduction of brain water by the intravenous injection of ANP.36 Intravenous infusion of ANP effectively blocked the development of the capillary damage and reduced brain water.

In conclusion, we have shown in an animal model of cerebral hemorrhage that both an AVP V1 receptor antagonist and ANP reduce cerebral edema. Our results indicate that endogenous neuropeptides are important in brain fluid and electrolyte homeostasis and that antagonists to AVP and ANP may be beneficial in the treatment of brain edema.

Acknowledgments
Margaret O'Neal, Debbie Heuser, and Milo Navratil provided technical assistance. Agnes Truske helped prepare the manuscript.

Table 5. Effect of Intravenous Atrial Natriuretic Peptide on Brain Uptake of Intravenous [14C]Sucrose 30 Minutes After Collagenase-Induced Intracranial Hemorrhage

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Left hemisphere</th>
<th>Right hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 IU collagenase</td>
<td>17.3±2.0</td>
<td>1.7±0.35</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 IU collagenase + 50 μg/kg per hour</td>
<td>5.1±0.40</td>
<td>1.3±0.05</td>
</tr>
<tr>
<td>IIv ANP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.0004</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ANP, atrial natriuretic peptide.
* Determined by Student's t test.

References
Humoral mechanisms, regulated by arginine vasopressin and atrial natriuretic peptide, appear to play an important role in fluid balance in the central nervous system. The purpose of the present study was to examine the effects of inhibition of arginine vasopressin, using a V1 receptor antagonist, and atrial natriuretic peptide on cerebral edema. The authors measured brain water and sodium contents and [14C]sucrose brain uptake after intracerebral injection of bacterial collagenase to produce cerebral hemorrhage and subsequent cerebral edema. The authors report that inhibition of V1 receptors and atrial natriuretic peptide reduced brain water and sodium contents and brain uptake of [14C]sucrose after injection of collagenase. Thus, arginine vasopressin and atrial natriuretic peptide are important in regulation of cerebral water and electrolyte homeostasis during pathophysiological conditions.
Arginine vasopressin V1-antagonist and atrial natriuretic peptide reduce hemorrhagic brain edema in rats.
G A Rosenberg, O Scremin, E Estrada and W T Kyner

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