Effect of Vasopressin on Intracranial Pressure of Rabbit

TADASHI NOTO1, TERUO NAKAJIMA1*, YOSHIKI SAJI2 and YUJI NAGAWA2

1Department of Neuropharmacology and Neurochemistry, Institute of Higher Nervous Activity, Osaka University Medical School, Fukushima-ku, Osaka, 553 and 2Medicinal Research Laboratory, Takeda Chemical Industry, Ltd., Yodogawa-ku, Osaka 532, Japan

Synopsis

Effect of vasopressin on intracranial pressure (ICP) was examined by an intraventricular administration of the hormone to a rabbit. ICP was determined at the cisterna magna with a manometer and recorded automatically with a recorder. An injection of over 150 μU of vasopressin lowered ICP, but there was no clear dose-response relationship of the effect of vasopressin on ICP. When vasopressin was injected intraventricularly after lowering ICP by an intravenous injection of acetazolamide which inhibits the production of cerebrospinal fluid (CSF), an additive effect of the hormone on ICP was observed. The effect of vasopressin on excretion of water in CSF was examined by the determination of drainage of tritiated water injected into the lateral ventricle of a rabbit. Drainage of radioactive water into vein was measured by collection of blood at the internal jugular vein and radioactivity of the plasma was counted. Vasopressin accelerated excretion of tritiated water into vein. These results indicate that vasopressin facilitated drainage of CSF into vein to lower ICP.

Although intracranial pressure (ICP) is mainly regulated by blood pressure, we have a clinical experience that a patient with pan-hypopituitarism had a normal ICP in spite of very low blood pressure. This experience led us to look for the other mechanisms for regulation of ICP. The activity of vasopressin, a regulatory hormone for water excretion, has been found in cerebrospinal fluid (CSF) (Heller et al., 1968; Vorherr et al., 1968; Pavel, 1970). Recently, neurophysins, the carrier proteins for the neuro-pituitary hormones, were also detected in CSF (Robinson and Zimmerman, 1973). Therefore, there is a possibility that vasopressin or vasopressin-like protein is excreted into CSF together with neurophysins and may regulate ICP.

This paper reports an effect of vasopressin on ICP of a rabbit and the data on its facilitatory effect on excretion of CSF are also presented.

Materials and Methods

Drugs and reagents
Arginine-vasopressin (Grade VII, 100 IU/mg) was purchased from Sigma Chemical Co. Acetazolamide sodium salt was available from Lederle Co. Ltd. Tritiated water was purchased from New England Nuclear Co. and diluted with water to make its specific activity of 38.8 mCi/ml.

Animals and procedures
Male rabbits (Japanese White), weighing 2.7 to 3.4 Kg, were used. A rabbit was slightly anesthetized with ethyl ether and a tracheal cannula was inserted. Following injections of a muscle relaxant (Gallamine triethiodide, 2 mg/Kg intravenously and 3 mg/Kg intramuscularly), the cannula was connected to a respirator. Under an artificial respiration, the head was

Received April 24, 1978.
* To whom all correspondence should be addressed.
fixed on a stereotaxic brain holder (Todai-Noken type, Tokyo) in the way that Bregma was 1.5 mm higher than Lambda. According to the stereotaxic atlas of a rabbit brain (Sawyer et al., 1954) a stainless cannula (the inner diameter, 0.6 mm) was inserted into the left lateral ventricle for injections of the drugs. The insertion of the cannula into the ventricle was confirmed by the overflow and the respiratory fluctuation of CSF at the top of the cannula and by cutting the brain injected with methylene blue into the ventricle through the cannula after the experiment was over.

ICP was measured at the cisterna magna with a pressure transducer (Nihon Kohoden LPU-0.1) and recorded automatically with a recorder (Nihon Kohoden RM-5 Power Unit, 4 Channels Recticorder RJG-3024) as follows. A sharp-edged stainless cannula was inserted into the cisterna magna and connected to the transducer with a Polyethylene tube filled with saline.

Blood pressure was measured at the right femoral artery. A polyethylene tube (the inner diameter, 0.8 mm) filled with 50 units of heparin sodium solution was inserted into the artery and then it was tied at the peripheral site of the insertion. The polyethylene tube was connected to a pressure transducer.

When the excretion of tritiated water injected intraventricularly was examined, blood was collected at the internal jugular vein. The subclavian vein were tied and a polyethylene tube (the inner diameter, 0.8 mm; the length, 6.5 cm) filled with 30 units of heparin sodium solution was inserted in the direction of heart into the external jugular vein until the top of the tube reached the confluence of the internal and the external jugular vein. The tube was fixed and the external jugular vein was tied at the cranial site of the insertion of the tube. Two tenth ml of blood was sampled at the indicated periods.

Measurement of radioactivity in blood

Two tenth ml of blood sampled from the internal jugular vein was centrifuged at 3,000 rpm for 4 min and plasma was pooled. Fifty μl of plasma each was transferred into a vial and 5 ml of a scintillator solution of xylene-polyalkylene surfactant system was added to it. Radioactivity was counted with a liquid scintillation counter (Nuclear Chicago, Mark II). Counting efficiency of the samples was about 25%.

Results

Effect of vasopressin on ICP

Vasopressin dissolved in 10 μl of saline was injected into the lateral ventricle of a rabbit. ICP and blood pressure were monitored with a recorder for more than 1.5 hr. The 6 cases out of total 24 rabbits, in which blood pressure of the animals dropped during the experiment, were excluded, since ICP is dependent on blood pressure. Individual variations from 20 to 60 mm H2O of ICP of rabbits were observed under the experimental condition described above. Fig. 1 shows the changes in ICP of rabbits after the intraventricular injections of various doses of vasopressin. 100 μU of vasopressin caused no change in ICP and the doses over 150 μU produced decrease in a pressure. The pressure dropped gradually until 60–90 min after the injection and then remained at the low level for the following 1 hr or more. There was no clear dose-response relationship of the effect of vasopressin on ICP (Table 1.)
Table 1. Decrease in ICP after intraventricular injections of various doses of vasopressin.

<table>
<thead>
<tr>
<th>Time after the injection (min)</th>
<th>Saline</th>
<th>100</th>
<th>150</th>
<th>Vasopressin (μU)</th>
<th>250</th>
<th>500</th>
<th>1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>3.7</td>
<td>(± 3.2)</td>
<td>2.0</td>
<td>2.3</td>
<td>1.7</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>9.0</td>
<td>(± 3.6)</td>
<td>5.3</td>
<td>3.7</td>
<td>4.3</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>0</td>
<td>11.7</td>
<td>(± 3.1)</td>
<td>6.3</td>
<td>6.3</td>
<td>7.3</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0</td>
<td>12.3</td>
<td>(± 1.5)</td>
<td>8.7</td>
<td>7.7</td>
<td>9.7</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
<td>0</td>
<td>18.3</td>
<td>(± 3.8)</td>
<td>11.7</td>
<td>9.7</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Values represent Means ± S.D. in the three experiments showed in Fig. 1.

Effect of vasopressin and acetazolamide on ICP

Acetazolamide, carbonic anhydrase inhibitor, has been reported to inhibit production of CSF at choroid plexus and then decrease ICP (Tschirgi et al., 1954; Davson and Luck, 1957). Therefore, the drug was used in order to determine the mechanism of the effect of vasopressin, whether the hormone acted on the production of CSF at choroid plexus or not.

Acetazolamide sodium salt (3.5 mg/Kg) was dissolved in 0.5 ml of saline and injected intravenously from the preauricular vein of a rabbit. ICP was gradually decreased by the injection of acetazolamide (Fig. 2). When the pressure remained constant at the low level at least for 30 min, 1 μU of vasopressin was injected intraventricularly into the animal. The pressure was further decreased by the injection of the hormone. The drug and the hormone were administered to a rabbit in the reverse order to the injections of these compounds in the above experiment. An additive effect of acetazolamide was also observed to the effect of vasopressin (Fig. 3). From these observations it was speculated that

---

Fig. 2. Changes in ICP of rabbits after the injections of acetazolamide and vasopressin. Acetazolamide sodium salt (3.5 mg/Kg) was injected intravenously into a rabbit. When ICP ceased from decreasing and remained constant at least for 30 min, 1 μU of vasopressin was injected intraventricularly into the animal. AZ, acetazolamide injection; VASO, vasopressin injection.

Fig. 3. Changes in ICP of rabbits after the injections of vasopressin and acetazolamide. One μU of vasopressin was injected intraventricularly into a rabbit. When ICP ceased from lowering and remained constant at least for 30 min, acetazolamide sodium salt (3.5 mg/Kg) was injected intravenously into the animal. VASO, vasopressin injection; AZ, acetazolamide injection.
vasopressin did not inhibit the production at choroid plexus but probably accelerated excretion of CSF.

**Effect of vasopressin on excretion of CSF**

Ten min after an intraventricular injection of vasopressin (1 mU) or saline, 5 μl of the diluted radioactive water was injected intraventricularly into a rabbit. Two tenth ml of blood was sampled from the internal jugular vein at the indicated intervals after the injection of radioactive water. Fig. 4 shows the increase and decline of radioactivity in blood during 60 min after the injection of radioactive water. In the case of the injection of vasopressin, radioactivity in blood from the vein increased rapidly by 5 min and then declined rapidly to reach the constant level 30 min after the injection.

![Graph](image)

**Discussion**

Robinson and Zimmerman (1973) reported on occurrence of neurophysins in CSF, the supraoptic and paraventricular nuclei and their tracts, the ependymal tanyctyes of the infundibular recess of the third ventricle, the external layer of the median eminence where capillaries drain into hypophysial portal vessels and posterior pituitary gland. From these findings as well as occurrence of the activities of neuropituitary hormones in CSF, they speculated that neurophysins as well as the neuropituitary hormones were secreted into CSF, reabsorbed by the tanyctyes, transported through their processes and then secreted again into the portal blood. However, there were no reports about physiological significances of the hormones and their carrier-proteins in CSF. The present data demonstrated a possible role of vasopressin in CSF in spite of the fact that the minimal effective dose of vasopressin was thought to be higher than the physiological concentration of vasopressin in CSF which was reported to be 4–5 μU/ml in rabbits. By anesthetization with pentobarbital sodium it was increased to 60 μU/ml in rabbits (Heller et al., 1968). Since a volume of CSF in a rabbit was reported to be about 2 ml (Bradbury and Davson, 1964), the concentration of vasopressin was calculated to be 80 U/ml when the minimal effective dose was injected. This value is not so...
far from that under anesthesia. Therefore, there is a possibility that vasopressin may act on the regulation of ICP under some condition, although the pressure is mainly regulated by blood pressure under a normal condition.

The effect of vasopressin on ICP lasted for a long time as showed in Fig. 1. This observation was coincident with the findings on water transport of membranes. Using abdominal skin of the green frog, Fuhrman and Ussing (1951) demonstrated that the effect of vasopressin on water uptake and on the potential difference lasted for 6 hr or more. Sawyer (1960) also reported that the effect of vasopressin on water movement from serosal to mucosal surface of the bladder of *Rana catesbina* continued for 4.5 hr or more. These long lasting effects of vasopressin were also confirmed by the binding experiment of vasopressin that when the toad bladder to which tritiated arginine vasopressin bound was incubated in the sodium bicarbonate buffer, pH 8.5, containing 0.1 M cystein for 8 hr, about 40% of radioactivity still remained on the bladder (Schwartz et al., 1960).

In the present experiment no clear dose-response relationship was obtained in the effect of vasopressin from 150 to 1,000 μU on ICP. This may be caused by the fact that there were individual variations from 20 to 60 mm H₂O of ICP of rabbits under the present experimental condition and the dose of vasopressin could not be increased stepwise in the same experimental animal. Therefore, a more delicate design is required to demonstrate a dose-response relationship in the effect of vasopressin on ICP. Indeed, in our preliminary experiment using the isolated meninges of a cat, a clear dose-response relationship was observed in the effect of vasopressin on water transport.

The major physiological role of vasopressin has been known to regulate water balance in animals. The hormone regulates transportation of water to toad bladder (Hays and Leaf, 1962; Rasmussen et al., 1960; Sawyer, 1960), toad skin (Fuhrman and Ussing, 1951; Koefoed-Johnsen and Ussing, 1953; Whittenbury, 1962) and mammalian distal nephrons (Morel et al., 1965). The *in vitro* experiments using renal tubules collected from rabbit kidney (Grantham and Burg, 1966) and medullary collecting ducts from renal papillae of rat kidney (Morgan et al., 1968) indicated that vasopressin increased the permeabilities of the tubules and the ducts to water. Therefore, the effect of vasopressin on excretion of CSF must be caused by its effect on the permeability to water. From this viewpoint, an interesting finding is that the arachnoid villi from which more than 2/3 of CSF excretes into the sinus are composed of many tubules structurally resembling to the glomerulus of kidney (Welch and Friedman, 1964).

Mechanisms of the effects of vasopressin on excretion of CSF and its clinical application to hydrocephalus caused by disturbance of excretion of CSF are now under investigation.

**References**


