Central Injections of Capsaicin Cause Antidiuresis Mediated Through Neurokinin-1 Receptors in Rat Hypothalamus and Vasopressin Release

Hiromi Tsushima and Mayumi Mori

Department of Pharmacology, Nagoya City University Medical School, Kasugai, Mizuho-ku, Nagoya 467-8601, Japan

Received July 17, 1998 Accepted November 27, 1998

ABSTRACT—Intracerebroventricular injections of capsaicin at 100–500 nmol elicited dose-dependent decreases in urine outflow volume in anesthetized, hydrated rats. The capsaicin (500 nmol)-induced antidiuresis was inhibited by pretreatment with CP96345 (30 nmol, a neurokinin-1-receptor antagonist), but not by that with phenoxybenzamine (20 nmol, an alpha-adrenoceptor antagonist), timolol (100 nmol, a beta-adrenoceptor antagonist) or atropine (300 nmol, a muscarinic antagonist) into the hypothalamic supraoptic nucleus (SON). Intravenous injections of d(CH2)2-α-Tyr(ET)VAVP (50 μg/kg, a vasopressin-receptor antagonist) completely blocked the antidiuresis. In intra-SON microdialysis experiments, acetylcholine concentration in the perfusate of the capsaicin-injected rats was not different from that of the vehicle-injected rats. These findings suggested that capsaicin stimulated substance P release in the SON and caused the antidiuresis as a result of the increased release of vasopressin into the circulation from the neurohypophysis mediated through neurokinin-1 receptors in the SON.

Keywords: Supraoptic nucleus, Vasopressin, Substance P, Capsaicin, Neurokinin-1 receptor

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 280–360 g (Kitayama Labes, Ina), which were housed under a 12-hr light/dark cycle at a room temperature of 22–23°C, were used. The animal care and research protocols were in accordance with the guidelines of our university.

Experiment 1: Measurement of urinary volume

After they were starved for approximately 15 hr, the rats were orally loaded with a volume of tap water equivalent to 5% of their body weight followed 45 min later by the same volume of 12% ethanol. In the rats anesthetized with ethanol, three catheters were placed in the urinary bladder, the trachea and the jugular vein. They were fixed in a stereotaxic apparatus (Takahashi Co., Tokyo), and then stainless steel cannulae were inserted into the right SON (6.3 mm anterior to the lambda, 1.3 mm lateral to the midline, 8.9 mm ventral from the dural surface) and the left lateral ventricle (5.7 mm, 1.7 mm, 3.8 mm) according to the atlas of König and Klippel (12). The drugs were injected into the ventricle or the nucleus with a microsyringe connected to the stainless
Urine outflow from the bladder catheter was measured with an electric drop counter (DCT 102; Unique Medical, Inc., Tokyo) every 10 min. The volume of one drop was 20 μl. The changes of urinary volume after drug administrations were expressed as a percentage of the control urinary volume (urinary volume for 10 min before drug administration). The jugular vein catheter was used for infusion of Locke's solution (154 mM NaCl, 3.4 mM KCl, 1.4 mM CaCl₂, 5.6 mM glucose) containing 3% ethanol at 0.1 ml/min. Ethanol-anesthesia and -infusion brought a stable anesthetic condition and a constant level of urinary volume during the experiments. After urinary volume became constant for at least 20 min, phenoxybenzamine, timolol, atropine, CP96345 (intra-SON injection), d(CH₂)₇-ß-Tyr(ET)VAVP (i.v.) or vehicle were pretreated and 30–40 min later, capsaicin was given i.c.v.

Experiment 2: microdialysis

A few days before the experiment, rats anesthetized with pentobarbital (40 mg/kg, i.p.) were implanted with a guide cannula (G-12; Eicom, Kyoto) in the right SON using dental cement and three small screws. On the experimental day, under the same experimental conditions as those for measurement of urinary volume, a stainless steel cannula was inserted into the left lateral ventricle and a microdialysis probe (A-1-12-02, Eicom) was put in the guide cannula. The microdialysis probe was perfused with saline (containing 10⁻⁴ M physostigmine) at a rate of 2 μl/min, which was collected every 5 min. Acetylcholine (ACh) concentration in the perfusate was measured with an ACh Analysis System (HPLC combined with an enzyme-immobilized column and an electrochemical detector (BAC-304, Eicom)). ACh concentration in the perfusate was constant for 2–3 hr from approximately 2 hr after perfusion of the microdialysis probe. The recovery of ACh (3 × 10⁻⁷ M) was 9.8±1.4% (n=5). Urine outflow volume was measured simultaneously.

Identification of injection site

At the end of the experiment, methylene blue was injected into the SON and the ventricle to verify the injection sites of the drugs (33). After the brain was cut into 15-μm coronal sections with a microtome (Tissue-Tek II; Miles Inc., Erkhardt, IN, USA), the stained sites were determined under a microscope. Methylene blue injected into the left lateral ventricle spread all over the right and left lateral and the third ventricle. The sites of the microdialysis probes in the sections were found under a microscope without injection of methylene blue.

Statistic analysis

The results were represented as the mean±S.E.M. Statistic analysis between two values was performed by Fisher's PLSD test following one-way ANOVA. P value less than 0.05 was significantly different.

Drugs

The following drugs were used: capsaicin and atropine sulfate (Sigma Chemical Co., St. Louis, MO, USA) and phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto). Timolol maleate (Sankyo Co., Tokyo), 1-(ß-mercapto-ß-ß-cyclopentamethylene propionic acid) 2-(O-ethyl)-ß-Tyr, 4-Val, Arg-vasopressin [d(CH₂)₇-ß-Tyr(ET) VAVP; Prof. K.G. Hofbauer, Ciba-Geigy, Ltd., Basel, Switzerland] and CP96345 hydrochloride (Pfizer Inc., Groton, CT, USA) were generous gifts. The other chemicals were of the highest analytical grade available. Capsaicin was dissolved in a minimum volume of dimethylsulfoxide (DMSO), and then diluted with saline to the concentration used. The other drugs were dissolved in saline.

RESULTS

Effects of capsaicin on urinary volume

As shown in Fig. 1, i.c.v. administration of capsaicin produced antidiuretic effects in a dose-dependent manner. Saline containing DMSO injected into the ventricle showed 20% increases in urine outflow volume for 20–50 min after the administration, compared with the control.
urine outflow level. The effect of capsaicin at 100 nmol on urinary volume was not statistically different from the two vehicle-injected groups. Capsaicin at 500 nmol elicited potent antidiuretic effects with a slow time-course, showing the maximum decrease in urinary outflow volume at 20 min and the recovery to the control level at 90 min after the administration.

Effects of the antagonists on the capsaicin-induced antidiuresis

Intravenous injections of the vasopressin antagonist, d(CH₂)₇-d-Tyr(Et)VAVP at 50 μg/kg completely blocked the capsaicin (500 nmol)-induced antidiuresis (Fig. 2A). We have previously shown that this dose of d(CH₂)₇-d-Tyr-(Et)VAVP inhibited the vasopressin (4 mU, i.v.)-induced antidiuresis to an extent similar to its inhibition of the capsaicin-induced one under the same experimental condition (14). The vasopressin antagonist itself did not change urine outflow (data not shown).

To examine neurotransmitters involved in the capsaicin-induced antidiuresis, the various antagonists were microinjected into the SON before i.c.v. administration of capsaicin. The results were shown in Fig. 2B. The muscarinic antagonist atropine (300 nmol) did not influence the antidiuresis at all. The alpha-adrenoceptor antagonist phenoxybenzamine (20 nmol) showed a tendency to inhibit the capsaicin-induced antidiuresis and significantly inhibited it at only 10 min after administration. The beta-adrenoceptor antagonist timolol (100 nmol) appeared to strengthen the effects for 40–60 min after the administration of capsaicin, but not significantly. On the other hand, capsaicin following the neurokinin-1-receptor antagonist CP96345 did not induce any significant change in urinary outflow volume. When timolol alone was microinjected into the SON, urine outflow increased to 115±6%, 167±18%, 179±24% and 184±19% of the control level at 10, 20, 30 and 40 min after the administration, respectively. The diuresis continued for more than 60 min. The other antagonists themselves did not influence urine outflow (data not shown).

ACh content in the microdialysis perfusate

After capsaicin (500 nmol) was injected into the ventricle, ACh concentration was measured in the perfusate of the microdialysis of the SON (Fig. 3). Microinjection of the vehicle for capsaicin (saline containing DMSO) into the lateral ventricle caused 35% decreases in ACh release of the SON, compared to the control level, for 0–80 min after the administration. When saline was injected

![Fig. 2. Effects of the various antagonists on capsaicin-induced antidiuresis. The abscissa and ordinate are the same as those in Fig. 1. Control urine outflow volume: 0.80±0.05 ml/10 min. A: ○: 500 nmol capsaicin, n=8; ●: 50 μg/kg d(CH₂)₇-d-Tyr-(Et)VAVP + capsaicin, n=5. The vasopressin antagonist d(CH₂)₇-d-Tyr(Et)VAVP was intravenously injected prior i.c.v. administration of capsaicin. B: ○: 500 nmol capsaicin, n=8; ●: 30 nmol CP96345 + capsaicin, n=3; △: 20 nmol phenoxybenzamine + capsaicin, n=6; ▲: 100 nmol timolol + capsaicin, n=3; □: 300 nmol atropine + capsaicin, n=6. Capsaicin was administered into the lateral ventricle at 30–40 min after intra-SON injection of the antagonists. The values are the mean±S.E.M. *P<0.05 vs values of the capsaicin-injected group without the pretreatment at each corresponding time-point (○).](image-url)
into the ventricle, ACh release stabilized for more than 120 min (15). Following the administration of capsaicin, ACh release did not show any significant change, compared with the saline-containing-DMSO-injected rats or the pre-injected value in the capsaicin-injected group. The control ACh content was 0.0267 ± 0.0067 pg/μl.

DISCUSSION

The present study demonstrated that an intravenously injected vasopressin antagonist can inhibit the antidiuresis induced by i.c.v. administered capsaicin. This suggested that capsaicin-induced antidiuresis results from an increase in plasma vasopressin concentration. Probably, capsaicin indirectly activates the vasopressin-containing neurons in the SON and/or PVN, which are known to contain the cell bodies of vasopressin-containing neurons. Capsaicin-induced antidiuresis was also inhibited by CP96345 microinjected into the SON. CP96345 is a specific antagonist of neurokinin-1 receptors, one of the three neurokinin-receptor subtypes (16, 17). Capsaicin is suggested to promote substance P release in the hypothalamus from existence of binding sites for capsaicin in the hypothalamus and decreased content of hypothalamic substance P after capsaicin treatment (10, 11). We have previously reported that microinjection of substance P (a specific neurokinin-1-receptor agonist, 17) at 1 nmol into the SON produced an 80% reduction in urinary volume and a 50-fold increase in urinary vasopressin concentration at 20–30 min after the injection, compared with the vehicle-injected group (6). Taken together, we consider that capsaicin promotes release of substance P in the SON and substance P stimulates vasopressin release, resulting in antidiuresis, which is mediated through neurokinin-1 receptors. Neurokinin-1 receptors probably exist in the SON or at a site less than 1 mm from the nucleus because methylene blue injected into the nucleus did not diffuse more than 1 mm from the injection site (unpublished data, H. Tsushima and M. Mori). Furthermore, norepinephrine and oxotremorine, which produced antidiuretic effects after microinjections into the nucleus, did not show any significant effects on urine outflow after microinjections at several sites 1 mm from the nucleus (18, 19).

On the other hand, the effects of neurokinin-receptor agonists injected into the ventricle are reported to be prevented by i.c.v. administration of neurokinin-3-receptor antagonists (20–22). In this case, the agonists seem to affect neurokinin-3 receptors on/in the ventricle wall or its vicinity. Recently, neurokinin-3 receptors in the PVN adjacent to the ventricle were found to contribute urine production (3). Massi et al. (5) conclude that the PVN is the action site for the antidiuretic effects induced by i.c.v. administration of neurokinin-receptor agonists. Therefore, in the two nuclei, the SON and PVN, neurokinin-receptor subtypes involved in the regulation are different.

Although substance P-containing nerve projections and substance P binding sites are present in the SON, synapses of substance P-containing neurons with cell bodies of vasopressin-containing neurons have not been found (1, 2). Therefore, the antidiuretic effects and vasopressin release by substance P are possibly mediated through intra-SON interneurons. Although the neurotransmitter is not defined yet, it is not ACh, adrenaline or noradrenaline because the capsaicin-induced effects were not affected by atropine, timolol nor phenoxybenzamine as shown in this study.

ACh concentration in the dialysate did not change significantly after i.c.v. administration of capsaicin. This is consistent in the results that atropine did not inhibit the capsaicin-induced antidiuresis. On the other hand, vehicle containing DMSO appeared to decrease ACh release in the SON and increase urinary volume. ACh applied exogenously in the nuclei produced antidiuresis mediated through vasopressin release (18, 23). Intra-SON microinjection of atropine alone did not influence urine outflow. From these findings, it is supposed that the role of ACh in urine outflow regulation may be minor under the control condition, although ACh has the ability to regulate vasopressin release, resulting in antidiuresis.

In conclusion, i.c.v. administration of capsaicin elicited antidiuresis involving substance P and vasopressin.
release. Endogenously-released substance P in the SON regulates urine production due to increased secretion of vasopressin, and substance P probably binds to neurokinin-1 receptors in the nucleus. On the other hand, ACh does not play an important role under this experimental condition.

REFERENCES


