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Agonist and hypertonic saline-induced trafficking of the NK3-receptors on vasopressin neurons within the paraventricular nucleus of the hypothalamus

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Haley, Gwendolen E., and Francis W. Flynn. Agonist and hypertonic saline-induced trafficking of the NK3-receptors on vasopressin neurons within the paraventricular nucleus of the hypothalamus. Am J Physiol Regul Integr Comp Physiol 290: R1242–R1250, 2006. First published December 15, 2005; doi:10.1152/ajpregu.00773.2005.—The neurokinin 3 receptor (NK3R) is colocalized with vasopressinergic neurons within the hypothalamic paraventricular nucleus (PVN) and intraventricular injections of NK3R agonists stimulate vasopressin (VP) release. Our objectives were to test the hypotheses that intraventricular injections of the selective NK3R agonist, senktide ([Asp6, N-Me-Phe8] substance P, senktide), activate NK3R expressed by vasopressinergic neurons within the PVN, and see whether NK3R expressed by vasopressinergic neurons in the PVN are activated by hyperosmolarity. NK3R internalization was used as a marker of receptor activation. Immunohistochemistry revealed that NK3Rs were membrane-bound on VP immunoreactive neurons in control rats. Following senktide injection, there was a significant increase in the appearance of NK3R immunoreactivity within the cytoplasm and a morphological rearrangement of the dendrites, indicating receptor internalization, which was reversible. Furthermore, pretreatment with a selective NK3R antagonist, SB-222200, blocked the senktide-induced VP release and internalization of the NK3R in the PVN. These results show that the trafficking of the NK3R is due to ligand binding the NK3R. In a subsequent experiment, rats were administered intragastric loads of 2 or 0.15 M NaCl, and NK3R immunohistochemistry was used to track activation of the receptor. In contrast to control rats, 2 M NaCl significantly increased plasma VP levels and caused the internalization of the NK3R on VP neurons. Also, NK3R immunoreactivity was located in the nuclei of vasopressinergic neurons after senktide and 2 M NaCl treatment. These results show that hyperosmolarity stimulates the local release of an endogenous ligand in the PVN to bind to and activate NK3R on vasopressinergic receptor internalization; endosomes; neurokinin B; hyperosmolarity

THE MAMMALIAN TACHYKININ FAMILY consists of the neuropeptides substance P, neurokinin A, and neurokinin B (NKB) that preferentially bind to the neurokinin 1 receptor (NK1R), the neurokinin 2 receptor (NK2R), and the neurokinin 3 receptor (NK3R), respectively. The NK3R is widely distributed throughout the rat central nervous system, with particularly high concentrations of NK3R immunoreactivity and mRNA found within the magnocellular neurons of the supraoptic nuclei (SON) and the paraventricular nuclei (PVN) of the hypothalamus (12, 28, 35). Subsequent studies have shown that NK3R are colocalized extensively with vasopressinergic neurons within the PVN (11, 16). An estimated 72% of vasopressinergic neurons express NK3R in the PVN (10). This anatomical relationship suggests that NK3Rs play a significant role in vasopressin (VP) release from VP neurons. Indeed, exogenous central injections of NK3R agonists, such as senktide, cause a systemic VP release and an antidiuresis in water-loaded rats (30, 34). In addition, intraventricular injections of NK3R agonists induce c-Fos expression in magnocellular vasopressinergic neurons within the PVN (11, 38). While intraventricular injections of NK3R agonists stimulate the release of VP, the site of transduction is not specified. Intraventricular administration of senktide could spread through the ventricular system to activate NK3R distant from the PVN. NK3Rs are widely distributed in the brain and are present in several nuclei, such as the SON, nucleus of the solitary tract, septum, and the bed nucleus of the stria terminalis, all of which project to the PVN (12, 28, 35, 37, 41). Activation of NK3Rs within these nuclei could initiate a synaptic activation of the vasopressinergic neurons within the PVN, which may or may not involve the local release of an endogenous tachykinin to bind the NK3Rs. To determine whether the senktide-stimulated release of VP involves the activation of NK3Rs expressed by the VP-producing neurons themselves, we imaged the trafficking of NK3Rs from the membrane to organelles within the intracellular compartment of VP immunoreactive VP neurons. The translocation of NK3Rs requires the binding of its agonist and is a specific pharmacological marker of receptor activation (3, 8, 17, 25).

Exogenous ligands for the NK3R stimulate the release of VP into the circulation. The physiological stimulus that causes the local synaptic release of tachykinin ligands, presumably NKB (20, 33) to activate NK3Rs in the magnocellular VPN is not known. One candidate stimulus is osmolarity, because changes in plasma osmolality and plasma sodium concentration affect tachykinin gene expression (31) and NKB immunoreactive terminals are described in the PVN (26). Also, it is well established that hyperosmolarity induces c-Fos expression in VP neurons as well as a systemic release of VP (7, 10, 14). We

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used immunohistochemistry to track the internalization of NK3Rs to test the hypothesis that hyperosmolarity causes the local release of tachykinins that bind and activate the NK3R expressed by VP neurons in the PVN.

MATERIALS AND METHODS

Animals

All procedures were approved by the Animal Care and Use Committee at the University of Wyoming. Rats (male, 300–400 g, n = 36; Charles River Laboratory) were housed individually in standard wire mesh cages in a temperature-controlled room with a 12:12-h light-dark cycle. Rats had ad libitum access to Purina chow and water.

Surgical Procedures

Rats were anesthetized with a mixture of ketamine HCl and acepromazine maleate (0.07 ml/100 g ip) and were secured in a stereotaxic device using blunt earbars with the skull level. A midline incision was made, and a hole was drilled through the skull at 0.9 mm posterior to bregma and 1.8 mm lateral to the midline. The dura was cut and a stainless steel guide cannula (26 g; Plastics One) was lowered 4.5 mm from the dura. The guide cannula was anchored to the skull using four jewelry screws and dental acrylic, and was sealed with an obturator.

The following day, rats were reanesthetized and implanted with femoral arterial and venous catheters constructed from polyethylene (PE) tubing. Arterial catheters were constructed from 3 cm of PE-10 tubing superglued to a 20-cm length of PE-50 tubing. Venous catheters were a 25-cm length of PE-50 tubing. One 2-in. hindlimb incision was made and the femoral artery and vein were exposed using blunt dissection. Catheters were inserted into position through punctures in the vein and artery, and were secured by ligatures around the blood vessels. Once secured, the tubing was filled with heparinized saline (50 U/ml), and the distal ends were drawn subcutaneously to an exit incision between the scapulae and heat sealed until testing. Rats were returned to their cages to recover with ad libitum access to food and water for 24 to 48 h.

NK3R Agonist Experiment

Rats were removed from their home cages, and the arterial and venous catheters were connected to lengths of PE-50 tubing that were attached to syringes filled with heparinized saline. An injector, which was connected to PE tubing, was inserted into the intraventricular cannula. The distal end of the PE tubing was attached to a syringe mounted on an infusion pump. The rats were placed in a clear plastic chamber. Femoral arterial and venous catheters were connected to lengths of PE-50 tubing that were attached to syringes filled with heparinized saline. The infusion pump was turned on and the rats were administered intraventricular infusions of 250 pmol of the NK3R agonist SB-222200 (5 μl). Upon completion of the agonist infusion, the liquid was removed. Rats were then administered an intraventricular injection (5 μl) of either senktide (400 μg, n = 4) or 0.15 M isotonic saline (n = 4). Blood samples (2 ml) were taken at 0, 10, and 20 min after the start of the infusion of the agonist. With each blood sample taken from the femoral artery a simultaneous infusion of donor blood (2 ml) was infused into the femoral vein (1, 2, 9). After the final blood sample was collected, the rats were killed with a lethal injection of pentobarbital sodium and the brains were processed for VP and NK3R immunoreactivity. Plasma VP was extracted using 1% TFA buffer and C-18 column technique. Each sample was analyzed in triplicate on a VP ELISA (Assay Designs, Ann Arbor, MI).

Intragastric Hypertonic Saline Experiment

Rats were anesthetized with a mixture of ketamine HCl and acepromazine maleate (0.07 ml/100 g ip) and fitted with femoral arterial and femoral vein catheters. One day after surgery, each rat was gently held and a feeding tube was inserted into the mouth and advanced to the stomach. Rats were administered either an intragastric load (6 ml) of 2 M NaCl (n = 3) or 0.15 M (n = 3) NaCl. The tube was then withdrawn, the arterial and venous lines were connected to lengths of tubing, and the rat placed into a clear plastic chamber. An initial blood sample (2 ml) was taken before the rat was administered the intragastric load for a baseline. Blood samples (2 ml) were then collected from the femoral artery at 15, 30, and 45 min after the intragastric load. As blood was being drawn from the femoral artery, an equal volume of replacement blood collected from an etherized donor rat 1–2 h earlier, was infused into the femoral vein (1, 2, 9). Forty-five min after the intragastric load, rats were killed and the brains were processed for NK3R and VP immunoreactivity. This time point was chosen because pilot experiments showed that both plasma osmolality and VP levels were higher than baseline levels following 2 M NaCl at this time. Plasma osmolality (Multi-Osmette, Precision Systems) and VP were determined for each sample.

Immunohistochemistry

Rats were administered a lethal injection and perfused with 300 ml of cold PBS followed immediately by 300 ml of cold Zambonis fixative. Brains were extracted, postfixed for 2 h in the Zamboni’s fixative at 4°C, and stored in 30% sucrose/PBS for 24 h. Brains were blocked for the PVN and alternate 30-μm sections were cut on a cryostat and mounted directly on slides. The sections were incubated simultaneously in antibodies against the NK3R (1 μg/ml, raised in rabbit; Novus Pharmaceuticals) and VP (2 μg/ml, raised in guinea pig;
Peninsula Laboratories) for 48 h at 4°C. Sections were then washed with PBS and incubated in secondary antibodies conjugated to either Texas Red (1.5 μg/ml, anti-rabbit; Vector Laboratories) or FITC (1.5 μg/ml, anti-guinea pig; Vector Laboratories). In addition, sections were incubated in 4,6-diamidino-2-phenylindole (DAPI), a DNA stain (1.0 μg/ml; Pierce Biotechnology) for 3 min.

Triple immunohistochemical labeled sections were imaged using a Bio-Rad Confocal microscope and Bio-Rad Laser 2100 software. Sections were imaged using the following lasers: blue diode with a 405 nm (DAPI), argon with an excitation wavelength of 488 nm (FITC), and green He/Ne with an excitation wavelength of 658 nm (Texas Red). Images were taken with a ×60 objective and a digital zoom of 3. Each optical section was scanned a total of three times through Kalman filtering every 0.05 μm for a total of 12 sections utilizing fast-speed scanning. Each flurochrome was registered in a separate channel. In addition, the software enabled the merging of all three channels to create a composite image. A two-dimensional reconstruction of specific z-series could be generated to quantify NK3R on VP neurons. In the composite image, neurons that were immunoreactive for both VP and NK3R were yellow in color. Twenty images per rat were taken at random through the PVN.

**NK3R Internalization**

Internalization of NK3R was used to track the activation of the receptor on dendrites and soma of VP immunoreactive neurons. When receptors were internalized on dendrites, the dendrites took on a beaded appearance (25). This was in contrast to unstimulated neurons in which receptors are membrane bound and the dendrites are uniform in diameter with NK3R immunoreactivity outlining the perimeter of the neurons’ processes. After NK3R is internalized into the cell, the immunoreactivity appears as bright spots ~0.5 μm in diameter within the intracellular compartment of the soma. Conversely, control neurons show NK3R immunoreactivity that is membrane bound and outlines the perimeter of the neuron (3, 25).

NK3R internalization on dendrites and soma was quantified in composite confocal images using NIH ImageJ software. With the ×60 objective and a digital zoom of 3, each image was 100 μm². VP and NK3R immunoreactive dendrites spanned the entire PVN area. To quantify NK3R internalization, a 50-μm² square was placed over the image and dendrites with NK3R internalization that intersected one of the four sides were counted (13, 32).

Data were analyzed using SPSS software. All values are means ± SE. Means were compared using independent Student’s t-tests and one-way ANOVAs.

**RESULTS**

**NK3 Agonist Experiment**

**Plasma VP.** Intraventricular injections of senktide significantly increased plasma VP levels. Plasma VP levels increased from a baseline of 3.3 ± 0.4 pg/ml to 10.7 ± 1.6 pg/ml (t₀ = 4.66; P < 0.001) at 10 min following senktide injection. Plasma VP levels increased further to 16.2 ± 3.3 pg/ml at 20 min following senktide injection (t₀ = 4.11; P < 0.001). Plasma VP levels returned to preinjection levels 120 min after the senktide injection, 5.3 ± 1.0 pg/ml (t₀ = 1.53; P > 0.15). Plasma VP levels of control rats did not significantly change during the 20-min time period after the saline injection, and VP levels stayed between 2.4 ± 0.2 and 2.6 ± 0.4 pg/ml (Fig. 1).

Receptor internalization. Following saline injection, a small number of VP immunoreactive dendrites displayed morphological changes consistent with NK3R receptor internalization (0.4 ± 0.5 intersections/50 μm²). In contrast, following senktide injection, significantly more NK3R immunoreactive dendrites of VP neurons took on a beaded appearance, which is indicative of receptor internalization (3.3 ± 0.4 intersections/50 μm²; Fig. 2). Senktide induced significantly more dendritic NK3R internalization than saline injection at 5 min (t₀ = 4.14; P < 0.006) and 20 min (t₀ = 6.46; P < 0.001). In senktide-treated rats, NK3R recycled to the dendritic membrane by 120 min and at this time there was no significant difference between the saline and senktide treatment, P > 0.3 (Figs. 2 and 3).

A significant amount of NK3R internalization was seen within the somas as early as 5 min after the senktide treatment.
NK3R internalization was seen in 61% of the soma of rats treated with senktide and 20% of soma from rats treated with saline after 20 min (Figs. 4 and 5; $t_0 = 9.84; P < 0.001$). NK3R internalization and recycling within the soma did not follow the same time course observed for the dendrites. NK3Rs recycled to the dendritic membrane within 120 min, but NK3Rs that were sequestered into organelles within the soma following senktide injection were still present at 120 min.

Additionally, by optically sectioning images at 0.05 μm, the NK3R immunoreactivity was detected in the nuclear compartment, defined by the DAPI stain, of individual VP neurons (Fig. 4). NK3R immunoreactivity within the nucleus was concentrated in a few immunoreactive “hot spots” scattered within the nucleus. Although NK3R was localized within the soma 5 min after senktide injection, the nuclear localization of NK3R immunoreactivity was not present until 20 min after the senktide injection. While the number of hot spots within the nucleus varied, no more than 10 spots were seen. NK3R immunoreactive spots were detected in roughly 50% of vasopressinergic cells. Furthermore, the translocation of NK3R from the cytoplasm to the nucleus following senktide injection, was not specific to VP neurons. NK3R immunoreactivity was detected in the nucleus of nonvasopressinergic cells (Fig. 4). For example, in Fig. 4F, there are six NK3R immunoreactive spots detected within the nucleus of a non-VP neuron and one immunoreactive spot in a VP neuron.

**NK3R Antagonist Experiment**

Plasma VP. Compared with the effects of senktide on plasma VP release, pretreatment with SB-222200 significantly blocked the effects of senktide on plasma VP release ($F_{1,8} = 51.7; P < 0.003$, Fig. 1). Following intraventricular injection of SB-222200, plasma VP levels of saline (4.4 ± 0.4 pg/ml) and senktide-treated rats (5.8 ± 0.8 pg/ml) were not significantly different 20 min after injection ($P > 0.5$).
Receptor internalization. Pretreatment of SB-222200 blocked senktide-induced NK3R internalization (Fig. 3). NK3R internalization on dendrites from senktide injected rats pretreated with SB-222200 was not significantly different from those of saline-treated rats ($P > 0.6$). Additionally, compared with senktide treatment alone, NK3R internalization within organelles in the somas following senktide injection was prevented by pretreatment with SB-222200 ($t_6 = 18.75; P < 0.001$). NK3R immunoreactivity was seen within 60% of the somas of senktide-treated rats but only in 25% of the somas from rats pretreated with SB-222200. The number of somas with NK3R internalization after a SB-222200 was not significantly different from rats treated with saline ($P > 0.4$).

Hypertonic NaCl Experiment

Plasma osmolality and VP. An intragastric load of 2 M NaCl altered plasma osmolality and VP levels. Plasma osmolality significantly increased from baseline after 2 M NaCl by 34 ± 5.3 mOsm ($P < 0.002$), whereas after 0.15 M NaCl, there was very little change with levels remaining ~3 mOsm above baseline. The VP levels of 0.15 M NaCl-treated rats did not significantly change and remained between 3.1 pg/ml and 4.5 pg/ml. Following a 2 M NaCl treatment, plasma VP levels significantly increased from a baseline of 5.5 ± 0.6 pg/ml to 14.6 ± 6.3 pg/ml after 45 min ($t_{10} = 2.91; P < 0.01$, Fig. 6).

Receptor internalization. Following an intragastric load of 2 M NaCl, NK3R and VP immunoreactive dendrites within the PVN appeared beaded, indicating receptor internalization. Conversely, following an intragastric load of 0.15 M NaCl, NK3R remained membrane bound and dendrites appeared uniform in shape (Fig. 7). Internalization of NK3R on dendrites of VP immunoreactive neurons following intragastric loads of 2 M NaCl was three times greater than that observed following 0.15 M NaCl treatment ($t_{4} = 7.06; P < 0.002$, Fig. 8).

Intragastric loads of 2 M NaCl caused significantly more NK3R internalization within VP soma than did 0.15 M NaCl
(t₀ = 4.61; P < 0.01). Approximately 60% of VP immunoreactive somas in the PVN of 2 M NaCl-treated rats had NK3R immunoreactivity within the intracellular compartment, while only 30% of somas in the images of 0.15 M NaCl-treated rats showed NK3R immunoreactivity within the intracellular compartment. Also, NK3R immunoreactivity was observed in the intracellular compartment of non-VP immunoreactive cells.

Following intragastric loads of 2 M NaCl NK3R immunoreactivity was detected within the nucleus and was concentrated in a few “hot spots” scattered within the nucleus. The number of NK3R immunoreactive spots within the nucleus was similar to the observations in the senktide experiment, ranging from 0 to 10 spots. For example, in Fig. 7F, five NK3R immunoreactive spots were detected within the nucleus. NK3R immunoreactive spots were detected in roughly 25% of vasopressinergic cells. This was similar to that observed following senktide treatment. However, senktide caused the translocation of NK3R immunoreactivity to the nucleus of both VP and non-VP immunoreactive neurons.

In saline-treated rats, NK3Rs remained on the membrane surface. In contrast, senktide caused translocation of the NK3Rs from the membrane to the intracellular compartment of both dendra and soma of VP neurons. NK3Rs were internalized and then recycled to the membrane surface in a time-dependent manner. Following senktide treatment, internalization on dendrites was detected at 5 min and had recycled to the membrane at 120 min. The time course of dendritic recycling of NK3R is similar to other reports that show tachykinin receptors are recycled back to the plasma membrane on dendrites within 60 min of being activated (24, 25, 39).

The time course of activation and inactivation of NK3Rs expressed on dendra of VP neurons seemed to follow the time course of VP in plasma. Senktide caused a significant increase in NK3R internalization and plasma VP levels within 10 min of the injection. Plasma VP levels increased further at 20 min after senktide injection and then returned to baseline within 120 min of the injection. In a parallel fashion, NK3R were recycled to the dendrite membrane by 120 min following senktide injection.

NK3R expressed on the soma of VP immunoreactive neurons were also activated by senktide injection. There was significantly more NK3R localized within the soma of senktide-treated rats than in saline-treated rats. However, whereas NK3R expressed on dendra were recycled to the membrane within 120 min following senktide injection, NK3R immunoreactivity remained in the cytoplasm for 120 min. Once NK3R were activated, there was no decrease in the number of NK3R

FIG. 6. Plasma VP levels (means ± SE) following an intragastric load of 0.15 M NaCl and 2 M NaCl. #P < 0.01.
immunoreactive soma during the 120 min after senktide injection. Thus recycling of NK3R expressed on the cell body appears to follow a different time course than NK3R on the dendrites.

Rats were pretreated with the selective NK3R antagonist to further test the hypothesis that the effects of senktide on both VP levels and neuron morphology were mediated through the NK3R. Pretreatment with the NK3R antagonist SB-222200 prevented the senktide-stimulated release of VP. Although the senktide rats pretreated with SB-222200 had a higher baseline than saline-treated rats, there was no significant change in plasma VP levels from baseline levels. In addition, senktide-induced NK3R internalization was blocked by pretreatment with SB-222200. NK3R immunoreactivity remained membrane-bound when SB-222200 was injected before senktide. These results illustrate that agonist binding to the NK3R was required for VP release and NK3R internalization.

The results of the NK3R agonist experiment show that injections of NK3R selective ligands cause the internalization of NK3R expressed on VP neurons. We are confident that this anatomical rearrangement reflects a ligand-mediated translocation of the NK3R from the membrane to the intracellular compartment for several reasons. First, the antibody is selective for the NK3R. Second, senktide is a selective agonist for the NK3R (19). Third, pretreatment with a selective antagonist (21) before senktide, prevented both NK3R internalization and a peripheral release of VP. Therefore, the ligand binding to the NK3R on the vasopressinergic neurons themselves causes the systemic release of VP and the morphological change in dendrites and soma.

Fig. 7. Photomicrographs from the PVN illustrating the effects of intragastric load of 0.15 M NaCl (A and B) or 2 M NaCl (D and E) on NK3R in VP neurons. VP immunoreactive neurons are labeled in green and NK3R immunoreactive neurons are labeled in red. Arrows point to dendrites that are both immunoreactive for both VP and NK3R. # Indicates NK3R immunoreactivity within the intracellular compartment of the soma. Merged images (C and F) include the blue image which stains all the cell nuclei within a given image. Carets indicate NK3R immunoreactivity within the nucleus of the cell. Scale bar = 15 μm.
The osmotic challenge experiment tested the hypothesis that hyperosmolarity, a potent stimulus for VP release (7, 10), stimulates the intra-PVN release of an endogenous tachykinin, which then activates NK3Rs on VP neurons. In agreement with previous studies, a significant increase in plasma VP levels was measured after an intragastric load of hypertonic saline (7). When examining PVN neurons, the same morphological change in dendrite appearance caused by senktide injection was seen after an intragastric load of 2 M NaCl. In contrast, NK3Rs remained membrane-bound in saline-treated rats. Furthermore, the number of soma that showed NK3R immunoreactivity within the intracellular compartment was greater after a 2 M NaCl treatment than a 0.15 M NaCl treatment. NK3R internalization requires ligand binding, and the observed trafficking of the NK3R following hypertonic NaCl loads shows that hyperosmolarity stimulated the release of an endogenous NK3R ligand in the PVN that then activated NK3Rs expressed by VP neurons.

The endogenous ligand that binds to the NK3R expressed by VP neurons is hypothesized to be NKB because it has the highest affinity for the NK3R (20, 33). The source of release of NKB within the PVN is not known. One possibility is a release from NKB terminals that have been described in the magnocellular portions of the PVN (26). The origin of these NKB terminals remains unknown. Nuclei that project to the PVN from the subfornical organ (6, 40), do not contain NKB immunoreactive soma (23, 26, 27). The ipsilateral SON and amygdala are two nuclei that project to the PVN and contain NKB immunoreactive soma (23, 26, 36, 40) and may be the origin of NKB terminals in the PVN. An alternative to afferent projections being the source of release of NKB is an intracellular release of NKB. A recent study showed that NKB is colocalized with VP in magnocellular neurons, and the authors suggest that the magnocellular neurons themselves are the source of the NKB (16).

In both experiments, we quantified NK3R internalization on VP neurons because agonists stimulate the systemic release of oxytocin (4). However, this release is not due to a direct action of senktide on NK3R in the PVN but instead is mediated by senktide stimulating the local release of norepinephrine in the PVN (4). Alternatively, senktide may activate NK3R expressed by CRF expressing neurons in the PVN because agonists stimulate the central release of ACTH (29).

In both senktide and 2 M NaCl-treated rats, NK3R immunoreactivity was localized within the cytoplasm for over 2 h, whereas dendritic NK3R had recycled to the dendrite membrane in this time. One possible explanation is that a NK3R within the soma is directed to the nucleus where it may serve a possible role as a nuclear transcription factor (18). With the use of the nuclear stain DAPI, NK3R immunoreactivity was demonstrated within the nuclear compartment of the PVN neurons after an intraventricular injection of senktide and an intragastric load of hypertonic saline (Fig. 4). By optically sectioning the images at 0.05 μm we show that the NK3R immunoreactivity is localized within the nucleus of the particular cell and not due to the superimposition of NK3R immunoreactivity localized in the cytoplasm of neurons below or above the nucleus. The nuclear translocation of the NK3R is attributed to the presence of a nuclear localization signal, located on the COOH-terminal cytoplasmic tail of the receptor. The nuclear localization signal is necessary for entry through the nuclear pore (22). This amino acid sequence is found within the Angiotensin II type I receptor sequence as well as the NK3R sequence (18, 22). This observation is consistent with a recent study that found NK3R nuclear localization in the SON in response to senktide injection and hydralazine-induced hypotension/hypovolemia (18). The function of the nuclear localization of the NK3R is unknown. However, using steroid receptors as a model, because they are well established to enter the nucleus to act as transcription factors, nuclear localization of NK3R can be suggested to play a role in genomic activity by altering the excitability of the neuron, increasing the production of certain proteins, or changing the membrane properties.

Senktide injection caused the translocation of NK3R to the nucleus of both VP and non-VP immunoreactive neurons in the PVN. However, 2 M NaCl treatment caused nuclear translocation of NK3R in only VP immunoreactive neurons. Because senktide is an exogenous ligand, it bound all NK3R present and activated the neurons that expressed the NK3R regardless of their phenotype. In contrast, the endogenous stimulus of 2 M NaCl activated the NK3R and caused a specific translocation of the NK3R into the nucleus of only the VP immunoreactive cells. The difference between these two stimuli shows that the translocation of the NK3R into the nucleus has specific functions dependent upon the stimulus given.

It has been well established that two potent stimuli of vasopressinergic neurons are hypotension/hypovolemia and hyperosmolarity. Previous studies show that hypotension/hypovolemia stimulate VP release through activation of the NK3R (18). Similarly, a hyperosmotic stimulus causes the same activation of NK3R and morphological rearrangement of the dendrites. These two findings suggest that afferents to VP neurons relaying information related to bodily fluid volume and osmolarity activate tachykinin transmission and NK3R activation.
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