Ionotropic Glutamate Receptors in Hypothalamic Paraventricular and Supraoptic Nuclei Mediate Vasopressin and Oxytocin Release in Unanesthetized Rats

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We report changes in plasma arginine vasopressin (AVP) and oxytocin (OT) concentrations evoked by the microinjection of L-glutamate (L-glu) into the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) of unanesthetized rats, as well as which local mechanisms are involved in their mediation. L-Glu microinjection (10 nmol/100 nl) into the SON increased the circulating levels of both AVP and OT. The AVP increases were blocked by local pretreatment with the selective non-N-methyl-D-aspartate (NMDA) receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (2 nmol/100 nl), but it was not affected by pretreatment with the NMDA-receptor antagonist LY235959 (2 nmol/100 nl). The OT response to L-glu microinjection into the SON was blocked by local pretreatment with either NBQX or LY235959. Furthermore, the administration of either the non-NMDA receptor agonist (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA) (5 nmol/100 nl) or NMDA receptor agonist NMDA (5 nmol/100 nl) into the SON had no effect on OT baseline plasma levels, but when both agonists were microinjected together these levels were increased. L-Glu microinjection into the PVN did not change circulating levels of either AVP or OT. However, after local pretreatment with LY235959, the L-glu microinjection increased plasma levels of the hormones. The L-glu microinjection into the PVN after the local treatment with NBQX did not affect the circulating AVP and OT levels. Therefore, results suggest the AVP release from the SON is mediated by activation of non-NMDA glutamate receptors, whereas the OT release from this nucleus is mediated by an interaction of NMDA and non-NMDA receptors. The present study also suggests an inhibitory role for NMDA receptors in the PVN on the release of AVP and OT. (**Endocrinology** 153: 2323–2331, 2012)

Arginine vasopressin (AVP), also known as antidiuretic hormone, and oxytocin (OT) are peptides synthesized by two different types of hypothalamic neurons: magnocellular and parvocellular neurons. Magnocellular neurosecretory cells are mainly located in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus (1, 2). Each magnocellular neuron in either the PVN or SON gives rise to a single axon into the neurohypophysis, where its neurosecretory endings release AVP and OT (2, 3). Because the capillaries within the pituitary gland do not have a blood-brain barrier, the AVP and OT that are released in close proximity to the capillaries easily enters the bloodstream (2, 3).

The classical actions of OT are the stimulation of uterine smooth muscle contraction during labor and milk ejection during lactation (4–6). Other functions of oxytocin

Abbreviations: ACSF, Artificial cerebrospinal fluid; AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide; AVP, arginine vasopressin; L-glu, L-glutamate; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA, N-methyl-D-aspartate; OT, oxytocin; PVN, paraventricular nucleus; SON, supraoptic nucleus.

are not well established, but some evidence suggests that this peptide is important for the control of gastrointestinal, renal, and cardiovascular functions (4, 5, 7, 8). AVP plays an important role in the control of extracellular fluids and blood volume maintenance due to its action in the kidney, promoting water retention, and in arterioles where it acts as a potent vasoconstrictor agent (2, 3, 9). Physiological stimuli such as hypotension, hypovolemia, and increase in plasma osmolality promote the release of AVP (2, 10).

The amino acid L-glutamate (L-glu) is the main excitatory neurotransmitter in the mammalian central nervous system. Glutamate receptors are divided into ionotropic and metabotropic receptor subfamilies (11). The ionotropic receptors are further classified as *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, according to agonist selectivity and amino acid homology. OT and AVP neurosecretory cells located in the SON and PVN receive a dense glutamatergic innervation, which accounts for approximately one fourth of the total number of synaptic contacts to these neurons (12). Furthermore, it is well documented that magnocellular neurons in the hypothalamus express glutamate receptors (13–17).

It has been demonstrated that the activation of either NMDA or non-NMDA glutamate receptors promote AVP and OT release from rat hypothalamo-neurohypophysial explants (18). Blockade of metabotropic glutamate receptor did not alter AVP and OT release from hypothalamo-neurohypophysial explants evoked by osmotic stimulation (19). We have previously reported that L-glu microinjection into the SON and PVN of unanesthetized rats caused increase arterial pressure, which was mediated by an acute vasopressin release into the circulation (20, 21). However, electrophysiological evidence has suggested that OT and AVP neurons differ in their control by glutamate (22–25). For example, *in vivo* local application of NMDA agonists in the SON strongly activated putative AVP but not OT neurons (22, 26). Although these data indicate that glutamate plays a key role in regulating the activity of magnocellular neurosecretory cells and that the control of AVP and OT cells by glutamate may differ, direct evidence of a glutamate contribution in the control of AVP and OT release in unanesthetized animals is sparse. Therefore, information on the subtype of glutamate receptor involved in AVP and OT release from magnocellular neurosecretory cells in either the PVN or SON of unanesthetized rats has yet to be addressed in a systematic fashion.

Thus, the aim of the present study was to evaluate the effects of L-glu microinjection into either the SON or PVN on the plasma AVP and OT content in unanesthetized rats. We also investigated which subtype of ionotropic gluta-

mate receptor is involved in the mediation of AVP and OT release.

Materials and Methods

Animal care

Male Wistar rats weighing 250–270 g were used. Animals were kept in the Animal Care Unit of the Department of Pharmacology of the School of Medicine of Ribeirão Preto, University of São Paulo. Rats were kept under a 12-h light, 12-h dark cycle (lights on between 0600 and 1800 h) and had free access to water and standard laboratory food, except during the experimental period. Housing conditions and experimental procedures were approved by the University of São Paulo Animal Ethical Committee, which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society.

Surgical preparation

Rats were anesthetized with tribromoethanol (Aldrich Chemical Co. Inc., Milwaukee, Wi) (250 mg/kg, ip). After local anesthesia with 2% lydocaine, the skull was surgically exposed and a stainless steel guide cannula (0.6 mm outer diameter, 23 gauge) was implanted 1 mm above the injection site using a stereotaxic apparatus (Stoelting, Wood Dale, IL). Stereotaxic coordinates for cannula implantation into the SON or PVN areas were selected from rat brain atlas (27). Stereotaxic coordinates for cannula implantation into the SON and PVN were as follows: SON, anteroposterior = +6.9 mm ahead from the interaural line, lateral = +1.8 mm from the medial suture, and ventral = −8.1 mm deep from the skull; PVN, anteroposterior = +7.2 mm ahead from the interaural line, lateral = +0.6 mm from the medial suture, and ventral = −6.8 mm deep from the skull at an angle of 12° (20, 21, 28). Cannulas were fixed to the skull with dental cement and one metal screw. A tight-fitting mandrel was kept inside the guide cannula to avoid its occlusion. After surgery, the animals received 100,000 U im polyantibiotic (Pentabiotic; Fontoura-Wyeth, São Paulo, Brazil; 80,000 IU im), with streptomycin and penicillin, to prevent infection and a nonsteroidal antiinflammatory flunixin meglumine (2.5 mg/kg, sc) (Banamine; Schering Plough, São Paulo, Brazil) for postoperative analgesia.

Drug microinjection into the central nervous system

Needles (33 gauge; Small Parts, Miami Lakes, FL) were used for microinjection of drugs into the PVN or SON and were 1 mm longer than the guide cannulas. The injection needle was connected to a 1-μl syringe (7001KH; Hamilton, Reno, NV) through PE-10 tubing, and bolus injections of 100 nl were made into the SON or PVN (20, 21, 28). Microinjections were performed within a 5-sec period. After microinjection, the needle was left within the guide cannula for 1 min before being removed. Drugs were prepared before the experiments and stored at −20 C. On the day of the experiment, the drugs were thawed and kept at 0 C during experiments.

RIA

After decapitation, trunk blood was collected in chilled plastic tubes containing heparin (10 μl heparin per milliliter of collected blood). Samples were centrifuged at 4 C and 3000 rpm for 20 min, and plasma was stored at -70 C until OT and AVP levels were determined. Plasma AVP and OT levels were measured by specific RIA after previous extraction from plasma using acetone and petroleum ether (29, 30). The recovery rates were greater than 87%. The assay sensitivity and intra- and interassay coefficients of variation were 0.9 pg/ml and 7.7 and 11.9% for AVP and 0.9 pg/ml and 12.6 and 7% for OT, respectively. All samples from a single experiment were assayed in duplicate in the same assay.

Experimental procedure

Three days after the stereotactic surgery, animals were transported to the experimental room in their own home cage and were allowed 60 min to adapt to the conditions of the room, such as sound and illumination, before starting experiments. The experimental room had temperature control (25 C) through an air conditioning unit and was acoustically isolated from other rooms. An air exhauster was used to generate a constant background noise to minimize external sound interference within the experimental room.

Plasma AVP and OT levels after L-glu microinjection into the SON of unanesthetized rats

These experiments aimed to study local mechanisms involved in plasma OT and AVP level changes induced by L-glu microinjection into the SON. Animals were divided into four experimental groups: 1) vehicle (100 nl) microinjected into the SON [artificial cerebrospinal fluid (ACSF) group, n = 6] (31, 32); 2) L-glu (10 nmol/100 nl) microinjected into the SON (L-glu group, n = 6) (20); 3) L-glu microinjection into the SON 10 min after local treatment with the selective NMDA glutamate receptor antagonist LY235959 (LY+L-glu group, 2 nmol/100 nl, n = 6) (20); and 4) L-glu microinjection into the SON 10 min after local treatment with the selective non-NMDA glutamate receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (NBQX+L-glu group, 2 nmol/100 nl, n = 6) (20). A last experiment was performed to verify whether the microinjection of each selective non-NMDA or NMDA receptor agonist into the SON increases OT plasma levels alone or whether they need to be combined. For this, animals were divided into four additional groups: 5) vehicle (100 nl) microinjected into the SON (ACSF group, n = 5); 6) selective non-NMDA glutamate receptor agonist ($\pm$)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA) (5 nmol/100 nl) microinjected into the SON (AMPA group, n = 5) (33); 7) selective NMDA glutamate receptor agonist NMDA microinjected into the SON (NMDA group, n = 5) (33); and 8) microinjection of both AMPA and NMDA into the SON (AMPA+NMDA group, n = 5). Animals were decapitated and blood samples were collected 1 min after the last microinjection into the SON (20).

Plasma AVP and OT levels after L-glu microinjection into the PVN of unanesthetized rats

This protocol aimed to investigate local mechanisms involved in plasma OT and AVP level changes induced by the L-glu microinjection into the PVN. Animals were divided into four ex-

perimental groups: 1) vehicle (100 nl) microinjected into the PVN (ACSF group, n = 6) (21, 28); 2) L-glu (10 nmol/100 nl) microinjected into the PVN (L-glu group, n = 6) (21, 28); 3) L-glu microinjection into the PVN 10 min after local treatment with the selective NMDA glutamate receptor antagonist LY235959 (LY+L-glu group, 2 nmol/100 nl, n = 6) (21, 28); and 4) L-glu microinjection into the PVN 10 min after local treatment with the selective non-NMDA glutamate receptor antagonist NBQX (NBQX+L-glu group, 2 nmol/100 nl, n = 6) (21). One minute after the last microinjection into the PVN, animals were decapitated and blood samples were collected (20).

Histological procedure

After decapitation, 100 nl filtered 1% Evan's blue dye was injected into the brain as a marker of the injection site. The brains were postfixed for 48 h at 4 C, and 40-μm sections were cut with a cryostat (CM1900; Leica, Wetzlar, Germany). Brain sections were stained with 0.5% cresyl. The actual placement of the injection needles was verified in serial sections. Placements of the injection needles were verified in serial sections using the rat brain atlas of Paxinos and Watson (27) as reference.

Drugs

L-Glutamic acid monosodium salt (L-glu; Sigma, St. Louis, MO), LY235959 ([3S-(3a,4aa,6b,8aa)]decahydro-6-(phosphonomethyl)-3-is oquinolinecarboxylic acid) (Tocris, Ellisville, MO), NBQX (Tocris), AMPA (Sigma) and NMDA (Sigma) were dissolved in ACSF, which had the following composition: 100 mM NaCl, 2 mM Na_3PO_4, 2.5 mM KCl, 1.0 mM $MgCl_2$, 27 mM $NaHCO_3$, and 2.5 mM $CaCl_2$ (pH 7.4).

Tribomoethanol (Sigma) and urethane (Sigma) were dissolved in saline (0.9% NaCl). Flunixin meglumine (Banamine; Schering Plough) and polyantibiotic preparation of streptomycin and penicillin (Pentabiotic; Fontoura-Wyeth) were used as provided.
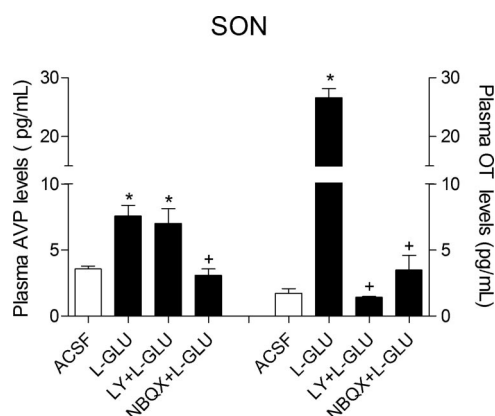
Statistical analysis

The statistical analysis was performed using commercial software (Prism version 3.0; GraphPad, San Diego, CA). Data are represented as mean $\pm$ SEM. One-way ANOVA was used to compare effects of the PVN or SON treatments on plasma AVP and OT levels. The Newman-Keuls *post hoc* test was used for multiple comparisons. Significance level was set at $P < 0.05$.

Results

Plasma AVP and OT levels after L-glu microinjection into the SON of unanesthetized rats

Vasopressin

Microinjection of L-glu (10 nmol/100 nl, n = 6) into the SON increased plasma AVP concentration [ACSF 3.6 $\pm$ 0.2 pg/ml $vs.$ L-glu 8.0 $\pm$ 0.8 pg/ml, $F_{(3, 20)} = 9.3, P < 0.05$] when compared with vehicle-treated animals (100 nl, n = 6) (Fig. 1). SON pretreatment with the selective NMDA glutamate receptor antagonist LY235959 (2 nmol/100 nl, n = 6) did not affect the increase in plasma AVP content
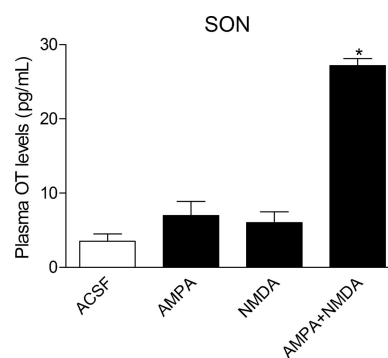
FIG. 1. Plasma AVP or OT concentration in response to the microinjection into the SON of ACSF (100 nl, n = 6), ʟ-glu (10 nmol/100 nl, n = 6), ʟ-glu after local pretreatment with the selective NMDA glutamate receptor antagonist LY235959 (LY+ʟ-glu, n = 6), or ʟ-glu after local pretreatment with the selective non-NMDA glutamate receptor antagonist NBQX (NBQX+ʟ-glu, n = 6). *Columns* represent the mean and *bars* the sᴇᴍ. *, $P < 0.05$ when compared with ACSF group; +, $P < 0.05$ when compared with ʟ-glu group.

FIG. 2. Plasma OT concentration in response to the microinjection of ACSF (n = 5), selective non-NMDA glutamate receptor agonist AMPA (n = 6), selective NMDA glutamate receptor agonist NMDA (n = 5), or both AMPA and NMDA (n = 5) into the SON. *Columns* represent the mean and *bars* the sᴇᴍ. *, $P < 0.05$ when compared with ACSF group.

evoked by local ʟ-glu microinjection (ʟ-glu 8.0 ± 0.8 pg/ml *vs.* LY+ʟ-glu 7.0 ± 1.1 pg/ml, $P > 0.05$) (Fig. 1). However, SON treatment with the selective non-NMDA glutamate receptor antagonist NBQX (2 nmol/100 nl, n = 6) inhibited the increase in plasma AVP levels induced by local ʟ-glu microinjection [ʟ-glu 8.0 ± 0.8 pg/ml *vs.* NBQX+ʟ-glu 3.1 ± 0.5 pg/ml, $F_{(3, 20)} = 9.3$, $P < 0.05$] (Fig. 1). Circulating AVP content was not statistically different in animals treated in the SON with ACSF or NBQX+ʟ-glu (ACSF 3.6 ± 0.2 pg/ml *vs.* NBQX+ʟ-glu 3.1 ± 0.5 pg/ml, $P > 0.05$) (Fig. 1).

Oxytocin

Microinjection of ʟ-glu (10 nmol/100 nl, n = 6) into the SON increased plasma OT concentration [ACSF 1.7 ± 0.3 pg/ml *vs.* ʟ-glu 27 ± 1.6 pg/ml, $F_{(3, 20)} = 158.1$, $P < 0.0001$] when compared with vehicle-treated animals (100 nl, n = 6) (Fig. 1). SON pretreatment with LY235959 (2 nmol/100 nl, n = 6) inhibited the increase in plasma OT content evoked by local ʟ-glu microinjection [ʟ-glu 27 ± 1.6 pg/ml *vs.* LY+ʟ-glu 1.4 ± 0.1 pg/ml, $F_{(3, 20)} = 158.1$, $P < 0.05$] (Fig. 1). SON pretreatment with NBQX (2 nmol/100 nl, n = 6) also inhibited the increase in plasma OT content evoked by local ʟ-glu microinjection [ʟ-glu 27 ± 1.6 pg/ml *vs.* NBQX+ʟ-glu 3.5 ± 1.1 pg/ml, $F_{(3, 20)} = 158.1$, $P < 0.05$] (Fig. 1). Plasma OT content was not statistically different in animals treated into the SON with ACSF, LY+glu, or NBQX+ʟ-glu (ACSF 1.7 ± 0.3 pg/ml, LY+ʟ-glu 1.4 ± 0.1 pg/ml, NBQX+ʟ-glu 3.5 ± 1.1 pg/ml, $P > 0.05$) (Fig. 1).

The microinjection of the selective non-NMDA glutamate receptor agonist AMPA (5 nmol/100 nl, n = 5) or the selective NMDA glutamate receptor agonist NMDA (5 nmol/100 nl, n = 5) alone into the SON did not change the

baseline plasma OT levels (ACSF 3.5 ± 1.0 pg/ml *vs.* AMPA 6.9 ± 1.9 pg/ml *vs.* NMDA 6.0 ± 1.5 pg/ml, $P > 0.05$) (Fig. 2). However, when the agonists were microinjected together into the SON, they caused a significant increase in plasma OT levels [ACSF 3.5 ± 1.0 pg/ml *vs.* AMPA+NMDA 27 ± 1.0 pg/ml, $F_{(3, 12)} = 84.6$, $P < 0.05$] (Fig. 2).

A photomicrograph of a coronal brain section depicting the microinjection site in the SON of one representative animal is presented in Fig. 3. A diagrammatic representation showing microinjection sites in the SON of all animals used in this study is also presented in Fig. 3.

Plasma AVP and OT levels after ʟ-glu microinjection into the PVN of unanesthetized rats

Vasopressin

Microinjection of ʟ-glu (10 nmol/100 nl, n = 6) into the PVN did not change plasma AVP concentration (ACSF 3.2 ± 0.1 pg/ml *vs.* ʟ-glu 3.0 ± 0.2 pg/ml, $P > 0.05$), when compared with vehicle-treated animals (100 nl, n = 6) (Fig. 4). The pretreatment with 2 nmol/100 nl LY235959 (n = 5) into the PVN had no effect on baseline plasma levels of AVP (ACSF 1.2 ± 03 pg/ml *vs.* LY 1.7 ± 0.3 pg/ml, $P > 0.05$). However, ʟ-glu microinjection into the PVN after local pretreatment with LY235959 (2 nmol/100 nl, n = 6) increased circulating AVP level [ACSF 3.2 ± 0.1 pg/ml *vs.* LY+ʟ-glu 9.0 ± 1.9 pg/ml, $F_{(3, 20)} = 9.1$, $P < 0.05$] when compared with vehicle-treated animals (Fig. 4). ʟ-Glu microinjection into the PVN after local pretreatment with NBQX (2 nmol/100 nl, n = 6) did not affect plasma AVP level (ACSF 3.2 ± 0.1 pg/ml *vs.* NBQX+ʟ-glu 3.0 ± 0.2 pg/ml, $P > 0.05$) (Fig. 4).

Oxytocin
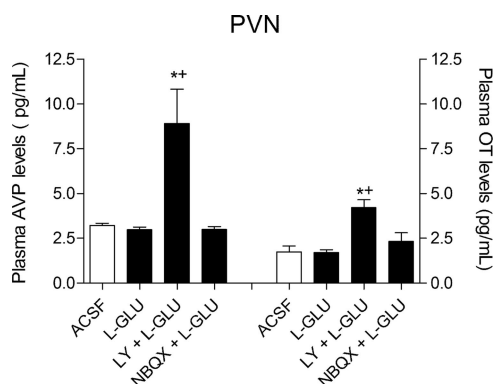
Microinjection of ʟ-glu (10 nmol/100 nl, n = 6) into the PVN did not change plasma OT concentration (ACSF
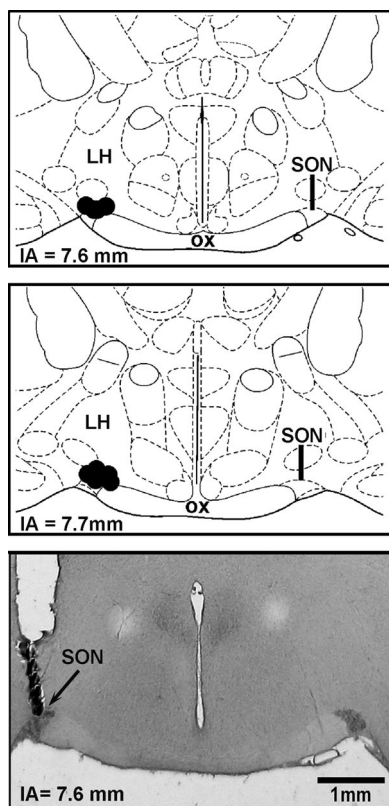
FIG. 3. Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (27), showing the microinjection sites into the SON and photomicrography of a rat brain coronal section depicting the site of microinjection into the SON of one representative animal. The *arrow* indicates the center of the microinjection. IA, Interaural; LH, lateral hypothalamus; ox, optic chiasm.

1.7 ± 0.3 pg/ml *vs.* L-glu 1.7 ± 0.2 pg/ml, $P > 0.05$) when compared with vehicle-treated animals (100 nl, n = 6) (Fig. 4). The pretreatment with 2 nmol/100 nl of LY235959 (n = 5) into the PVN had no effect on baseline


FIG. 4. Plasma AVP or OT concentration in response to the microinjection into the PVN of ACSF (100 nl, n = 6), L-glu (10 nmol/100 nl, n = 6), L-glu after local pretreatment with the selective NMDA glutamate receptor antagonist LY235959 (LY+L-glu, n = 6) or L-glu after local pretreatment with the selective non-NMDA glutamate receptor antagonist NBQX (NBQX+L-glu, n = 6). *Columns* represent the mean and *bars* the SEM. *, $P < 0.05$ when compared with ACSF group; +, $P < 0.05$ when compared with L-glu group.

plasma levels of OT (ACSF 4.9 ± 1.4 pg/ml *vs.* LY 6.3 ± 1.4 pg/ml, $P > 0.05$). However, L-glu microinjection into the PVN after local pretreatment with LY235959 (2 nmol/100 nl, n = 6) increased plasma OT level [ACSF 1.7 ± 0.2 pg/ml *vs.* LY+L-glu 4.2 ± 0.4 pg/ml, $F_{(3, 20)} = 10\ P < 0.05$] when compared with vehicle-treated animals (Fig. 4). L-Glu microinjection into the PVN after local pretreatment with NBQX (2 nmol/100 nl, n = 6) did not affect plasma OT level (ACSF 1.7 ± 0.2 pg/ml *vs.* NBQX+L-glu 2.3 ± 0.5 pg/ml, $P > 0.05$) (Fig. 4).

A photomicrograph of a coronal brain section depicting the microinjection site in the PVN of one representative animal is presented in Fig. 5. A diagrammatic representation showing microinjection sites into the PVN of all animals used in this study is also presented in Fig. 5.

Discussion

AVP and OT release from magnocellular neurosecretory cells in the hypothalamus is controlled by a large number of synaptic inputs involving a wide variety of neurotransmitters. L-Glu is the main excitatory transmitter involved
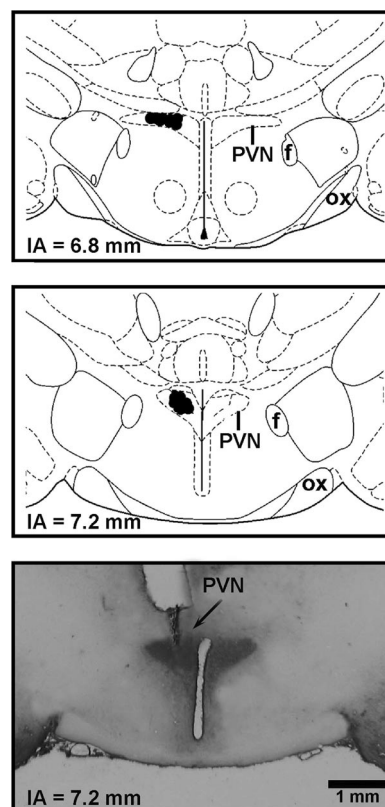
FIG. 5. Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (27), showing the microinjection sites into the PVN and photomicrography of a rat brain coronal section depicting the site of microinjection into the PVN of one representative animal. The *arrow* indicates the center of the microinjection. f, Fornix; IA, interaural; ox, optic chiasm.

in the control of magnocellular cells in the hypothalamus, as demonstrated by extensive morphological, electrophysiological, and pharmacological studies (12, 17, 20, 21, 34). L-Glu acts via two major classes of receptors, namely ionotropic and metabotropic (11). Ionotropic glutamate receptors are coupled to ion channels, which allow the flow of Na^+, K^+, and Ca^{2+} through membrane (35). These receptors are classified according to their selectivity to agonist and the amino acid homology into NMDA and non-NMDA glutamate receptors (35, 36). The latter group is further subdivided into AMPA and kainite receptor subtypes (36).

We report in the present study that L-glu microinjection into the SON increased circulating AVP and OT levels in unanesthetized rats. The OT response to L-glu was markedly reduced after SON pretreatment with either the selective non-NMDA glutamate receptor antagonist NBQX or the selective NMDA receptor antagonist LY235959. Moreover, plasma OT levels were increased only after combined administration of NMDA and the non-NMDA glutamate receptor agonist AMPA into the SON. These results suggest that both subtypes of ionotropic glutamate receptors modulate OT release from SON magnocellular neurons. Electrophysiological studies have suggested the presence of functional NMDA as well as non-NMDA receptors in SON OT neurons (22–24, 37, 38). Also, it was shown that OT release from explants of the hypothalamo-neurohypophysial system is stimulated by activation of both NMDA and non-NMDA glutamate receptors (18). Activation of either NMDA or non-NMDA receptors in the SON also evoked OT release in lactating rats (39). However, it has been suggested that OT release does not require coactivation of these receptors but may result from a synergistic interaction between NMDA and non-NMDA glutamate receptors (18, 39), because individual activation of either the NMDA or non-NMDA glutamate receptor evoked OT release from SON magnocellular neurons (18, 39, 40). We observed that the blockade of only NMDA or non-NMDA receptors in the SON abolished plasma OT release evoked by local L-glu microinjection. Also, administration of AMPA or NMDA alone was not able to change plasma OT levels. Therefore, in contrast to previous studies, our results suggest that the modulation of OT release by glutamate terminals in the SON require a coactivation of NMDA and non-NMDA glutamate receptors.

The AVP response to L-glu microinjection into the SON was blocked after the local pretreatment with NBQX but was not affected by local pretreatment with LY235959, thus suggesting that glutamate terminals in the SON modulate AVP release through the activation of non-NMDA glutamate receptors. These results led to the interesting observation that AVP and OT release are modulated by different glutamatergic mechanisms in the SON. Present results corroborate with previous data suggesting that the excitatory amino acid regulates SON cells activity by acting primarily on non-NMDA glutamate receptors (12). Moreover, it has been reported that L-glu microinjection into the SON increases arterial pressure in unanesthetized rats through an acute AVP release into the circulation (20), and the response is markedly reduced after the SON pretreatment with NBQX but not after a local treatment with LY235959 (20). However, our results contrast with those from *in vitro* studies showing that AVP release from explants of the hypothalamo-neurohypophysial system is stimulated by activation of both NMDA and non-NMDA glutamate receptors (18, 41). In addition to the magnocellular neurons SON, these explants also included the organum vasculosum of the lamina terminalis, the suprachiasmatic nucleus, and the arcuate nucleus (18, 41). Therefore, differences between results obtained from explants and those presently reported may result from a broad pharmacological stimulation when using explant preparations.

Another goal of the present study was to evaluate the involvement of glutamate in the control of AVP and OT release from the PVN. The microinjection of L-glu into the PVN did not change plasma AVP levels. However, L-glu microinjection into the PVN after local pretreatment with the selective NMDA glutamate receptor antagonist LY235959 caused an increase in circulating AVP levels. L-glu did not change plasma AVP content after the PVN pretreatment with NBQX. Busnardo *et al.* (21) have reported that the microinjection of L-glu into the PVN caused arterial pressure and heart rate increases, which were mediated by changes in autonomic activity. In the same study, it was observed that a local treatment with LY235959 did not affect the L-glu pressor response but changed the tachycardiac response into a bradycardiac one. The pressor and bradycardiac responses observed after the microinjection of LY235959 into the PVN were blocked when the local treatment with LY235959 was associated with the systemic treatment with a V_1-vasopressin receptor antagonist or the local treatment with the selective non-NMDA receptor antagonist NBQX into the PVN. Together, these results suggested the existence of two glutamatergic mechanisms involved in the cardiovascular modulation within the PVN: one autonomic mechanism that is mediated by NMDA receptors and a vasopressinergic mechanism that is mediated by non-NMDA receptors (21). Present data corroborate these results. Moreover, this previous evidence suggests that an increase in plasma AVP levels evoked by L-glu microinjection into

the PVN after local treatment with LY235959 is mediated by activation of non-NMDA glutamate receptors.

Effects of PVN pharmacological treatments on plasma OT levels were similar to those observed on circulating AVP levels. The microinjection of L-glu into the PVN did not change circulating OT levels. These results contrast with those reported by Hattori *et al.* (42), which indicated that a perfusion of the PVN with L-glu using a dialysis probe increased plasma OT levels with no effect on dialysate peptide levels. However, concentrations of L-glu (0.2 and 0.5 M) that evoked OT response were higher than that used in the present study (0.1 M) (42), whereas PVN perfusion with the same concentration used in the present study did not affect circulating OT levels (42). However, the difference among data could not be due to the use of an insufficient dose of L-glu in the present study, because we have observed that the microinjection of L-glu into the PVN increased circulating OT levels after local pretreatment with LY235959. The injection of higher doses of L-glu may evoke OT release though the activation of other signaling mechanisms in the PVN. For example, with the use of the push-pull technique, it has been reported that a perfusion of either glutamate or NMDA into the PVN induced an increase in the local nitric oxide (NO) release (43). It has been suggested that NO formation mediates, at least in part, the L-glu actions into the PVN (44). Interaction between glutamatergic and endocannabinoid system in control of magnocellular neurons of the SON and PVN has also been reported (45). Therefore, differences in experimental procedure can also explain the opposite results. It has been previously reported that the injection of kainic acid activates OT neurons in the PVN, and this activation is blocked by local treatment with a selective non-NMDA glutamate receptor antagonist (46). Therefore, similar to glutamatergic mechanisms involved in AVP release from PVN, OT release evoked by the L-glu microinjection into the PVN after the local treatment with LY235959 may be mediated by the activation of non-NMDA glutamate receptors.

Interestingly, NMDA glutamate receptor blockade was necessary to unveil the excitatory effect of L-glu on AVP and OT release from PVN magnocellular neurons. This result suggests an inhibitory influence of the activation of NMDA receptors in PVN magnocellular cell activity. The mechanism of inhibitory action of NMDA glutamate receptor is not totally understood. Microinjection of LY235959 into the PVN had no effect on the baseline values of AVP and OT plasma levels, thus suggesting that NMDA glutamate receptors have no tonic inhibitory influence in PVN magnocellular neurons. However, electrophysiological studies have suggested that L-glu may elicit hyperpolarizing postsynaptic potentials through ac-

tivation of local GABAergic inputs to PVN neurons (47, 48). Therefore, the excitatory effect of L-glu in the PVN may be the result of action in multiple cells.

It has been reported that the dose of LY235959 (2 nmol) used in the present study effectively inhibited cardiovascular responses evoked by L-glu microinjection into the lateral hypothalamus (49), whereas the same dose of NBQX (2 nmol) was shown to block the response to L-glutamate microinjection into the SON (20). In addition, microinjection of equimolar doses of NBQX into the lateral hypothalamus and LY235959 into the SON had no effect on cardiovascular responses to L-glu microinjection into those areas (20, 49). These observations indicate that the doses of LY235959 and NBQX used in the present study are effective and selective enough to evidence the predominance of either NMDA or non-NMDA glutamate receptors in responses involving a glutamatergic neurotransmission.

The AVP has been shown to play an important role during the decompensatory phase of hemorrhage and increase in plasma osmolality through its action in the kidney, promoting water retention, and in arterioles where it acts as a potent vasoconstrictor agent (2, 10, 50, 51). Besides, it has been demonstrated that an increase in circulating vasopressin content may mediate the hypertension observed in ethanol-treated rats (52). Therefore, it is possible that L-glu-mediated vasopressin release from SON and PVN magnocellular neurons may be relevant during hemorrhage, changes in plasma osmolality, and hypertension. OT is released in response to suckling during lactation (4). Although the central pathway involved in this reflex is not totally understood, the present results suggest that glutamate receptors in the PVN and SON may be involved. Also, the neuroendocrine system could be a marker of stress (53, 54). The AVP and OT have been associated with stressful situations (54–56). Thus, the glutamate receptors activation in AVP and OT magnocellular neurons could stimulate the release of both hormones during stressful situations. Despite the above evidence, additional studies are necessary to elucidate the physiological role of the control of AVP and OT release by glutamate receptors within the PVN and SON.

In summary, our findings provide evidence on the subtype of glutamate receptors involved in AVP and OT release by magnocellular neurons of the SON and PVN. The present results led to the interesting observation that AVP and OT secretion from SON differ in their control by glutamate. OT release from the SON requires coactivation of NMDA and non-NMDA glutamate receptors, whereas AVP secretion is mediated by activation of non-NMDA glutamate receptors. In the PVN, the present results suggest an inhibitory influence of the NMDA glutamate receptor on AVP and OT release. AVP and OT responses

were unveiled only after the blockade of local NMDA receptors and are possibly mediated by the activation of non-NMDA glutamate receptors.

Acknowledgments

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