Oxytocin actions within the supraoptic and paraventricular nuclei: differential effects on peripheral and intranuclear vasopressin release

Inga D. Neumann,1 Luz Torner,1 Nicola Toschi,2 and Alexa H. Veenema1

1Institute of Zoology, Department of Behavioural Neuroendocrinology, University of Regensburg, Regensburg, Germany; 2Max-Planck-Institute of Psychiatry, Munich, Germany

Submitted 31 October 2005; accepted in final form 17 January 2006

Oxytocin actions within the supraoptic and paraventricular nuclei: differential effects on peripheral and intranuclear vasopressin release. Am J Physiol Regul Integr Comp Physiol 291: R29–R36, 2006. First published January 19, 2006; doi:10.1152/ajpregu.00763.2005.—In response to forced swimming (FS), AVP is released somato-dendritically within the supraoptic nucleus (SON) and paraventricular nucleus (PVN), but not from neurohypophysial terminals into blood. Together with AVP, oxytocin (OXT) is released within the SON and PVN. Here, we studied the role of intra-SON and intra-PVN OXT in the regulation of local AVP release and into the blood in male rats. Within the SON, bilateral retrodialysis of an OXT receptor antagonist (OXT-A) increased local AVP release in response to FS [60 s, 21°C, vehicle twofold, not significant (ns); OXT-A: 15-fold increase, P < 0.05] without significantly affecting basal AVP release. In addition, local OXT-A elevated plasma AVP secretion under basal conditions (twofold increase, P < 0.05) without further elevation after FS. Within the PVN, exposure to FS elevated local AVP release, reaching significance only in the OXT-A group (vehicle: 1.4-fold, ns; OXT-A: 1.6-fold increase, P = 0.050). Bilateral OXT-A into the PVN did not affect peripheral AVP secretion either under basal or stress conditions. Basal ACTH concentrations tended to be elevated by local OXT-A within the PVN (1.7-fold increase, P = 0.076). In contrast, the swim-induced ACTH secretion was attenuated after retrodialysis of OXT-A within both the SON (at 5 min) and PVN (at 15 min) (P < 0.05 both) compared with vehicle. The results demonstrate a receptor-mediated effect of OXT within the SON and PVN on local and neurohypophysial AVP release, which depends upon the activity conditions. Further, while exerting an inhibitory effect on hypothalamic-pituitary-adrenal axis activity under basal conditions, hypothalamic OXT is essential for an adequate acute ACTH response.

plasma; hypothalamic-pituitary-adrenal axis; dendritic release; microdialysis; swim stress

Similarly, such local release within the SON and PVN has been described for both peptides, for instance, in response to various psychological stressors, such as forced swimming in male and female rats (for a review, see Ref. 16). However, upon stressor exposure, only OXT is simultaneously released from the dendrites within the hypothalamic nuclei and from neurohypophysial terminals into blood, as revealed by intracerebral microdialysis in conjunction with blood sampling in conscious rats. In contrast, AVP secretion into blood remains unchanged despite its release within the PVN, SON, and/or septum, for example, during forced swimming or exposure to social defeat (13, 14, 44, 60, 61).

The phenomenon of independent release patterns of AVP from dendrites within the SON/PVN and from neurohypophysial terminals, in particular, in response to stress, has attracted substantial scientific attention (15, 42; for a review, see Refs. 16 and 33), but it is far from being elucidated. It demonstrates the ability of neuronal cells to independently regulate neuropeptide release from various neuronal elements (axon terminals vs. dendrites and somata). With respect to dendritic release, it has been revealed that intrahypothalamic neuropeptide release can occur independently of the electrical activity (11, 36), which is required for axonal neuropeptide secretion. Furthermore, locally released neurotransmitters/neuromodulators, including AVP itself (21, 63), GABA (17, 28, 29) and taurine (15, 26, 52) were recently considered to regulate basal and stimulated activity of neurohypophysial AVP (and also OXT) neurons and, thus, AVP secretion into blood. Here, we want to test the hypothesis that OXT locally released within the SON/PVN is another candidate involved in the control of the somato-dendritic and neurohypophysial release of AVP under both basal and/or stress conditions. Although electrophysiological studies indicate that OXT predominantly acts on OXT, but not AVP, neurons within the SON or PVN in an autocrine-paracrine fashion (10, 18, 21, 27, 40), evidence from conscious animals under conditions of psychological stress is lacking.

The stress response of the organism is characterized not only by an activation of the OXT/AVP system, but mainly by stimulation of the hypothalamo-pituitary-adrenal (HPA) axis, as well as the sympathetic nervous system. Brain OXT has been shown to generally inhibit the activity of the HPA axis.
Acute intracerebroventricular administration of the OXT receptor antagonist (OXT-A) elevated corticotropin (ACTH), and corticosterone plasma concentrations in virgin female and male rats (46, 47). Moreover, chronic intracerebroventricular infusion of OXT in ovariectomized females diminished corticosterone secretion (58) and attenuated the stress-induced neuronal activation in several brain regions, including the PVN (59). Within the PVN, corticotropin-releasing hormone (CRH) neurons producing the main ACTH secretagogue are located (55). As OXT is locally released within the SON and PVN, for example, during swim stress (57, 61), and OXT receptors have been identified within these hypothalamic nuclei (2, 5, 19, 31), we aim to more specifically reveal local OXT actions on basal and stress-induced HPA axis activity. We hypothesize OXT actions on HPA axis functions would occur predominantly within the PVN, but likely also within the SON, as a significant contribution of magnocellular SON neurons to the regulation of HPA axis activity has repeatedly been shown (12, 20, 37, 62, for a review, see Ref. 16).

Thus, to reveal “acute” OXT actions within the SON and PVN on local and neurohypophyseal AVP release and on ACTH secretory responses, a selective OXT-A was administered bilaterally into the respective brain area of male rats via reversed microdialysis, while, simultaneously, either local or peripheral release patterns of AVP and ACTH, respectively, were monitored.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (300–350 g body wt; Charles River, Sulzfeld, Germany) were housed in groups of four under standard laboratory conditions (12:12-h light-dark cycle, lights on at 0600, 22°C, 6% humidity and free access to water and standard rat chow) in standard rat cages (40 × 60 × 20 cm) at least 6 days before surgery. All surgical, sampling, and behavioral protocols were approved by the Committee on Animal Health and Care of the local government.

Surgery

Microdialysis. Under halothane anesthesia (Hoechst, Frankfurt am Main, Germany) using semisterile procedures, rats were stereotaxically implanted with U-shaped microdialysis probes (dialysis membrane: molecular cutoff of 18 kDa; Hemophan, Gambo Dialsatoren, Hechingen, Germany; for details, see Refs. 42 and 43) in the left and right SON (0.6 mm caudal to bregma, 1.8 mm lateral to the midline, 9.4 mm below the surface of the skull; 50) or the left and right PVN (1.6 mm caudal, 1.8 mm lateral, 9.1 mm deep, angle of 10° to avoid sagittal sinus damage). The probes were flushed and filled with sterile Ringer solution (147.1 mM Na⁺, 2.25 mM Ca⁺, 4 mM K⁺, 155.6 mM Cl⁻, pH 7.4; Fresenius, Bad Hamburg, Germany) and secured in place with dental cement to two stainless steel screws inserted into the skull. Afterward, the two ends of the probe were attached to PE-20 polyethylene tubings (adapters, each 5 cm long) filled with Ringer solution.

Jugular vein catheter. After stereotaxic implantation of the microdialysis probes, the jugular vein was exposed by blunt dissection via a ventral approach, and a small incision was made into the vessel using iridectomy scissors, as described before in detail (44). The silicone catheter filled with sterile saline (0.9%) containing gentamicin (30,000 IU/ml; Centravet, Bad Benthem, Germany) was inserted ~3.5 cm into the vessel in the cardiac direction, ligated to the vessel, and exteriorized dorsally in the cervical region. All animals received 0.2 ml of the antibiotic gentamicin solution after completion of surgery.

After surgery, animals were housed singly in experimental poly-carbon cages (38 × 22 × 35 cm) and handled carefully each day to familiarize them with the microdialysis and blood sampling procedures and to reduce nonspecific stress responses during the experiment.

Microdialysis

At 0800 on the day of experiment, and 2–3 days after surgery, one adapter of each of the two microdialysis probes was connected to a syringe mounted onto a microinfusion pump via pieces of PE-20 tubing. The other adapter was equipped with a tube holder that allowed sample collection into a 1.5-ml Eppendorf tube (containing 10 μl 0.1 N HCl). The microdialysis probes were perfused at 3.3 μl/min with sterile Ringer solution (pH = 7.4) for 2 h before the initiation of sampling. During this period, sample collection was simulated every 30 min to adapt the animal to the experimental procedure. During the experiment, four consecutive 30-min microdialysates were collected (see below) and immediately stored on dry ice until lyophilization. Lyophilized samples were stored at −20°C until quantification of AVP.

Blood Sampling

Simultaneously, at 0800, the end of the jugular vein catheter was attached to a 1-ml plastic syringe filled with sterile heparinized saline via 60-cm pieces of PE-50 tubing, allowing blood sampling without disturbance of the conscious, unrestrained animal. Blood samples (0.2 ml for ACTH; 0.6 ml for ACTH, AVP, and OXT) were collected into EDTA-coated Eppendorf tubes containing 10 μl Trasylol (Bayer, Leverkusen, Germany) and replaced with sterile saline. Blood samples were immediately kept at 4°C until centrifugation (4,000 rpm, 4°C, 5 min), and plasma samples were stored at −20°C until quantification of ACTH, corticosterone, and AVP.

Forced Swimming

Forced swimming represents an ethologically relevant combined physical and emotional stressor for rats (1) shown to increase plasma ACTH and corticosterone after an exposure time of either 60 s or longer (21, 40, 45, 62), and AVP and OXT release within the SON and PVN after an exposure time of 10 min (58, 62). With the microdialysis tubing and the extension tubing of the venous catheter still attached, rats were forced to swim for 60 s in a clear plastic cylinder (30 cm in diameter and 70 cm in height) filled with tap water (21°C) to a depth of ~60 cm. After the swim, the rats were gently dried using towels for 10 s and returned to their home cages.

Experimental Protocol

Four 30-min microdialysates were collected simultaneously from the SON or the PVN: samples 1 and 2 were taken under basal conditions with sterile Ringer as perfusion fluid. Sample 3 was collected under basal conditions while either Ringer (vehicle group), Ringer containing the selective OXT-A (des Gly-NH₂ d(CH₂)₅ [Tyr(Me)₂, Thr⁷] OVT; 10 μg/ml; Ref. 39) or an AVP V₁ receptor antagonist [Manning compound, d(CH₂)₅ Tyr(Me)AVP, 10 μg/ml] was perfused. The concentration of the receptor antagonists was chosen to reach a local substance delivery into the retrodialyzed area of about 4–6 ng over 30 min (45, 47). During sampling period 4, rats were forced to swim for a short period (60 s) with ongoing microdialysis. Blood samples were withdrawn 5 min before termination of each of the first three 30-min microdialysis sampling periods, as well as 5 and 15 min after swimming during microdialysis period 4.

Histology

At the end of the experiments, the rats were killed with an overdose of halothane. The brains were removed, frozen in prechilled n-methylbutane on dry ice, and stored at −20°C. To histologically...
verify the placement of the microdialysis probes according to the criteria defined before (43, 47, 60, 62), brains were cut into 25-μm coronal sections in a cryostat, and stained with cresyl violet.

Radioimmunoassays

AVP content in lyophilized dialysates and in extracted plasma was measured by highly sensitive and selective radioimmunoassay (detection limit: 0.1 pg/sample; cross-reactivity of the antisera with other related peptides, including OXT was <7%; for details, see Ref. 32). Plasma ACTH was measured using commercially available immunoradiometric assay kits (ICN Biomedicals, Costa Mesa, CA), according to the respective protocols. The detection limit for ACTH was 6 pg/ml.

Statistics

For the microdialysis study, experimental animals were included in the statistical analysis only if the microdialysis probes had been localized in the respective target brain area. All statistics were performed using computer software packages (GB-Stat 6.0, Dynamic Microsystems, Silver Spring, MD; SPSS, version 12). A two-way ANOVA (factor treatment, ratio, and delta values) was performed where appropriate. Significance was accepted at \( P < 0.05 \).

RESULTS

Effects of OXT-A Within the SON on Local AVP Release

Retrodialysis of OXT-A bilaterally into the SON significantly affected local, intra-SON release of AVP (treatment \( \times \) time, \( F_{3,69} = 2.99 \), \( P = 0.037 \)). Specifically, blockade of local OXT receptors resulted in a fourfold rise in local AVP release under basal conditions which, however, did not reach statistical significance (ratio sample 3/sample 2, \( P = 0.18 \) vs. vehicle, Fig. 1). Importantly, whereas exposure to 60 s of swimming did not significantly elevate local AVP release in vehicle-treated rats [from 0.17 ± 0.10 to 0.36 ± 0.20 pg/sample, not significant (ns)], AVP release within the SON was further augmented by local OXT-A treatment (\( F_{1,23} = 5.79 \), \( P = 0.02 \), as additionally reflected by the respective ratio values (sample 4/sample 3; \( P < 0.05 \) vs. vehicle, Fig. 1).

Effects of OXT-A Within the SON on AVP Secretion Into Blood

In all vehicle-treated control rats bilaterally implanted with microdialysis probes in the SON, neurohypophyseal secretion of AVP into the bloodstream remained unchanged throughout the entire sampling period, that is, also in response to swim stress. In contrast, bilateral administration of OXT-A into the SON significantly elevated AVP secretion into the blood under basal conditions (from 2.6 ± 0.58 to 4.7 ± 1.1 pg/ml, \( P < 0.05 \)), as additionally reflected by respective delta (sample 3/sample 2, \( F_{1,21} = 6.07 \), \( P = 0.022 \) vs. vehicle; Fig. 2) and a tendency in ratio (sample 3/sample 2, \( F_{1,21} = 3.99 \), \( P = 0.058 \) vs. vehicle; Fig. 2) values. No further rise in plasma AVP concentration was found in response to forced swimming (Fig. 2). Separate statistics did not reveal an effect of bilateral administration of the AVP V1 antagonist directly into SON on AVP secretion into blood under basal or stress conditions compared with the vehicle group (Fig. 2).

Effects of OXT-A Within the PVN on Local AVP Release

Retrodialysis of OXT-A into the PVN did not significantly alter local, intra-PVN release of AVP under basal or stress-induced conditions compared with the vehicle group (treatment \( \times \) time, \( F_{3,42} = 0.155 \), \( P = 0.925 \); Fig. 3A). Further, exposure to a short period of forced swimming (60 s) only tended to increase AVP release within the PVN in the vehicle group (from 1.02 ± 0.14 to 1.42 ± 0.47 pg/sample, ns). In contrast, the stress-induced rise of local AVP release reached statistical significance in the OXT-A group (from 0.90 ± 0.13 to 1.44 ± 0.26 pg/sample, \( P = 0.050 \)).

Effects of OXT-A Within the PVN on AVP Secretion Into Blood

In contrast to the SON, bilateral administration of OXT-A into the PVN did not change AVP secretion into blood either under basal or swim stress conditions (treatment \( \times \) time, \( F_{3,42} = 0.380 \), \( P = 0.76 \); Fig. 3B). Neurohypophyseal secretion of AVP...
into blood was not found to be altered in response to forced swimming in either vehicle- or OXT-A-treated rats.

Effects of OXT-A Within the SON and PVN on HPA Axis (Re-)Activity

Forced swimming (60 s) induced a significant increase in plasma ACTH concentrations (P < 0.01) in all groups of rats implanted with microdialysis probes within the SON or PVN.

Retrodialysis of OXT-A bilaterally into the SON altered ACTH secretion into blood (treatment × time F_{4,76} = 3.51, P = 0.011, Fig. 4A). In more detail, the rise in plasma ACTH was significantly attenuated in OXT-A vs. vehicle-treated rats at 5 min after exposure to the swim stress (P = 0.043). This is also reflected by a reduced ACTH ratio in OXT-A rats (sample 4/sample 3, P = 0.045 vs. vehicle; Fig. 4A). Basal ACTH secretion (sample 3) was not altered by OXT-A.

Retrodialysis of OXT-A bilaterally into the PVN significantly altered ACTH secretion into blood (treatment × time F_{4,52} = 5.52, P = 0.001, Fig. 4B). Specifically, the stress-induced rise in plasma ACTH was significantly lower in OXT-A rats (P = 0.022, 15 min after swim stress exposure). This is also reflected by a reduced ratio after OXT-A treatment (P = 0.018; sample 4/sample 3 in Fig. 4B). Basal ACTH secretion 25 min after onset of local OXT-A treatment (sample 3) only tended to be elevated after OXT-A (ratio sample 3/sample 2, P = 0.076 vs. vehicle).

Fig. 2. Effects of OXT-A within the SON on plasma AVP. AVP concentration was quantified in plasma of conscious male rats (undergoing the procedure described in Fig. 1) sampled under undisturbed basal conditions (samples 2 and 3) and 5 min (sample 4), as well as 15 min (sample 5) after 60 s of forced swimming (left). Rats were fitted bilaterally with a microdialysis probe in the SON and locally perfused with either Ringer (vehicle) or OXT-A, 10 µg/ml, 3.3 µl/min) starting after blood sample 2 (gray bars). On the right side, the ratio between samples 3 and 2, and the delta of samples 3 and 2 indicate the effect of OXT-A on AVP release into blood under basal conditions. Dotted bars indicate data from OXT-A treated animals with the microdialysis probe located outside the SON, gray bars indicate data from rats locally treated with the AVP V1 receptor antagonist (AVP V1, 10 µg/ml). Numbers in parentheses indicate group size. Data are expressed as the means ± SE; *P < 0.05 vs. vehicle group, #P < 0.05 vs. sample 2.

DISCUSSION

The stress response of the organism is a fine-tuned physiological process, which is regulated by a concerted action of multiple factors, including classical neurotransmitters, as well as neuropeptides released as neuromodulators within the brain. Besides well-defined actions of neuropeptides such as CRH, the regulatory capacity of others is less extensively characterized. Among those, AVP and OXT are of particular interest, as their release within limbic and hypothalamic brain regions has been found in response to a variety of stressors. Interestingly, whereas OXT is also secreted into blood in response to exposure to most psychological and physical stressors in the rat, the neurohypophysial secretion of AVP remains unchanged or even decreases (13, 14, 44, 60, 61).

Here, we show that blockade of OXT receptor-mediated actions within the SON differentially disinhibits the AVP system. Administration of a selective OXT-A directly into the SON of conscious male rats via reversed microdialysis increased AVP secretion into blood under basal conditions, indicating an inhibitory tone of intra-SON released OXT upon AVP neurons projecting to the neurohypophysis in the conscious rat. This is surprising as electrophysiological studies failed to demonstrate OXT actions on AVP cells within the hypothalamic magnocellular nuclei in vitro (10, 27, 40). A negative feedback effect of locally released AVP on peripheral

Fig. 3. Effects of OXT-A within the paraventricular nucleus (PVN) on local AVP release reflected by AVP content in 30-min microdialysates (A), and on AVP secretion into blood reflected by AVP plasma concentrations (B). Conscious male rats were sampled under undisturbed basal conditions (dialysate samples 1–3, blood samples 2–3) and after 60 s of forced swimming (arrow, dialysate sample 4, and blood samples 4 and 5). Rats were fitted bilaterally with a microdialysis probe in the PVN and locally perfused with either Ringer (vehicle) or OXT-A (10 µg/ml, 3.3 µl/min) starting after microdialysis sample 2, and blood sample 2 (gray bars). Data are expressed as the means ± SE; #P = 0.050 vs. sample 3 in the OXT-A group only.
AVP secretion could be excluded, as retrodialysis of the AVP V1 receptor antagonist into the SON did not alter plasma AVP concentrations, which is in line with recent observations (62). Somato-dendritically released AVP was shown to activate quiescent AVP cells (10, 21, 40). In contrast to AVP secretion under resting conditions, the stress-induced secretion of AVP into blood was not found to be further enhanced after local blockade of OXT receptor-mediated actions. Thus, from these results, we may conclude that the unchanged AVP secretion into blood in response to swim stress found in both male and female rats (44, 61) is not primarily due to the inhibition by locally released OXT within the SON. Among the factors that may contribute to the inhibition of neurohypophyseal AVP secretion during stress are amino acids. During forced swimming, glutamate, aspartate, and taurine are released within the SON, whereas the release of GABA remains unchanged (for a review, see Ref. 16). Taurine, which mainly originates from the ventral glial lamina in close vicinity of AVP neurons of the SON, has recently been revealed to inhibit the electrical and secretory activity of AVP neurons (26, 52), also during swim stress (15). Another possibility of regulation of AVP secretion is the release of endocannabinoids from OXT neurons. Endocannabinoids act as mediators of presynaptic inhibition and may have been altered by local treatment with the OXT-A (24).

Using local microdialysis and a highly sensitive radioimmunoassay for vasopressin, we were able to detect AVP release within the SON both under basal and stress conditions. It has recently been shown that 10 min of exposure to forced swimming elevated local AVP release within the SON (62). In our study, exposure to only 60 s swimming doubled the release of AVP within the SON, although the increase did not reach statistical significance. Thus a longer period of stressor exposure seems to be essential to detect acute neuronal activation. In this study, the short period of swimming was used to reveal possible inhibitory effects of endogenous OXT on the AVP and the HPA axis systems and to avoid “ceiling effects”.

However, administration of the OXT-A directly into the SON did not significantly alter local somato-dendritic AVP release under basal, undisturbed conditions of the male rat. Similarly, in acute hypothalamic slice preparations from juvenile rat brains, OXT (1 μM) did not affect AVP release from isolated SON under basal conditions (10). In contrast, we showed that under conditions of stress, local treatment with the OXT-A affected the release of AVP within the SON, as the stress-induced rise in local AVP release was significantly more pronounced in OXT-A compared with vehicle-treated rats. Thus OXT released in a paracrine fashion from dendrites and somata within the SON, in particular during exposure to stress, may diffuse to AVP neuronal elements to inhibit local AVP release. From these observations, we may conclude that an inhibitory effect of OXT on AVP dendritic release within the SON seems to be restricted to a state of increased activation. Such effects may, for example, depend on the concentration of OXT in the extracellular fluid, which is subject to changes in response to stress (for a review, see Ref. 33). Moreover, during stressor exposure, various neuroactive factors (classical neurotransmitters, amino acids, neuropeptides) are simultaneously released within the SON, which may interact with OXT to
regulate local AVP release in a fine-tuned manner. Thus intra-SON OXT seems to independently regulate central and peripheral AVP release patterns, as the OXT-A affected local somato-dendritic release of AVP preferentially in a state of stress, but, in contrast, altered AVP secretion into blood only under basal resting conditions.

Although OXT receptors could be localized within the hypothalamus, including the SON (2, 5, 18, 31), their presence on AVP neurons has not been demonstrated. Nevertheless, different local modes of OXT action and different neuronal target elements could be hypothesized: 1) OXT could inhibit the activity of AVP neurons at the level of the soma. However, electrophysiological studies did not consistently reveal OXT effects on the electrophysiological activity of AVP neurons, and neurohypophyseal AVP secretion was not further altered after stress. 2) Although experimental evidence from in vitro or electronmicroscopic studies is missing, OXT may directly act at the dendritic level to locally inhibit excitocytic processes related to AVP release. A dissociation of dendritic release and the electrical activity of neuropeptidergic neurons has been established (11, 36). 3) Alternatively, OXT could modulate excitatory or inhibitory inputs to AVP neurons identified pre- or postsynaptically within the SON (7, 8; for a review, see Ref. 33). In light of the ability of neuropeptides to diffuse over long distances (for a review, see Ref. 33), OXT molecules are likely to occupy remote receptors on neuronal structures that indirectly modulate release or secretion patterns of AVP.

In contrast to the SON, effects of endogenous OXT released within the PVN on the AVP system were only found to be marginal. Administration of the OXT-A into the PVN did not alter AVP secretion into blood under basal or stress conditions. Further, stress-induced AVP release within the PVN was only found to be disinhibited to a lesser extent compared with the OXT-A effect seen in the SON. Thus site-specific effects of OXT controlling the activity of hypothalamic AVP neurons have to be considered, which are far from being understood.

We could at least partly confirm our recent results, indicating that locally released OXT within the PVN of male rats inhibits the “basal” activity of the HPA axis (47). Blockade of OXT actions within the PVN by retrodialysis of the OXT receptor antagonist elevated ACTH secretion into blood under resting conditions, although in the present study, this effect did not reach statistical significance, probably because ACTH concentrations were estimated only 25 min after starting local retrodialysis with the OXT-A. Also, in this particular experiment, the number of animals with bilateral retrodialysis of the OXT-A within the PVN was relatively low (n = 6). Generally, it is assumed that brain OXT reduces the activity of the HPA axis (45, 48, 58), specifically within the PVN (47, 59). In contrast, after blockade of OXT receptors within the SON basal ACTH secretion remained virtually unchanged. However, after intra-PVN administration of the OXT-A, the “stress-induced” rise in plasma ACTH 5 min (ratio) and 15 min after forced swimming was significantly reduced. This clearly indicates that OXT acutely released within the PVN during exposure to swim stress (57, 61) promotes the acute stress-induced rise in ACTH. Consequently, a local regulatory circuitry involving locally released OXT and AVP (60) seems to be essential during an acute challenge to mount an adequate acute stress response (48).

Importantly, in the SON, we could reveal a similar facilitating effect of endogenous OXT on the stress-induced secretion of ACTH. Bilateral retrodialysis of the OXT-A significantly blunted the swim-induced rise in plasma ACTH. Consequently, OXT released within the SON in response to swim stress also contributes to the stimulation of ACTH secretion from pituitary corticotrope cells. This finding is supported by others showing the involvement of magnocellular SON neurons in the regulation of the activity of the HPA axis. For example, electrical stimulation of the SON has been demonstrated to increase plasma corticosterone in anesthetized rats (37). Also, an attenuated stress-induced rise in plasma ACTH was found in hypotremic rats (12) and after peripheral immunoneutralization of OXT (20), indicating the involvement of magnocellular AVP and/or OXT in HPA axis regulation. Similarly, local osmotic stimulation of the SON of conscious rats increased not only local and peripheral AVP and OXT release but also elevated ACTH secretion into blood (62; for a review, see Ref. 16).

However, peripheral AVP receptor blockade by intravenous administration of the AVP V1 receptor antagonist indicated a predominant involvement of AVP (originating from SON neurons) in the regulation of ACTH secretion (62). The neuroanatomical basis for these findings could be that both AVP and OXT are potentially released from axons of magnocellular SON neurons at the level of the eminentia mediana (en passant release, 9, 25, 51). In addition, vascular connections via short portal vessels between the adeno- and neurohypophysis may allow diffusion of these neurohypophyseal neuropeptides to regulate adenohypophyseal corticotrope cells (4, 6). Also, neurons synthesizing CRH or urocortin have been identified in the SON (41, 49), which can potentially participate in HPA axis activation and be regulated by local OXT. Moreover, the possibility should also be considered that PVN neurons projecting to the pituitary stalk or to the neurohypophysis and passing the SON are antidromically activated by OXT-A treatment within the SON. Taken together, although the precise mechanisms of action need to be revealed, our findings indicate that the acute rise in local OXT within both the PVN and SON during stressor exposure contributes to an appropriate activation of the HPA axis during an acute stress response. Our study further indicates that we need to differentiate between acute and chronic effects of OXT on stress-induced activation of the HPA axis. An example for a possible chronic inhibitory effect is the blunted HPA axis response in the peripartum period (pregnancy, parturition, lactation) (23, 34, 35, 44, 53, 54, 56), that is, at a time of elevated brain OXT system activity. In support of this, it was recently demonstrated that chronic OXT infusions into the lateral ventricle inhibit the synthetic activity of CRH neurons within the PVN and the stress-induced secretion of ACTH and corticosterone in ovarioectomized virgin female rats (59). The same treatment also reduced stress-induced Fos expression in other limbic brain areas known to be involved in the regulation of the hypothalamic neuroendocrine stress response. Moreover, an elevated corticosterone response to a psychogenic stressor was recently described in female OXT knockout mice (3), further indicating that the chronic presence of OXT within the brain restricts HPA axis responses. However, comparable studies performed in male mice are missing.
The results of the present study demonstrate differential inhibitory effects of local OXT on neurohypophysial and somato-dendritic AVP release within the SON and PVN. These actions are site specific and depend on the state of stress-related activity of the animal. Moreover, hypothalamic OXT exerts a bimodal action on the activity of the HPA axis with a chronic inhibition of the basal ACTH secretion, but acute facilitation of the HPA axis response to swim stress. Future studies need to reveal the molecular basis of the functional complexity of neuropeptide-regulated cell responsivity within the hypothalamus, which seems to be dependent on the level of physiological activity.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R. Landgraf (Munich) for radioimmunoquantitative estimation of vasopressin and constructive discussions, and to Dr. M. Manning (Toledo) for the generous gift of the oxytocin antagonist. Supported by VW-Stiftung (IDN) and Bayerische Forschungsförderung (AV).

REFERENCES


