Noradrenergic control of central oxytocin release during lactation in rats

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Noradrenergic control of central oxytocin release during lactation in rats. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E453–E458, 1998.—Noradrenergic systems regulate the systemic release of oxytocin (OT) in lactating rats. However, a role for norepinephrine (NE) in release of OT within the magnocellular nuclei during suckling has not been established. These studies were designed to determine 1) if suckling induces NE release in the supraoptic (SON) and paraventricular (PVN) nuclei of conscious rats and 2) the role of NE in the central, intranuclear release of OT within these nuclei. Female Holtzman rats were implanted with microdialysis probes adjacent to the PVN or SON on lactation days 8–12. The following day, the pups were isolated from the dams for 4 h. Microdialysis probes were perfused with artificial cerebrospinal fluid (ACSF) or with ACSF containing an α- or β-adrenergic receptor antagonist. Dialysate was collected before, during, and after suckling and analyzed for NE or OT. In an additional experiment, an α- or β-adrenergic agonist was administered via the microdialysis probes into the PVN in nonsuckled, lactating rats. Extracellular NE increased in the PVN during suckling but was not detectable in the SON. OT concentrations in dialysates from the PVN and SON significantly increased during suckling. Blockade of either α- (in both PVN and SON) or β- (PVN) adrenergic receptors prevented the suckling-induced increase in central OT release. OT release was increased in nonsuckled, lactating rats by central application of either an α- or β-adrenergic agonist. These data demonstrate that intranuclear NE release is increased in the PVN by suckling and that subsequent stimulation of both α- and β-noradrenergic receptors mediates intranuclear OT release.

OXYTOCIN (OT) is released from neurosecretory terminals in the neurohypophysis in response to suckling in the lactating female to induce the milk-ejection reflex (13, 37). OT release into the systemic circulation results from the activation of OT-containing magnocellular neurons located in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. In addition to this systemic release of OT, release of the peptide occurs locally within the PVN and SON in response to a number of stimuli (4). For example, both peripheral hypertonic stimulation (15, 22) and direct administration of hypertonic solutions into the PVN and SON (6, 7, 21, 22) increase extracellular OT concentration within these nuclei. In addition, electrical stimulation of the pituitary stalk increases OT concentration in the extracellular fluid of the SON (17), and intranuclear release of OT occurs during both parturition and lactation (17, 20, 22, 24). There is growing evidence that OT acts within these structures to facilitate and coordinate the excitation of the OT magnocellular neuronal population in these conditions (11, 19).

There is strong evidence that norepinephrine (NE) is an important excitatory neurotransmitter in regulation of OT secretion. For example, noradrenergic fibers, arising predominantly from the region of the nucleus tractus solitarius in the medulla, innervate magnocellular OT neurons (4, 9). Moreover, depletion of hypothalamic NE decreases the systemic release of OT in response to 1) footshock (26), 2) peripheral administration of cholecystokinin and hypertonic saline (25), and 3) suckling during lactation (5). Peripheral administration of an α-adrenergic antagonist significantly increases the latency of the milk-ejection reflex (34), which is mediated by OT, and completely prevents the suckling-induced increase in plasma OT concentration (31).

These data suggest that the suckling stimulation provided by the offspring activates an ascending noradrenergic system that acts via adrenoreceptor stimulation in the magnocellular nuclei to stimulate systemic OT release. A role for NE in controlling the central release of OT during suckling appears likely but has not been demonstrated directly. Therefore, the objectives of the present studies were to test 1) whether suckling increases the release of NE within the magnocellular nuclei, as determined by in vivo microdialysis, and 2) whether local blockade of noradrenergic receptors alters the suckling-induced release of OT in the PVN and SON.

MATERIALS AND METHODS

Animals. Pregnant Holtzman rats were obtained from a commercial supplier (Harlan) and were housed individually in temperature-controlled (22°C) rooms with a 12:12-h light-dark cycle. Animals had free access to food and water. After parturition, litters were culled to eight pups on postpartum day 5, and animals were tested on lactation days 8–12. The procedures used in these studies were approved by the University of Tennessee Animal Care and Use Committee.

Microdialysis probes. Loop-style microdialysis probes (35, 36) with an exchange area 1.5 mm long × 0.7 mm wide × 0.3 mm deep were used in these studies. The exchange membrane consisted of cuproammonium-rayon with a 40,000 molecular weight cutoff (Asahi Medical, Japan). Under the conditions used in these experiments, these probes have typical in vitro recovery rates of between 25 and 30% for NE and 8 and 10% for OT.

Surgery. On the day before testing, animals were anesthetized with methohexital sodium (Brevital Sodium, 60 mg/kg) and implanted with a microdialysis probe with the tip aimed immediately adjacent to the PVN (∼1.9 mm posterior to bregma; ±0.6 mm lateral to the midline; −8.2 mm ventral to the surface of the skull) or the SON (∼1.1 mm posterior; ±1.6 mm lateral; −10.0 mm ventral to the skull). Dialysates were secured with small screws placed in the skull and dental

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acrylic. The dams were returned to their pups and recovered overnight.

Protocol. In all experiments, pups were isolated from the dams on the morning of testing and were returned exactly 4 h after removal. The dams were placed in plastic cages, and the dialysis probe was connected to a remote 3-ml syringe in a syringe pump with polyethylene tubing. Tubing from the output port of the dialysis probe was led from the cage, and dialysate was collected in chilled plastic tubes. Perfusion of dialysis probes with artificial cerebrospinal fluid [ACSF; (in mM) 126.5 NaCl, 4 KCl, 0.5 KH2PO4, 1.1 CaCl2, 2.5 dextrose, 0.83 MgCl2, and 0.5 Na2SO4] was initiated 130–160 min before the return of the pups. Dialysate ACSF used in studies to measure NE release contained 0.1% ascorbic acid and the NE reuptake inhibitor desmethylimipramine (1 µM). Microdialysis probes placed in the PVN were perfused at a rate of 2 µl/min, whereas dialysis probes aimed at the SON were perfused at 1 µl/min. The initial dialysate was discarded. Dialysate was collected during the final 45 (PVN) or 100 min (SON) before return of the pups (control period [Cont]), during two 45-min periods (PVN), or one 100-min period (SON) of suckling [experimental (Exp) period], and for a final 45 (PVN) or 100 min (SON) after the pups were again removed [recovery period (Rec)]. Dialysate was collected in chilled tubes containing 10 µl of 1.0 N HCl. Other animals were similarly treated, except that the pups were not returned during the Exp period.

Animals used to evaluate central OT release were similarly prepared. Microdialysis probes in both the PVN and SON were perfused at a rate of 1 µl/min with ACSF containing bacitracin (20 µM). Dialysate was collected during the final 100 min before return of the pups (Cont), during 100 min of suckling (Exp), and during 100 min after removal of the pups (Rec). Samples were collected in chilled tubes, frozen, and stored at −80°C. A separate group of animals was treated similarly, except that the pups were not returned during the Exp period. In addition, separate groups of animals were prepared with dialysis probes in the PVN or SON and similarly tested, except the dialysate contained the α1- and α2-adrenergic receptor antagonist phentolamine (3.15 mM), or were prepared with dialysis probes adjacent to the PVN and perfused with the β-adrenergic receptor antagonist propranolol (3.44 mM).

To validate the role for NE in stimulating intranuclear OT release, separate animals were prepared with dialysis probes adjacent to the PVN and perfused with ACSF containing the α2-receptor agonist, phenylephrine (24.6 mM), or the β-adrenergic receptor agonist, isoproterenol (13.8 mM). The concentrations of these agonists are lower than the typical concentrations of NE administered centrally via dialysate in previous studies, which range from 31.3 (27–29) to 50 mM (1).

Histology. After the experiments, the animals were anesthetized with pentobarbital (Nembutal; 60 mg/kg) and the brains were removed after transcardial perfusion with saline and Formalin solution. The brains were placed in sucrose Formalin (30%) and subsequently blocked, frozen, sectioned (40 µm), and stained with cresyl violet. These sections were observed under the light microscope for determination of proper placement of the dialysis probe.

Assays. All dialysate samples were frozen and stored at −80°C for subsequent analysis of NE concentration using a radioenzymatic assay (8, 35, 36) that has a detection limit of 0.4 pg (8). OT concentrations were determined by a radioimmunoassay described previously (5), except that an OT antibody from Arnel Products (New York, NY) was used; this antibody cross-reacts 0.0001% with vasopressin and has a detection limit of 0.7 pg. All samples from a single experiment were measured in the same radioimmunoassay to avoid interassay variability.

Data analysis. Only animals that had detectable levels of NE or OT in the dialysate sample collected during the Cont period and histological verification of probe tip placement <800 µm from the PVN or SON were included in the data analysis. If an animal had detectable neurochemical levels during the Cont period but nondetectable levels during the Exp and/or Rec periods, the concentrations were recorded as zero for the Exp and/or the Rec period. No animal with probe placement farther than 800 µm from the PVN or SON had detectable levels of NE or OT in the dialysates.

Within-group data were analyzed using a single-factor, repeated-measures analysis of variance (ANOVA), whereas differences between groups were evaluated with a two-factor ANOVA. Significant differences between individual means were determined using a Scheffé a posteriori analysis. A probability of <0.05 was considered significant.

RESULTS

Properly placed dialysis probe tips were positioned immediately adjacent to the PVN or SON. Data obtained from animals in which the tip of the dialysis probe was >800 µm from the nucleus (PVN, n = 16; SON, n = 9) or in which the probe destroyed the nucleus (PVN, n = 3; SON, n = 3) were eliminated from analysis, because neither NE nor OT was detectable in dialysate samples.

NE could not be reliably detected in dialysates from animals with dialysis probes placed adjacent to the SON. Measurable NE concentrations were found in only two of eight animals with dialysis probes correctly placed next to the SON. This is in contrast to detectable NE concentrations found in all animals with dialysis probes properly placed adjacent to the PVN.

Figure 1 shows NE concentrations in dialysates obtained from the PVN in animals that were suckled during Exp periods 1 and 2. Suckling induced a significant increase in NE concentration in the PVN, whereas dialysate NE concentrations did not change during the Exp or Rec periods in animals that were not exposed to

![Graph](http://ajpendo.physiology.org/DownloadedBy/10220.33.3.4/June25,2017)
pups. Dialysate NE concentrations in animals that were suckled during the Exp period remained significantly elevated during the Rec period.

The effect of suckling on the release of OT in the PVN is shown in Fig. 2A. Dialysate OT concentration significantly increased during suckling, whereas dialysate OT concentrations remained constant in animals whose pups were not returned. The administration of either phentolamine or propranolol in the dialysate completely prevented the increase in PVN OT concentration during suckling. Similarly, the intranuclear release of OT in the SON was increased by suckling (Fig. 2B), but the concentration of OT in the SON dialysate did not change during the protocol in nonsuckled animals. Furthermore, similar to the PVN, perfusion of the SON with dialysate containing phentolamine completely prevented the increase in intranuclear OT release during suckling.

Figure 3 shows dialysate OT concentrations before, during, and after dialysate probe perfusion with phentolamine or isoproterenol in the PVN. Administration of either the α- and the β-receptor agonist increased dialysate OT in the PVN.

DISCUSSION

These studies demonstrate for the first time that suckling increases NE release in the PVN and confirm earlier observations that OT is released within both the PVN and SON during suckling (10, 17, 22, 24). The present studies extend the prior work by demonstrating a functional relationship between suckling-induced NE and OT release in these magnocellular nuclei. Specifically, these findings support the proposition that the suckling stimulus evokes NE release in the PVN and that subsequent stimulation of both α- and β-adrenergic receptors is necessary for release of OT in these nuclei. Furthermore, the blockade of central OT release by phentolamine in the SON observed in the present studies and the previously reported increase in NE turnover evident in the SON in lactating rats (5) suggest that NE is also released in the SON during suckling.

Previous studies have shown that suckling increases the turnover rate of NE in the PVN and SON of lactating rats, suggesting increased synthesis and/or release of this transmitter in response to this physiological stimulation (5). That NE release is indeed elevated by suckling, at least within the PVN, is strongly suggested by the present studies, using the more direct approach of microdialysis to measure changes in the extracellular concentration of the catecholamine. The available anatomical evidence (reviewed in Ref. 4) strongly implicates the noradrenergic perikarya in the A2 cell group in the region of the nucleus tractus solitarius as the source of the noradrenergic innervation of OT magnocellular neurons. It is noteworthy that systemic administration of cholecystokinin, which, like suckling, selectively releases OT into the systemic circulation via noradrenergic mechanisms (25), activates the A2 cell group, as evidenced by expression of c-fos (25), but to date there have been no reports of similar studies testing the effects of suckling.
In these experiments, NE could not be consistently detected in the SON dialysates. This may have been due to low concentrations of NE in the SON compared with the PVN and/or to the lack of accessibility of NE released within the SON to dialysis probes placed adjacent to the nucleus proper. Alternatively, it is possible that NE is selectively released in the PVN in response to suckling. However, because previous work has demonstrated an increase in NE turnover rate in the SON in response to suckling (5) and because, in the present studies, local blockade of α-adrenergic receptors prevented the suckling-induced increase in OT release in the SON, it appears highly likely that NE release is increased in the SON, as well as the PVN, by the suckling stimulus.

The intranuclear OT obtained from the PVN may have been derived from parvo- and magnocellular components of this nucleus. The microdialysis procedures used in these studies do not permit determination of the contributions of these neuronal populations to extracellular OT. However, in view of the electron-microscopic evidence for direct innervation of OT perikarya by noradrenergic fibers (9), it seems likely that OT magnocellular neurons are directly targeted by the NE systems arising from the brain stem and have a predominant role in mediating this critical component of the milk-ejection reflex, with a possible contribution from the parvo- and magnocellular neurons in the PVN.

Previous studies have demonstrated that peripheral or central administration of α- or β-adrenergic antagonists impairs the milk-ejection reflex and prevents the suckling-induced increase in plasma concentration of OT (3, 31, 34), whereas β-adrenergic receptor stimulation decreases peripheral OT release (18, 31). These findings suggest that stimulation of central α-adrenergic receptors mediates systemic OT secretion in response to suckling, whereas a β-adrenergic system might exert inhibitory effects. The present studies extend these results by demonstrating that release of central OT during suckling is also dependent on α-adrenergic receptor stimulation, because the α-adrenergic antagonist phentolamine completely prevented the suckling-induced increase in extracellular OT concentrations within the PVN and SON.

However, an unexpected finding of the present studies was that, in contrast to the differential effects of α- and β-adrenergic antagonists on systemic OT release, the central OT release in response to suckling was abolished by treatment with either an α- or β-adrenergic antagonist, at least within the PVN. Consistent with this observation, we also found that intranuclear OT release could be reliably stimulated by either an α- or a β-adrenergic agonist. This suggests that the adrenergic receptors mediating the stimulatory action of NE on intranuclear OT release may be either α- or β-subtype. It is noteworthy that β-adrenergic mechanisms have been implicated in some of the plastic morphological changes occurring within the neurohypophysis on activation of posterior pituitary hormone secretion (14). Thus it may be the case that β-adrenergic mechanisms exert multiple effects within this neurosecretory system. Alternatively, the high concentrations required for administration of these agents via dialysis probes might reduce the selectivity of these drugs for one or the other receptor type. However, the opposite effects of both the α- and β-adrenergic agonists and antagonists on intranuclear OT release argue against the possibility that these drugs are acting nonselectively.

The functional significance of intranuclear OT release has not been completely defined, but there is considerable evidence that, when released centrally during lactation, OT exerts a number of important actions related to expression of the milk-ejection reflex. For example, central release of OT appears to be involved in morphological reorganization of the magnocellular hypothalamic nucleus before parturition (33) and in the upregulation of OT gene expression during early lactation (32). Moreover, centrally released OT has been implicated in the process whereby the entire OT neurosecretory population is recruited and activated synchronously so that a bolus of peptide is delivered into the systemic circulation to evoke a milk ejection (11, 17, 24). For example, administration of an OT antagonist significantly decreases systemic OT release during suckling (20), suggesting that the central release of OT is required for OT to be released into the systemic circulation. Furthermore, intranuclear OT facilitates its own release in the SON (16, 20), and electrophysiological studies indicate that this may occur by enhancing the amplitude of OT neuronal bursting activity (11). In addition, milk-ejection reflexes are induced by suckling only when intranuclear OT increases (17), and administration of OT antisense oligonucleotide into SON decreases milk-ejection reflexes and the increase in plasma OT evoked by suckling (23). Taken together, these studies demonstrate the importance of central OT release during suckling in facilitating the milk-ejection reflex.

The basal and suckling-induced levels of OT measured in dialysates of the PVN and SON are very similar to those reported from other laboratories (12, 20, 24). The sources of the neurochemicals measured in these studies are most likely to be the PVN and SON, since both NE and OT are released in these sites (6, 10, 17, 22, 24). Furthermore, detectable concentrations of these neurochemicals were obtained only from animals with dialysis probe tips within 800 µm of the target nucleus.

After removal of the pups after suckling, the concentration of NE in dialysates obtained from the PVN of suckled rats remained elevated during the Rec period, whereas intranuclear OT levels returned toward control values. This may be due to several factors. The dialysate used in the present studies to measure NE contained a reuptake inhibitor to increase the extracellular concentration of NE. As in previous studies using reuptake inhibitors (2, 30), the present results found that control dialysate concentrations of NE were stable and stimulation produced consistent increases in extracellular concentrations. Therefore, even though the
dynamics of local reuptake of NE may have been altered, the response of the noradrenergic nerve terminals appears normal. However, due to the presence of the reuptake inhibitor, NE would not have been cleared from the extracellular space as quickly as under normal conditions. Furthermore, the rec period in the NE experiments was 45 min, compared with 100 min for the rec period in studies measuring intranuclear release of OT. Therefore, it is likely that the high concentration of NE present immediately after the removal of the pups, at the initiation of the rec period, would contribute to an increased NE concentration, whereas the OT present at the initiation of rec would have been diluted by lower concentrations during the later portions of this period.

In summary, these studies have demonstrated that suckling induces NE release in the PVN and OT release in the PVN and the SON of the conscious, lactating rat. Furthermore, the release of intranuclear OT in response to suckling is dependent on stimulation of central α- and β-adrenergic receptors.

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