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The puzzle of pulsatile oxytocin secretion during lactation: some new pieces

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PROVIDING SUFFICIENT MILK to nursing young is necessary for infant survival and depends on periodic bolus secretion of oxytocin (OT) from the neurohypophysis during suckling. This pulsatile release maximizes myoepithelial cell contractions in the mammary gland by avoiding OT receptor desensitization (37, 44). Underlying the periodicity is the brief (4–6 s), synchronous, and explosive bursting of OT neurons in the supraoptic (SON) and paraventricular nuclei (PVN), the axons of which terminate at the neurohypophyseal neurohemal contact zone. These bursts (and the resultant OT release) appear with remarkably long intervals (5–20 min) despite the continual nipple stimulation provided by pups (30, 32, 38, 39), and are seldom observed during other periods of enhanced OT release. The bursting pattern maximizes frequency-dependent facilitation of OT release at neurohypophyseal terminals, and minimizes release fatigue (3–5). Understanding this periodicity remains one of the greatest challenges for OT neurobiologists. This system undergoes astonishing physiological plastic changes during pregnancy and lactation that should provide instructive clues to the process. It is now appreciated that OT itself clearly plays multiple roles during pregnancy and lactation, acting as a growth factor (7, 35) and as a central nervous system neuromodulator (10, 19–22, 29, 31), in addition to its peripheral endocrine actions.

Pregnancy and lactation are associated with a dramatic morphological reorganization of the SON and PVN, which includes the withdrawal of astrocyte processes in the nuclei, increased neuronal direct membrane apposition, and increased synaptic contact, especially shared synapses, where single terminals contact several distinct postsynaptic elements. The presence of gap junctions between these magnocellular neurons has been inferred from dye-coupling studies and the expression of connexin (the proteins that form gap junctions) mRNA (1, 25). Dye-coupling incidence among OT neurons increases during lactation (13, 14), giving rise to the hypothesis that gap junctional communication among local cell groups might be one of the factors facilitating the well-documented synchrony of OT neuronal firing during milk ejection bursts. Many of the salient aspects of this reorganization, as well as recent advances in understanding its potential underlying mechanisms and consequences during pregnancy and lactation, are reviewed in the May 2006 issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology by Theodosis et al. (35). Although a similar type of plasticity occurs in both vasopressin and OT neurons during osmotic challenge (24), changes associated with lactation are thought to be specific to the OT system. At least three types of synapses, including GABAergic, glutamatergic, and noradrenergic types, contribute to the plasticity. All three of these transmitters are known to participate in controlling OT neuronal activity during lactation.

Reproduction-related plasticity in the SON and PVN appears to be under the control of gonadal endocrine dynamics during late pregnancy. Estrogen and progesterone progressively increase until 2 days before birth, when progesterone levels fall precipitously while estrogen remains elevated until parturition (6). Excellent for the profound changes in the relationships between the neurosecretory terminals and their associate astrocytes in the neurohypophysis, which occur rapidly during but not before parturition (36), the reorganization is mostly complete by late pregnancy. The reorganized state persists during lactation, is rapidly reversed with early pup removal, and can be mimicked with high-dose steroid replacement therapy in ovariectomized rats. In addition, the central release of OT (presumably from the somato-dendritic region of SON and PVN) may also act in a paracrine manner during this period, as OT enhances the actions of progesterone and estrogen, and OT itself may induce morphological changes in the SON in vitro (35). Thus, as has been observed during developmental studies of magnocellular neurons (7), OT promotes synaptogenesis, probably by enhancing excitatory neurotransmission. Late pregnancy is associated with an upregulation of OT mRNA in the SON and PVN (43), again, a result mimicked with steroid replacement in ovariectomized rats (8). Although gestational changes in OT mRNA may anticipate the impending pituitary requirements of labor and lactation, an increase in somatodendritic release of OT within the SON and PVN has not been reported during pregnancy. However, the study by Bealer et al. (2) in this issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology demonstrates an increase in OT receptor binding in the SON and in related hypothalamic regions during pregnancy, and this change can be partially (i.e., not at all anatomical sites studied) mimicked with estrogen and progesterone treatment. Because...
central OT receptor blockade during pregnancy delays the response of suckling-induced OT release during lactation and leads to less total milk delivery to the pups (23), perhaps an increase in expression of this receptor would mediate actions of a constitutive release of OT (or a small regulated, pulsatile increase, undetectable by microdialysis) that is critical to the reorganization. Alternatively, OT receptor upregulation may increase the sensitivity of the system to constant levels of the peptide, getting more bang for the OT buck.

Interestingly, the paper by Kokay et al. (18) in the May 2006 issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology also demonstrates upregulation of another lactation-related molecule, the prolactin receptor, specifically in OT neurons during pregnancy and lactation. Although prolactin has been shown previously to stimulate OT release and increase OTmRNA in lactating rats (11, 12, 28), in the Kokay study (18), prolactin inhibited OT neuronal activity. However, because these studies were performed in nonpregnant rats and the prolactin was administrated into the lateral ventricle, it is possible that prolactin did not directly inhibit the OT neurons and may not reflect the effect of prolactin on OT neurons during lactation. Thus the precise role of centrally active prolactin during lactation, whether it derives from central nervous system sources or from adenohypophysis, remains to be determined.

OT also plays a significant role as a neuromodulator during lactation through paracrine actions. Françoise Moos and colleagues (22, 32) demonstrated that local, somato-dendritic release of OT during lactation is necessary for normal expression of the bursting pattern. This local OT release recruits neurons into the burst response and increases the number of spikes per burst. Although OT neurons possess an OT receptor that mediates an increase in intracellular [Ca^{2+}] donated largely from internal stores (21), the precise target for OT’s neurophysiological actions within the SON or PVN and the factors mediating its somato-dendritic release, are not yet known and are currently under intense investigation. Retrograde effects of OT are clearly observable in vitro, such that increased spiking from a single neuron suppresses excitatory postsynaptic activity onto that cell, and this suppression could be blocked with an OT receptor antagonist (20). Recently, it has been argued that this effect is mediated by OT’s ability to reduce the release of cannabinoids (CBs), presumably from OT neurons themselves. Thus retrograde effects are blocked equally well by CB1 or OT receptor antagonists (9, 15). The complexity of these local interactions is further demonstrated in the study by Sabatier and Leng (33), in which alpha-MSH stimulation of dendritic OT release ultimately leads to a decrease in peripheral OT secretion as a result of CB release by the OT neuron acting presynaptically to inhibit the electrical activity of OT neurons. Importantly, it is still unknown how, or even whether these effects of OT are altered during lactation. OT also provides a tonic modulation of GABA<sub>A</sub> receptor activity in the SON, preventing the ability of neurosteroids to prolong synaptic transients in neurons of rats that have recently given birth (19). These multiple actions of OT serve notice that it is a constellation of related changes, both pre- and postsynaptic, which underlies OT’s ability to modify its own activity during lactation.

Whereas phasic bursting in vasopressin neurons is largely expressed through intrinsic properties (though clearly activated by synaptic inputs) and therefore amenable to in vitro study, a similar analysis of OT bursting has been less tractable, perhaps because there is no obvious “suckling stimulus” for the hypothalamic slice or related in vitro preparations. Thus there is reason for excitement over recent developments of in vitro models for OT bursting. These include conventional hypothalamic slice preparations and the use of organotypic slice cultures. The organotypic slice culture is made from hypothalamic slices of early (unsexed) postnatal rats and cultured for weeks before analysis (16, 17). Unlike the normal SON, organotypic slice cultures contain a dearth of vasopressin neurons and large numbers of OT neurons, the latter of which are massively innervated by local, and spontaneously active, excitatory and inhibitory neurons. Remarkably, many OT neurons in this preparation exhibit synchronous, bursting activity that can be enhanced or even induced by exogenous OT. Like those during milk ejection, these bursts are brief and intense, and they can exhibit a slow (minutes) periodicity. Synaptic analysis indicates that the bursts are driven by synchronous glutamatergic inputs. Although providing a clue as to how OT neurons could be driven to burst during lactation, the question of how periodicity is realized must be moved a synapse back, to some as yet unidentified excitatory neurons. Thus the relevance of this preparation to understanding OT neuronal bursting in lactation may lie more in future studies demonstrating how a network of neurons, however dissimilar they are likely to be from the adult nervous system, can produce a behavior that so resembles that of an adult nursing female. This is critical, as the nervous system of lactating females has been carefully prepared and readied during pregnancy to preferentially deliver, during lactation, this behavior in response to suckling. Nevertheless, OT neuronal properties also deserve further examination, as these may decide the burst probability of individual neurons in the orchestration of whole networks.

Although synchronicity has yet to be demonstrated, OT-like bursting also can be induced with prolonged noradrenergic stimulation in hypothalamic slices, taken from lactating females (40) and, surprisingly, even from male rats (41). The paper by Wang and colleagues (42) further documents unique and biphasic actions of OT in acute slices from lactating rats. At low to intermediate doses, OT is excitatory and promotes spike clustering (which should lead to more efficient OT release), but when the concentration was progressively increased, OT promoted a pronounced reduction in spike frequency. Interestingly, these effects are largely dependent on the presence of excitatory synaptic transmission, sharing this dependence with the burst modulation in organotypic slice cultures considered above. Glutamatergic synapses, specifically on OT neurons, express a higher probability of release during lactation (27, 34). Because previous work (9, 15, 20) on retrograde OT actions suggests that the peptide suppresses excitatory inputs, it remains to be determined whether OT’s effects on excitatory transmission are substantially different during lactation and to what extent they participate in the regulation of the observed presynaptic activity changes. An intriguing speculation is that the plastic period of gestation and lactation may be similar to that of development, when OT promotes, rather than inhibits, excitatory synaptic activity. Locating the precise source of this excitatory input in the adult, lactating animal is another challenge which, when met, may
uncover the missing piece of the vexing puzzle that is OT pulsatility.

GRANTS
Authors supported by National Institutes of Health Grants NS-23941, HD-41002 (to W. E. Armstrong), and NS009140 (to G. I. Hatton)

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