MINIREVIEW

Oxytocin: A Neurohormone, Neuroregulator, Paracrine Substance

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In 1906, Dale demonstrated that the posterior pituitary gland contains a substance that has uterine stimulating properties [1]. Extract from 0.4 g dried ox pituitary was given to a cat in early pregnancy, producing a rise in blood pressure and contraction of the uterus. Interest in the neurohypophysis was further stimulated by Ott and Scott who demonstrated the milk ejection activity of posterior pituitary extracts [2]. They used goats with a cannulated nipple from which milk was withdrawn by a water aspirator. The milk was collected in a graduated flask and the volume was measured every 5 min. The rate of secretion increased from 5 to 405 drops per 5 min after the injection of the posterior pituitary extract into an ear vein. It was not until the classical studies of Scharrer that the concept of neurosecretion was established [3]. These workers and others demonstrated that transection of the neurohypophysial stalk led to a buildup of neurosecretory material proximal to the level of transection and to a loss of hormone distally [4], providing that the site of hormone synthesis was in the hypothalamic nuclei and that the secretory products were transported by axonal flow to the posterior pituitary gland where they are stored and released. Later studies were directed to understanding axonal flow and neurosecretion and the whole concept of packaging of hormones into neurosecretory granules was developed by studies of the neurohypophysis. The structure of oxytocin (OT) was determined by Du Vigneaud and his colleagues [5] as Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH₂ and shortly thereafter the hormone OT was synthesized. This was the first success of synthesizing a biologically active peptide in the test tube and the Nobel Prize was awarded for his work in 1955. Acher and colleagues demonstrated that the Van Dyke protein, a complex isolated from fresh-frozen bovine pituitary glands, could be separated into free peptide hormones and a carrier protein which he termed neurophysin [6]. A series of studies using infusion of ³⁵S-labeled cysteine into the third ventricle of dogs, followed by the isolation of radioactive vasopressin and neurophysin from hypothalamic and posterior pituitary tissue, led to the conclusion that the neurophysins are part of the prohormones for OT and vasopressin; a novel concept of protein synthesis originally suggested for neurohypophysial hormones by Sachs and Takabatake [7] prior to the identification of the prohormone for insulin. Meanwhile, studies on the mechanism controlling the release of OT was advanced by an electrophysiological technique. When action potentials are recorded extra-
OTA Gene

OT-Precursor

OT-Related Peptides

Fig. 1. Schematic presentation of the gene and biosynthesis of rat OT. SP, signal peptide; NP-OT, oxytocin associated neurophysin.

cellularly from the neurons in the supraoptic or paraventricular nucleus, if a neuron exhibits the characteristic burst firing pattern at the time of milk ejection, the cell can be identified as an oxytocinergic neuron [8]. Using this technique, subsequent electrophysiological studies established a precise correlation between the electrical activity of the OT neurons and hormone secretion to a physiological stimulus, providing the concept of excitation-secretion coupling.

More recently, OT and vasopressin genes have been isolated and their nucleotide sequences have been determined for the cow [9], rat [10], and human [11]. The OT gene has essentially the same structural organization as the gene for vasopressin, being divided into 3 exons by 2 small intervening sequences. Immunohistochemical studies revealed oxytocinergic neurons whose axons project to various areas in the brain other than hypothalamo-neurohypophyseal OT neurons [12], indicating other roles of OT in the central nervous system in addition to its function as a hormone. Specifically, OT has recently been implicated in the facilitatation of electrical activity of OT neurons at milk ejection reflex [13], maternal behavior [14], and sexual behavior. OT acts on the target cell via specific membrane receptor as with other peptide hormones and human OT receptor gene has also been determined using electrophysiological method with the frog oocyte as an expression system [15]. The OT receptor is a 389-amino acid polypeptide with seven transmembrane domains which is typical of G protein-coupled receptors. Besides the hypothalamus, OT mRNA and OT itself were found to be synthesized in the luteal cells in the ruminant corpus luteum [16] and in the pregnant rat uterus [17]. OT released from the corpus luteum functions as a classical hormone but that synthesized in the uterus seems to act within the uterus as a paracrine substance. The following minireview covers the works on OT under the three categories; OT as a neurohormone, as a neuroregulator and as a paracrine substance.
OT AS A NEUROHORMONE

OT released from the posterior pituitary gland reaches its target tissue via the general circulation. OT release is activated during parturition, by pups sucking stimulus, various kind of stress and raised plasma osmolality [18]. Since the physiological significance of the OT released in response to stress or high osmolality is not clear, OT secretion at parturition and nursing will be treated in this review.

1. *OT release at parturition*

Although OT was originally discovered as a substance with potent uterotonic activity and has been widely used in clinical obstetrics to facilitate parturition, the physiological role of OT in parturition is still relatively unclear. Increased release of OT from the neurohypophysis has not been observed before the onset of parturition in many species including the rat [19] and human [20]. The pregnant uterus is in a state of quiescence until the beginning of regular and rhythmic contractions of the uterus (the onset of parturition) which occurs on day 22 or 23 of pregnancy in the rat. The mean blood OT concentration was significantly raised 0–0.5 h after expulsion of the first fetus when compared with that 0–0.5 h before the expulsion. Blood levels of OT do not rise before but after parturition starts [19]. During the expulsive phase, in addition to an increase in basal OT levels, a slight but significant further increase in blood OT concentrations occurred [21]. To further examine the relationship between the rise in blood OT during parturition and the Ferguson reflex which brings about OT release by mechanical distension of the uterine cervix [22], OT release was examined in rats whose afferent pathway of the reflex (the pelvic nerve) was cut before they became pregnant. The pelvic neurectomized rats exhibited severe dystocia [23]. OT increase during parturition was lower but still present in pelvic neurectomized rats. These results indicate that the Ferguson reflex may be partially responsible for increased OT release during parturition, but other factor(s) may also be involved. Since replacement of OT in these rats failed to ameliorate the dystocia, the principal cause of dystocia in pelvic neurectomized rats is not the diminished rise in blood OT but an interruption of the reflex muscular contraction (fetus-expulsion reflex) itself which plays a critical role in removing the fetus from upper vaginal cavity against the resistance of the pelvic outlet [23]. It is not clear what causes the increase of OT during parturition except for the Ferguson reflex, but recently an interesting hypothesis has been proposed. They reported that there was changes in endogenous opioid input to the OT neurons around the time of parturition. The reduction of inhibitory influence of opioid control over OT neuronal activity may be involved in initiation and regulation of OT secretion at parturition [24].

In pregnant rats injected with anti-OT serum, the onset of labor was not delayed but the process of parturition was prolonged [25]. However, this same antiserum completely blocked the milk ejections, inducing no development of pups
despite the mother rats showing normal maternal behavior. On the other hand, an OT antagonist such as (β-mercapto-β, β-cyclopentamethylene propionic acid-D-Trp²-Phe³-Ile⁶-Arg⁷) OT has been reported to disrupt the process of labor in the rat [26] and inhibits the preterm labor contraction in humans [27]. Furthermore, OT receptors in the pregnant uterus dramatically increase in number just before and at the time of parturition in several species, including rats and human [28, 29]. The affinity of receptors for OT was the same throughout pregnancy and there did not appear to be any significant degradation of OT by the myometrial membrane. OT receptor concentrations in human myometria were low in the non-pregnant uterus, started to rise at 13–17 weeks of gestation and gradually increased towards term. The number of OT receptors per cell was more than 150 times greater in labor than in non-pregnant myometria. This increase of OT receptor is assumed to be caused by facilitated synthesis of OT receptor molecules caused by estrogen whose secretion is raised at the end of pregnancy. This increase in OT receptors brings about very high sensitivity of the uterus to OT and may be responsible for the initiation of labor without an apparent rise in the blood OT level. Recently, Lefebvre et al. [17] reported that OT mRNA and peptide were expressed in the rat uterus; uterine OT mRNA increases more than 150-fold at term and may play a role as a local mediator in the process of parturition. Uterine OT is discussed further below.

2. OT release at milk ejection

Compared with parturition, the role of OT in milk ejection is well documented [19, 20]. Survival of all newborn mammals is dependent upon an adequate supply of milk secreted from the mammary glands of the mother. To meet the great demand for milk, mammary tissue grows rapidly and acquires the ability to secrete milk through pregnancy and early lactation. Laboratory rats nurse their young for a total of 12 to 18 h a day, with frequent periods of nursing each lasting 20 to 30 min [30]. The synthesized milk is stored in the alveolar lumen and the duct system of the mammary gland. The mammals can be broadly divided into two groups: those species in which the duct system has become modified to provide a storage space, including the ruminants and the primates, and those species without obvious modification to increase storage, including the rat, rabbit, pig, and cat. The stored milk is expelled by the action of OT by contracting the myoepithelium which invests the alveoli and small ducts of the mammary gland. OT is released in response to the somatosensory stimuli applied to the nipples by pups. OT levels in plasma increase

Fig. 2. Pulsatile release of OT during suckling. Intrauterine pressure was recorded through a cannula inserted into one teat duct of mammary gland (upper panel) and plasma concentrations of OT was determined by radioimmunoassay (lower panel) in a urethane-anesthetized lactating rat. Photographs were taken at the time when the milk-ejection reflex was (right) or was not occurring (left). At milk ejection, the pups showed typical stretching reaction (from Higuchi and Negoro [84] with permission).
Fig. 2.
abruptly and uniformly by about 50 pg/ml and decline rapidly with a half-life of 1.5 min only at the time of milk ejection [21] (Fig. 2). This type of intermittent bolus release of about 0.5 to 1 mIU of OT during suckling has physiological significance: this hormonal message produces maximally efficient contractions of mammary myoepithelial cells and subsequent milk let-down. As the response of the mammary gland to OT is nonlinear, doses above 1 mU do not produce proportionately larger contractions, while doses below 0.2 mU have little effect. If a dose of 0.5 to 1 mU is given not as a bolus, but as an injection over 5 to 10 s, the contraction response of the myoepithelium is considerably reduced [31]. Moreover, this hormone release is achieved with a minimal number of action potentials; much more OT is released per action potential when the action potentials occur as a brief and high frequency train, than when they occur as a longer train at a lower frequency [32]. Neural impulses generated by suckling are transmitted to the central nervous system, where they are integrated to result in OT release from the posterior pituitary gland; this mechanism is typical of neuroendocrine reflexes.

The intermittent pattern of OT release during suckling may provide a good experimental model for analyzing the mechanism for synchronized activation of the electrical activity of a specific group of neurons which results in pulsatile release of many kinds of hormones such as ACTH and luteinizing hormone (LH) [33, 34]. Pulsatile release of LH-releasing hormone (LH-RH) is critical for maintaining responsiveness of the target tissues to the hormones by avoiding down-regulation [34]. LH-RH synthesized in the arcuate nucleus in the hypothalamus is released for short periods once every 30 min resulting in pulsatile release of LH. If LH-RH is replenished continuously in the monkeys whose LH levels are very low due to a lesion of the arcuate nucleus, serum LH levels rise at the commencement of the LH-RH perfusion but soon decline. However, if LH-RH is administered in a pulsatile fashion which mimics the normal secretory pattern of LH-RH, LH release can be maintained [34].

3. Neuronal pathway controlling milk ejection

The suckling stimulus applied to the nipples by the pups excites neurons in a wide area of the dorsal horn of the spinal cord [35]. Most neurons responding to stretching nipples showed considerable sensory convergence in that they responded to other stimuli such as stroking the skin overlying the mammary gland from several adjacent nipples. Since lesions of the lateral funiculi blocked the milk-ejection reflex whereas lesions of the dorsal and ventral funiculi were ineffective, the most likely route for the suckling stimulus is via the spinocervical tract [36]. The spinocervical tract ascends ipsilaterally within the lateral funiculus, relaying within the lateral cervical nucleus in the cervical segment of the spinal cord [37]. From the mesencephalon, the afferent pathway runs through the central area of the mid-hypothalamus to the supraoptic and paraventricular nuclei [38]. During the nursing period, basal OT level in the blood are not different before and during suckling, unless the milk-ejection reflex occurs. The occurrence of the milk-ejection
reflex is detected by observing stretching behavior of the pups which consists of a synchronous response from all the pups in which they pull strongly against the nipple with their legs outstretched and their backs arched [30] (Fig. 2) or the increase of the intramammary pressure in anesthetized animals through a cannula inserted into a teat duct. In the rat, OT-producing cells are distributed mainly in four discrete neuron groups, the bilateral paraventricular and supraoptic nuclei. Electrical activity of the magnocellular neurons projecting to the neurohypophysis can be monitored by extracellular recording of their action potentials [8]. Electrical recording from the OT neurons reveal that, preceding each milk ejection, probably all the OT neurons show a brief burst of firing: a 20- to 40-fold acceleration for 2 to 4s. Apart from these intermittent high-frequency bursts of firing, the OT cells are completely refractory to the suckling stimulus which is continuously applied by pups.

4. **Internuclear mechanism for synchronized burst firing of OT neurons**

The neuroanatomical basis for the synchrony between the OT neurons has received considerable attention. Since 10 or so pups suck randomly at the nipple, the afferent stimuli must be transmitted to the spinal cord randomly or continuously. Thus, there must be a gate or synchronizing mechanism that transfers the continuous stimuli to intermittent form at a location between the spinal cord and OT neurons. One hypothesis is that either the paraventricular or supraoptic nucleus act as an epileptic-like focus for excitation, which is then transmitted to the other nuclei by local pathways. However, simultaneous recordings from pairs of nuclei have shown that there is no evidence that one pair of nuclei acts as a pacemaker for the others [39]. Although there is some evidence for intersupraoptic connectivity [40], this appears not to control the onset of burst firing of OT neurons because electrical stimulation of the supraoptic nucleus does not generate a burst activity in the contralateral supraoptic nucleus. Thus, the trigger synchronizing burst onset must involve a common extrinsic input (pacemaker) along the afferent pathway. Recently, Takano et al. [38] discovered that destruction of the dorsomedial hypothalamic nucleus, the region just lateral to that nucleus and the posterior hypothalamus completely block milk ejections. They also found a new type of neuron in this area. These neurons send their axons to the supraoptic nucleus but not to the posterior pituitary gland. They display a brief high-frequency burst of spikes before each milk ejection in the same manner as OT neurons but differ from OT neurons in that their basal activity is also facilitated by sucking stimuli, which is in sharp contrast to OT neurons as mentioned above. These neurons may be a likely candidate for the synchronizer that changes the continuous stimuli from the nipple to intermittent stimuli.

5. **Intranuclear mechanism for synchronized burst firing of OT neurons**

Compared with the relatively poor evidence for internuclear connectivity, mechanisms for the synchronization of OT neurons within the individual magno-
cellular nuclei have received much more support [31]. In the supraoptic nuclei of non-lactating rats, glial cells have processes which separate neurosecretory cells from each other, and less than 10% of these cells are involved in direct soma-soma or soma-dendritic contacts. Interestingly, lactating rats show a reversible dramatic structural reorganization of the supraoptic nuclei. From late pregnancy, the glial cell processes begin to retract, leaving more OT cells in direct contact with other cells. In lactating rats, more than 30% of the cells were involved in direct cell-to-cell contacts [41], which may provide a mechanism for nonsynaptic mutual excitation either by field effects or by changes in extracellular potassium concentrations [42]. There is also some evidence that the number of gap junctions between OT cells is increased in lactating rats, enabling direct electrical coupling between OT neurons [43]. Moreover, there is evidence that the double synapses, which make synaptic contacts with each of two adjacent supraoptic neurons, is greatly increased in the lactating rat [41]. The rat hypothalamo-neurohypophysial system was found to continue to express high levels of polysialic acid neural cell adhesion molecule (PSA-N-CAM) immunoreactivity in adulthood, providing this system with the capacity of structural reorganization during lactation [44]. These morphological changes may provide a coupling of OT cells with each other during suckling in lactating rats.

6. **OT release from corpus luteum**

In ruminants such as sheep, goats, and cattle, the corpus luteum has been established as another source of OT secretion. OT has a number of possible functions during the ruminant estrous cycle including a role in luteolysis, regulation of reproductive tract muscular activity at estrus, and modulation of ovarian steroidogenesis. It has long been known that OT administration results in a shortening of the estrous cycle. The finding that immunization of sheep against OT resulted in a delay in luteal regression [45] stimulated investigation of the significance of OT in controlling the process of luteal regression. Active immunization of sheep against OT prolonged the luteal phase of the estrous cycle by 3.7 d and resulted in a 10-fold increase in circulating OT concentrations. Radioimmunoassay data reveal that OT levels in the blood were highest during the luteal phase of the cycle in non-pregnant ewes, and varied in parallel with progesterone during the estrous cycle and after ovariectomy [46]. Furthermore, the discovery of large amounts of OT in the corpus luteum and high OT levels in ovarian venous plasma led to the concept that the origin of the circulating OT was not the posterior pituitary gland but the large luteal cells of the ovarian corpus luteum. At the end of the luteal phase of the cycle when uterine OT receptor levels are high, OT will release prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) from the uterus and PGF$_{2\alpha}$ will in turn release OT from corpora lutea to form a positive feedback loop, resulting in regression of the corpus luteum due to the strong luteolytic action of PGF$_{2\alpha}$. In all animals in which the function of the corpora lutea is prolonged beyond the normal time of luteal regression (i.e. beyond 15 d), the concentrations of OT in the corpora lutea

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drops rapidly at the time when it would be expected to decline in non-pregnant animals. This is true whether luteal function is prolonged by pregnancy, hysterectomy or by immunization against PGF$_{2\alpha}$ or OT and presumably reflects the operation of a mechanism by which OT secretion is limited to a period of 15 d [47]. In pregnancy, protection against the effects of OT is ensured by a number of mechanisms, including the cessation of luteal OT secretion. This is the mechanism for maternal recognition of pregnancy.

**OT AS A NEUROREGULATOR**

In addition to magnocellular OT neurons whose axons terminate in the posterior pituitary gland, there are other OT axons projecting to many areas in the central nervous system such as the amygdala, dorsal vagal complex in the medulla, the organum vasculosum of the lamina terminalis, the bed nucleus of the stria terminalis, and the olfactory bulb [12]. These OT neurons were suggested to be involved in some nervous functions such as autonomic nervous function, maternal as well as sexual behavior. Recently, Insel proposed that OT is a neuropeptide for affiliation [48].

1. **OT involved in facilitation of milk-ejection reflex**

   Injection of OT, in amounts of 1 ng or less, into the lateral or third ventricle of the rat evokes a striking facilitation of the milk-ejection reflex [13]. Within a few minutes, the frequency of the intermittent bursts in the OT cells increases and the number of action potentials in each burst also increases. Conversely, intracerebroventricular administration of a selective OT antagonist during suckling temporarily interrupts the intermittent milk-ejection responses and OT-induced facilitation of the reflex. Similar facilitation of milk ejection can be induced by antidromic activation of hypothalamo-neurohypophysial neurons [49]. Stimulation of the neurohypophysis at 50–300 pulses/s for 0.5–6 s (current 1 mA) evoked additional bursts with amplitudes higher than those of spontaneously occurring bursts which recur at regular intervals. Thus, endogenous OT is released locally within the paraventricular and supraoptic nuclei probably from OT immunoreactive terminals which have synaptic contact with OT neurons [50], and potentiates its own local release [51]. Exogenous OT stimulates firing of the OT neurons in slice preparations [52] and the release of OT from magnocellular nuclei in vitro [51]. Exogenously added OT stimulated the release of endogenous OT from the isolated paraventricular or supraoptic nuclei without effect on vasopressin release. The effect was OT-specific and Ca$^{2+}$-dependent. These accumulated data strongly suggest that interaction with sucking stimuli of OT release from the axon terminals of recurrent collaterals are responsible for the induction of milk ejection-related bursts of OT neurons. On the other hand, Ingram et al. proposed that the bed nucleus of the stria terminalis (BNST) is the site of OT action in facilitating the milk-ejection reflex, following the demonstration that neurons of the BNST of
lactating rats are responsive to OT in vitro [53] and to electrical stimulation of the paraventricular hypothalamus [54]. Furthermore, they reported that beaded axons of the OT-immunoreactive perikarya running from the anterior parvoceellar paraventricular nucleus, entered to the BNST [54]. Thus, although this region is not crucial for burst generation, it exerts a facilitatory effect, perhaps acting through interaction with a coordinating pacemaker.

2. OT facilitates maternal behavior

The degree of development of the young at birth has important influence on the varying pattern of maternal care among mammals. Rodents, especially the rat, provide good examples of maternal care in species with altricial young. Nulliparous female rats display little interest in infants, and when presented with foster young they will avoid or cannibalize them. Most rodent young are essentially immobile and incapable of temperature regulation at birth. Sheep, goats, and other ungulates provide good examples of maternal care species that give birth to precocial young, which can follow their mother soon after birth. Pedersen and Prange [55] were the first to provide evidence for OT's role in maternal behavior. Virgin female rats were ovariecotomized, treated with estrogen and transferred to observation cages 45 to 46 h later. OT administered by intracerebroventricular but not intravenous injection, induced full maternal behavior (retrieving, nest building, crouching, and licking) within 2 h after foster pup presentation. Data from control subjects indicated that estrogen alone or OT alone was ineffective in stimulating this high level of maternal responsiveness. OT in addition to sex steroid hormone treatment brings about a dramatic shift in motivation from a lack of interest to full maternal behavior. Anti-OT antiserum, administered intracerebroventriculally, disrupts the short-latency maternal behavior induced by long-term estrogen treatment superimposed on progesterone withdrawal [56]. Either anti-OT antiserum or an analog antagonist of OT, when administered intracerebroventricularly, disrupted the short latency maternal behavior induced in rats that were hysterectomized and ovariecotomized on day 16 of pregnancy and treated with estradiol [57]. Since intracerebroventricular injections of an OT antagonist do not disrupt maternal behavior in postpartum lactating rats if the injections are performed after maternal behavior has become established [57], central oxytocinergic systems are needed to promote the immediate onset of maternal behavior at parturition but once the behavior is established this neurochemical influence is no longer necessary. The OT involved in the maternal behavior may come from the paraventricular nucleus, since lesion of the paraventricular nucleus on day 15 of gestation has been found to disrupt the onset of maternal behavior at parturition in rats. The same lesion on day 3 postpartum, after maternal behavior had been established, had no effect on the behavior [58]. The females lesioned during late pregnancy approached and explored pups quickly, but they failed to manifest all the higher order aspects of maternal care, including nest-building, licking, and crouching [58].

Recent work on the role of OT in maternal behavior in sheep is more
advanced. In this species, virgin females do not have maternal interest and the vaginal and cervical stimulation by pups at parturition is critically involved in stimulating the onset of maternal behavior [59]. The mechanism by which this stimulation to the vagina and cervix stimulate maternal behavior operates through the activation of central oxytocinergic systems. This is based on the following experimental evidence. In multiparous non-pregnant ewes primed with sex steroid hormones, intracerebrospinal but not peripheral injections of OT activate maternal behavior in a manner similar to that observed after vaginocervical stimulation [60]. OT levels in the cerebrospinal fluid increase during parturition and as a result of vaginocervical stimulation [61]. Peridural anesthesia, which blocks the afferent feedback from the vagina and cervix, disrupts the onset of maternal behavior in parturient ewes and also blocks the rise in cerebrospinal fluid levels of OT. Intracerebrospinal injections of OT reverse the disruption of maternal behavior caused by peridural anesthesia [62]. Thus, in sheep, OT released in the central nervous system by vagino-cervical stimulation during parturition, shifts the ewe’s behavior towards newborns from avoidance to exploration and caretaking.

In rats, the evidence shows that anosmia produced by bullectomy or lesions of the lateral olfactory tracts facilitate the maternal reactions of naive virgin females toward test pups [63]. These female rats show sensitization latencies to the onset of maternal behavior of 1 to 2 d rather than 4 to 5 d observed in female rats with an intact olfactory system. It is suggested that the virgin female rat typically finds the odor of novel test pups aversive and that she must overcome this fear in order to show maternal behavior. Similar phenomenon that olfactory inputs play an inhibitory role in the control of maternal responsiveness has also been observed in nonpregnant ewes and hamsters. From these results, it can be hypothesized that OT might act on the olfactory system to modulate maternal behavior. Thus, we have examined the effect of OT on neural activity of the olfactory bulb. The firing rate of the mitral cell which is the main output of olfactory signals through lateral olfactory tract, was identified by antidromic activation of the olfactory tract and was found to be suppressed by electrical stimulation of the paraventricular nucleus [64]. The same stimulation facilitates granule cells whose activity modulates the activity of the mitral cell through dendrodendritic reciprocal synapses [64]. These responses were mimicked by intracerebroventricular administration of OT or by iontophoretic application of OT to the olfactory bulb and blocked by OT antagonist. Moreover, if an OT antagonist is injected into the olfactory bulb through previously implanted cannula immediately after the expulsion of the first pup, the latency to showing retrieving was significantly prolonged compared with the control rats which received saline infusion [64]. This and previous evidence suggest that a fundamental change in the influence of olfactory input must occur at about the time of parturition to allow maternal behavior to occur. This change appears to include a modification in the valence of newborn-related odors from negative to positive.
Fig. 3. Ratemeter records of electrical activities of mitral (A, C) and granule (B, D) cells in response to electrical stimulation of the paraventricular nucleus (A, B) or to intracerebroventricular infusion of 200 ng of OT (C, D). The effect of paraventricular stimulation was blocked by intrabulbar infusion of OT antagonist (MTOV, 10 pmol) (Yu et al., unpublished).

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3. **OT facilitates sexual behavior**

Many studies have indicated that OT may have an important role within the central nervous system to regulate either the motivation for or the performance of sexual behavior. With moderate doses of estradiol benzoate priming, central administration of OT enhanced female sexual behavior measured as the amount of lordosis, but only if progesterone is available [65]. After prolonged priming with estradiol benzoate, lordosis has been reported to increase following OT injection, even in the absence of progesterone [66]. Lordosis quotients (number of lordosis/number of mounts by male), decreased by 75% following OT receptor antagonist administration into the cerebroventricle [67]. In this case, OT antagonist was effective only if it was given at the same time as progesterone, prior to the onset of receptivity, as in the case of inhibition by an OT antagonist of the initiation of, but not the maintenance of, maternal behavior. Further evidence indicating that central OT mediates some aspects of sexual behavior comes from a study reporting that OT immunoreactivity in the medial preoptic area increased following sexual activity in ovariectomized females primed with estradiol and progesterone [68]. There is also some evidence that central OT is implicated in the mediation of male sexual behavior. OT could induce penile erection when injected into the the ventricle or the paraventricular nucleus [69, 70]. As with female sexual behavior, central administration of OT receptor antagonist greatly reduced male sexual interest and performance as measured by declines in frequency of mounts, intromissions, and ejaculations [71].

4. **OT facilitates affiliative behavior**

Affiliation includes several different forms of social behavior which involve bringing conspecifics into close proximity for the formation of a social bond. Recently, Insel and colleagues have undertaken a series of experiments on OT involvement in affiliative behavior [72]. The genus *Microtus* includes several closely related species with an extraordinary range of affiliative patterns. Two of the most dichotomous are *M. ochrogaster*, the prairie vole, and *M. montanus*, the montane vole. These two closely related voles, with nearly identical morphology and from relatively similar habitats, have opposite patterns of social organization. The prairie vole is monogamous and highly parental; adults generally sit side-by-side, and the young show high levels of ultrasonic calls and glucocorticoid responses to social isolation [73]. The montane vole is polygamous, minimally parental, spends little time in contact with conspecifics, and has offspring which show little behavioral or physiological response to social isolation. Autoradiography of the OT receptors using an 125I-OT antagonist revealed heavy binding of the ligand in the BNST, lateral amygdala, cingulate cortex, and midline thalamus in the prairie vole. In the montane vole, little binding was seen in any of these areas. These differences in receptor distribution appear to be associated with species-specific patterns of social organization because most of the prairie-montane differences can be replicated with two other microtine rodents, the meadow vole and the pine vole, which are
similarly dichotomous for measures of affiliation [72]. Prairie and montane voles show few species differences in the distribution of vasopressin, benzodiazepine, and opioid receptors although each of these systems has been implicated in social behavior. There is one natural circumstance when the montane vole becomes parental: after parturition, the time the mother spends with the pups increases to almost the level observed in the prairie vole. At that time $^{125}$I-OT antagonist binding increases in the lateral amygdala in the montane vole to the level observed in the prairie vole. The lateral amygdala has been implicated in the formation of affective associations and the mediation of social affects.

OT AS A PARACRINE SUBSTANCE

As mentioned in the section describing the hormonal role of OT, the physiological significance of OT in parturition, especially in the initiation of delivery, is still controversial. OT levels in blood do not increase before the onset of labor [19] and anti-OT sera do not delay the onset of labor [24], but OT antagonists greatly affect the process of parturition [26, 27]. In 1992, OT was found to exist in the pregnant uterus and its expression is enhanced around the time of parturition [17]. Thus, it may not be the OT secreted from the posterior pituitary gland but the OT synthesized in the uterus that triggers the onset of parturition in the rat. The OT synthesized in the uterus may act at a location inaccessible to a circulating antibody but accessible to anti-OT analog which has a much smaller molecular weight. Similar expression of OT mRNA has been reported in human uterus at pregnancy [74, 75].

![Graph](Image)

Fig. 4. OT mRNA levels in the uterus of non-pregnant (Day 0) and pregnant rat of day 3 to 22 of pregnancy measured by dot hybridization analysis using 600-mer DNA comprising the 3rd exon as well as parts of the 2nd intron and 3’ non-coding region as a probe. PSL is a unit of density measured by the bioimage analyzer (BAS 2000, Fuji Photo Film Co., Ltd.) (from Higuchi et al. [76]; reproduced by permission of the Journal of Endocrinology Ltd.).

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1. Factors stimulating OT gene expression in the uterus

We have started to elucidate the factors controlling OT expression in the uterus, which may lead to a clearer understanding of the onset of parturition. As the most likely candidates for the controlling factors, the effect of ovarian sex steroid hormones on the expression of OT in the rat uterus was examined and found to be ineffective [76]. The rat OT promoter as well as human OT promoter contains the imperfect palindrome GGTGACCTTGACC that confers estrogen responsiveness in different heterologous expression systems [77] as in the human OT gene [78]. However, the ovarian steroid hormone does not seem to be responsible for the induction of OT mRNA in the hypothalamic supraoptic and paraventricular nuclei during gestation [79]. Burbach et al. [77] explained the result that despite the existence of an estrogen responsive element in the 5' flanking region in the rat OT gene, since the hypothalamic magnocellular neurons do not have estrogen receptors, the potential to respond to the steroid hormone is not utilized. In fact, recently Zingg et al. [80] reported that administration of estradiol led to a very strong increase in the uterine accumulation of OT mRNA in the immature female rat. In the uterine cells which have estrogen receptors, the estrogen responsive element works as real promoter in vivo. In this context, our result that the ovarian steroid hormone could not induce OT mRNA in the uterus of the ovariectomized rat is somewhat surprising. It is difficult to explain the discrepancy between the results of Zingg et al. [80] and ours. Although the dosage of steroid hormones used were similar, the duration of administration was longer in our experiment. OT might have been expressed during the earlier 2 or 3 d of estrogen treatment followed by a decline. A probably more significant difference may be that Zingg et al. used immature rats while we used mature ovariectomized rats. The effect of estrogen on uterine OT induction might be mediated by some factor of ovarian origin. OT production in the uterus must have been subtly regulated; it was reported in ovarian OT synthesis in the sheep that estradiol-17β might switch from having a stimulatory to an inhibitory action on OT synthesis shortly before ovulation [81].

In the medial preoptic area and anterior hypothalamus, the existence of neurons which are responsive to estrogen to induce OT has been reported [82]. This is in sharp contrast to the inability of estrogen to affect OT mRNA levels in the supraoptic and paraventricular nuclei. OT in these areas may have behavioral consequences such as female sexual as well as maternal behavior [83]. Detailed comparison of these neurons which both produce OT but are different in the responsiveness to estrogen is interesting in terms of steroid control of uterine OT in the pregnant uterus.

In contrast to the steroid hormones, the presence of fetus in the uterus seems to be essential for the large increase of OT mRNA at the late period of pregnancy. The gravid horn of the uterus had 3.6 times as much OT mRNA than the non-gravid horn on the 21st day of pregnancy in hemiovariectomized rats with one oviduct ligated [76]. Zingg et al. [80] also suggested that additional factors
contributed to the very high levels of OT mRNA found in the uterus at term, because the levels of OT mRNA induced by steroid hormone treatment were still much lower than those found in the uterus at term. Theoretically, it is natural to think that the stimulus triggering parturition comes from the well-grown fetus which is ready for extrauterine subsistence. Uterine OT is a possible mediator of the stimulus to initiate parturition. Present results indicate the importance of the conceptus or mechanical expansion of the uterus due to the conceptus to stimulate OT mRNA, expression. Further experiments are needed to identify the possible factors that brings about OT synthesized in the uterus during late pregnancy in the rat. The concentration of OT receptor also increases dramatically just before the onset of delivery [28]. This increased OT receptor is thought to be an important factor which initiates parturition, despite unchanged blood OT concentrations just before the onset of delivery [19]. The OT receptor might function as a signal mediator which responds not only to OT provided from the general circulation but also to OT from the endometrium. According to the in situ hybridization histochemistry of OT mRNA and immunohistochemistry of OT peptide, OT is present in the endometrium of the pregnant uterus [17]. It may give us an important clue for clearer understanding of the mechanism for the onset of parturition, and how the OT produced in the endometrium makes contact with OT receptors in the myometrium during late pregnancy.

CONCLUSION

Research on OT has produced new concepts which have great impact in other research areas, such as neuroendocrinology, the prohormone at the process of synthesis of peptide hormones, artificial synthesis of peptides. The role of OT was originally confined to parturition and milk ejection as a neurohormone released from the posterior pituitary gland, but now extends to a hormone secreted from the corpus luteum to regulate corpus luteum function, and even to a neuroregulator in controlling behavior and affiliation. Furthermore, OT may act as a paracrine substance in the uterus to locally regulate the onset of parturition. Physiological significance in these newly discovered roles is not completely established and even its role at parturition is not well defined. In addition, the milk-ejection reflex will continue to provide an ideal model for neuroendocrine mechanisms in general. Another new concept will be produced in the process of OT research to elucidate further the physiological roles of OT.

Key words: oxytocin, milk-ejection reflex, parturition, maternal behavior, luteolysis.

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