Possible neural mediation of the central effects of oxytocin on uterine motility

Anissa Benoussaidh, Yves Maurin, and Olivier Rampin
Analyse et Modélisation en Imagerie Biologique, NOPA-UR-Institut National de la Recherche Agronomique 1197, Jouy-en-Josas, France

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UTERINE MOTILITY IS CONTROLLED by sex steroid hormones, by peptidergic hormones such as oxytocin (OT), by some local factors such as prostaglandins and nitric oxide (NO), and by neurotransmitters released by the autonomic nervous system (20). Therefore, the central nervous system controls uterine motility through a variety of pathways. A simple, direct, and well-recognized control is exerted by OT released from the terminals of magnocellular neurons into the bloodstream (40, 44). From there, OT reaches the uterus and interacts with OT receptors to contract uterine smooth muscle fibers (45–47). The uterus also receives an important autonomic innervation. The motor limb of this innervation originates in the dorsal motor nucleus of the vagus (cervical parasympathetic nucleus) (13, 30), in the intermediolateral column of the thoracolumbar spinal cord (sympathetic nucleus), and in the sacral parasympathetic nucleus (50). These motor autonomic nuclei receive information from the periphery as well as from supraspinal structures (9, 35). Among the latter is the paraventricular nucleus, which sends OT fibers to the spinal cord (10, 38, 49).

It is noteworthy that OT affects uterine motility when it is administered intracerebroventricularly both at the thoracolumbar and at the lumbosacral levels of the spinal cord (8).

Preganglionic neurons of the motor autonomic nuclei establish synaptic contacts with postganglionic neurons in a variety of pre- and paravertebral autonomic ganglia, including the paracervical ganglion (33). Ganglionic synapses are nicotinic cholinergic and blocked by hexamethonium.

The parasympathetic cholinergic pathway plays an important role in the control of uterine motility (21, 42, 43). Parasympathetic postganglionic neurons synthesize and release not only acetylcholine (34, 48), whose stimulatory effects on the uterus are blocked by atropine, but also several other neuromediators such as NO (32), the synthesis of which is blocked by Nω-nitro-L-arginine methyl ester (L-NAME). Nitrergic transmission also affects uterine motility (16). Therefore, a series of pharmacological experiments have revealed the peripheral autonomic control of the uterus.

By contrast, data on the activation of motor autonomic nuclei are lacking. As mentioned above, the central nervous system may use one of several strategies to control uterine motility: activation of the neuroendocrine release of OT and/or activation of autonomic pathways.

The first aim of our study was to search for a central supraspinal mechanism controlling uterine motility. We have taken advantage of the observation that exogenous OT stimulates a positive feedback mechanism of OT release by the neurons of the paraventricular nucleus of the hypothalamus (28) to use intracerebroventricular (ICV) application of OT as a means of stimulating paraventricular neurons. The second aim of our study was to search for the pathways recruited by these mechanisms. To address these questions, we recorded intraterine pressure (IUP) changes on ICV administration of OT to female rats at the estrous stage. This stage was selected for two reasons: 1) female rats are preceptive and fertilized during estrus (6), and 2) OT administered intracerebroventricularly exerts its greatest effects on uterine motility at the estrous stage (8).

Arginine vasopressin (AVP) is a neuropeptide (structure of which is close to that of OT) synthesized in the same brain nuclei as OT and identified as a partial OT receptor agonist (for review, see Ref. 5). On the other hand, OT binds the AVP receptor (5). [Thr4,Gly7]OT is a specific agonist of OT receptors, exhibiting a higher affinity for them than OT itself, and
which does not bind the AVP receptor (15). We have tried to assess the role of central OT receptors in uterine motility by comparing the effects of ICV administration of OT or [Thr^4,Gly^7]OT or AVP.

MATERIALS AND METHODS

Animals. Adult female Sprague-Dawley rats weighing 250–300 g were purchased from René Janvier (Le Genest-St-Ise, France). Rats were housed in plastic cages containing wood-chip bedding. They had free access to commercial pelleted rodent chow (Scientific Animal Food & Engineering, Augy, France) and tap water. Cages were placed in an animal facility maintained at 21°C and kept in a 12:12-h light-dark cycle (lights on at 8:00 AM). Experiments were performed in accordance with the European Ecological Community Directive of November 24, 1986 (86/609/EEC) on the use of laboratory animals and with the National Research Council (NRC) publication Guide for Care and Use of Laboratory Animals (copyright 1996, National Academy of Science); the protocol was approved by the Local Ethics Committee (Agreement 04-003). All efforts were made to minimize animal suffering and to reduce the number of animals used. Estrus stage was identified by histological examination of daily vaginal smears according to the description by Long and Evans (26). After vaginal smears had indicated that regular 4-day cycles were present for at least two consecutive cycles, the animals were subjected to the experimental procedure.

Experimental procedures. Rats were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg in sterile water), and their temperature was maintained at 37°C using a homeothermic blanket.

To perform ICV catheterization, the rat head was fixed in a stereotaxic device (David Kopf, Tujunga, CA). Rats were implanted with a cannula directed toward the left lateral ventricle [coordinates: 1 mm anterior to bregma, 1.5 mm lateral to bregma, and 3.5 mm ventral to dura, according to Paxinos and Watson (36)]. The cannula was connected through a polyethylene tubing (PE-10) to a 25-μl Hamilton syringe filled with artificial cerebrospinal fluid (aCSF) (in mM: 148 NaCl, 3 KCl, 1.4 CaCl2, 2H2O, 0.8 MgCl2-6H2O, 0.8 NaH2PO4, and 0.2 Na2HPO4.

The jugular vein was catheterized with a polyethylene tubing (PE-10) filled with NaCl (0.9%) and connected to a Hamilton syringe also filled with NaCl (0.9%). Rats were tracheotomized to facilitate spontaneous breathing.

After an incision of the skin on the flank muscle was made, the left uterine horn was exposed. A small latex balloon (9 mm long, Ball Extrusion, Montmorency, France), attached to a 1.5 mm-diameter catheter, was inserted into the lumen of the uterus through a small incision and pushed caudally close to the cervix. The balloon and the catheter were filled with sterile water and connected to a pressure transducer (Elcomatic 750, Phymep, Paris, France) to monitor the IUP. Pressure of the balloon was increased to record a basal pressure level between 0 and 10 mmHg. Pressure signal was amplified (Bionic Ware, (Axon Instruments, Union City, CA).

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An experiment, carried out on one rat, consisted of ICV administration of 10 μl of aCSF followed by successive administrations of 0.3, 3, 30, and 300 ng of either OT or AVP or [Thr^4,Gly^7]OT suspended in a volume of 10 μl. Each administration (including the initial aCSF injection) lasted for 20 s and was followed by a flush of 10 μl of aCSF. Successive administrations were separated by a period of 15 min. In experiments with atosiban, hexamethonium, atropine, or l-NNAME, a single administration of either one of these compounds was performed before the first administration of aCSF. Table 1 displays the different experimental groups used in the present study.

At the end of the experiments, 10 μl of pontamine sky blue were injected ICV, followed by a flush of 10 μl of aCSF. Rats were then killed with an overdose of pentobarbital sodium (180 mg/kg ip) and perfused with saline through the left cardiac ventricle. The brain was rapidly removed and sectioned in the coronal plane. Only females that displayed a blue labeling of the ventricles (i.e., with a correct implantation of the cannula) were included in the results.

Drug injections. Stock solutions (10 μg/ml) of OT, [Thr^4,Gly^7]OT, and AVP (Sigma, Saint-Quentin Fallavier, France) in aCSF were prepared and stored at −70°C. The final solutions (0.3, 3, 30, and 300 ng in 10 μl) were freshly prepared on the day of the experiment and kept in ice until used. Atosiban (Ferring, Malmö, Sweden) was dissolved in aCSF for ICV injection (300 ng in 10 μl) or in NaCl 0.9% for intravenous injection (40 μg/kg). Hexamethonium, atropine and l-NNAME (all from Sigma) were injected intravenously at doses of 20, 0.5, and 30 mg/kg, respectively.

Data analysis. Uterine activity was characterized by contractions separated by periods of silence. A contraction was defined as a transient IUP rise, the amplitude of which was greater than 1 mmHg, and that corresponded to a clearly identifiable peak on the recording trace. The frequency of IUP rises (number/min), their amplitude (increase above the basal level expressed in mmHg), and their duration (s) were calculated. The effects of OT, [Thr^4,Gly^7]OT, and AVP on IUP rises were assessed, taking the pressure level after the initial injection of aCSF as a control value. The effects of the tested drugs on IUP rises were searched for with the use of one-way ANOVA with repeated measures in case of a normal distribution of the data or otherwise with a χ² test. We performed post hoc analysis using either the Holm-Sidak method following ANOVA or the Dunn’s test following the χ² test.

The effects of increasing doses of OT in the presence of a single dose of either atosiban or hexamethonium or atropine or l-NNAME were expressed as percent changes compared with the IUP measured after aCSF injection. We performed comparisons using one-way ANOVA with repeated measures in case of a normal distribution of the data or using a χ² test otherwise. We then performed comparisons with the control value using either the Holm-Sidak method following ANOVA or the Dunn’s test following χ² test. Statistical significance was at P < 0.05. We used two-way ANOVA to search for possible drug effects.

RESULTS

OT and OT agonists. The effects of ICV OT on IUP rises are illustrated in Fig. 1, which shows that the frequency of uterine contractions increased with increasing doses of OT. This result
administration, the dose-dependent effect of OT on the frequency of IUP rises (Fig. 4, top; P = 0.0012), and did not affect rise duration (Fig. 2, bottom) [F(4,24) = 1.211, P = 0.332]. The OT agonist [Thr⁴,Gly⁷]OT significantly increased the amplitude of IUP rises (\(\chi^2 = 19.130\), df = 4, \(P < 0.001\)) at the doses of 3, 30, and 300 ng (Fig. 2, middle; \(P < 0.05\) for each) but had no effect on their frequency (Fig. 2, top) [F(4,24) = 1.233, \(P = 0.323\)]. The duration of IUP rises was only affected at the dose of 0.3 ng (Fig. 2, bottom, \(\chi^2 = 10.949\), df = 4, \(P = 0.027\)). AVP did not affect either the frequency (Fig. 2, top, \(\chi^2 = 4.971\), df = 4, \(P = 0.290\)), amplitude (Fig. 2, middle) [F(4,24) = 2.836, \(P = 0.079\)], or duration of IUP rises (Fig. 2, bottom, \(\chi^2 = 6.324\), df = 4, \(P = 0.176\)).

Comparisons of the effects of OT, [Thr⁴,Gly⁷]OT, and AVP on IUP rises were performed with two-way ANOVA. The effects of these drugs on the frequency of IUP rises were significantly different [F(2,90) = 17.312, \(P < 0.001\)], with both [Thr⁴,Gly⁷]OT and AVP differing from OT (\(P < 0.001\) in both cases). Also significant was a drug effect on the amplitude of IUP rises [F(2,90) = 13.166, \(P < 0.001\)]. [Thr⁴,Gly⁷]OT differed from OT (\(P < 0.001\)), whereas AVP did not (\(P = 0.247\)). Finally, no drug effect was detected on the duration of IUP rises [F(2,90) = 2.509, \(P = 0.087\)].

**OT antagonist.** When atosiban was injected ICV before OT administration, the dose-dependent effect of OT on the frequency of IUP rises disappeared (Fig. 3, top) [F(4,16) = 2.272, \(P = 0.107\)], the amplitude of IUP rises diminished [F(4,16) = 5.098, \(P = 0.008\)] at the three highest doses (Fig. 3, middle) (\(P = 0.023\), \(P = 0.017\), and \(P = 0.001\), for 3, 30, and 300 ng, respectively), and a moderate decrease of their duration was recorded [F(4,16) = 4.084, \(P = 0.018\)], which reached statistical significance at the highest dose only (Fig. 3, bottom, \(P = 0.006\)). When injected intravenously, atosiban also antagonized the effects of OT on the frequency (Fig. 3, top) [F(4,16) = 1.695, \(P = 0.200\)] and on the amplitude of IUP rises (Fig. 3, middle) [F(4,16) = 1.879, \(P = 0.163\)].

**Antagonists of autonomic efferent pathways.** Hexamethonium (administered intravenously) antagonized the effects of OT on both the frequency (Fig. 4, top, \(\chi^2 = 4.896\), df = 4, \(P = 0.298\)) and the amplitude of IUP rises (Fig. 4, middle, \(\chi^2 = 8.343\), df = 4, \(P = 0.08\)). No effect on the duration of IUP rises was observed (Fig. 4, bottom).

Atropine (administered intravenously) abolished the effects of OT on the frequency of IUP rises (Fig. 4, top) [F(4,16) = 2.335, \(P = 0.100\)] but did not do so on amplitude (Fig. 4, middle) [F(4,16) = 7.856, \(P = 0.001\)]. Indeed, with atropine, 30- and 300-ng doses of OT significantly increased the amplitude of IUP rises (Fig. 4, middle, \(P = 0.0002\) and \(P = 0.0105\), respectively). No effect was observed on the duration of IUP rises.

**t-NAME (administered intravenously) antagonized the effects of OT on the frequency (Fig. 4, top) [F(4,16) = 1.262, \(P = 0.325\)] and on the amplitude of IUP rises (Fig. 4, middle, \(\chi^2 = 1.958\), df = 4, \(P = 0.743\)). t-NAME had no effect on the duration of IUP rises.

**DISCUSSION**

In line with our previous study (8) demonstrating the involvement of a spinal neural control of uterine motility, the aim of the present study was to search for an involvement of supraspinal structures in this control and, if present, to determine the routes through which this control could be exerted. We used ICV OT to activate supraspinal nuclei in anesthetized female rats at the estrus stage. It has been shown that ICV doses of OT in the range of 5–90 ng (≈5–90 pmol) are efficient to induce erections in the male rat (2) and that ICV doses of 400 ng (≈400 pmol) induce maternal behavior in virgin female rats (37) as well as induce their sexual receptivity (7). Similar doses, delivered by the intrathecal route, elicit penile erections in male rats (0.3–300 ng; ≈0.3–300 pmol) (19) and bladder contractions in conscious female rats (30–100 ng; ≈30–100 pmol) (31) and modulate uterine activity in anesthetized female rats (0.3–300 ng; ≈0.3–300 pmol) (8). The doses used in the present study comply with these experimental protocols. We were aware that these doses, although commonly used in all pharmacological experiments performed in the rat, are largely above (3 × 10⁴ times) the endogenous concentrations measured in CSF (41). Concerning other compounds (i.e., the OT agonist [Thr⁴,Gly⁷]OT and the partial agonist AVP), we have used the same doses for the sake of comparison. Because their molecular weights are close to that of OT ([Thr⁴,Gly⁷]OT = 940.1 and AVP = 1084.2), the injected doses are also close in terms of moles.

In this report, we show that, among the three parameters of uterine contractions, OT increased the frequency in the 3- to 300-ng dose range, whereas [Thr⁴,Gly⁷]OT increased the amplitude in the same dose range. [Thr⁴,Gly⁷]OT and OT itself display very similar affinities (Kₐ = 2 nM) for the OT receptor (15). However, although OT also binds AVP receptors (5, 39) with an affinity of ≈60 nM (3, 4), [Thr⁴,Gly⁷]OT either does not (27) or has a very low affinity (≈4 μM) (24). It is difficult to attribute the effects of OT on the frequency of
uterine contractions to the activation of AVP receptors, since AVP itself had no effect on either the frequency or the amplitude of uterine contractions, although the affinity of AVP for the OT receptor is in the nanomolar range (3). Another explanation for the discrepancy between OT and its agonist relies on the possible existence of different OT receptor subtypes. Such a possibility has been suggested (1, 11, 12), although only one type has been found so far (5, 18). Thus no clear-cut explanation for the discrepancy between the effects of

Fig. 2. Effects of OT and its agonists [Thr⁴, Gly⁷]OT and arginine vasopressin, administered icv, on IUP rises in anesthetized female rats. Histograms display the frequency (top, in number of IUP rises/min), amplitude (middle, in mmHg), and duration (bottom, in seconds) of IUP rises after administration of aCSF followed by either OT (0.3–300 ng, black bars), [Thr⁴, Gly⁷]OT (0.3–300 ng, light gray bars), or arginine vasopressin (0.3–300 ng, dark gray bars). Results are expressed as means ± SE of 7 rats/group. *Significantly different from corresponding aCSF value. See text for statistical significance.

Fig. 3. Antagonism of OT-induced increase of IUP rises by atosiban. Histograms display the percent variation of the frequency (top), amplitude (middle), and duration (bottom) of IUP rises in anesthetized female rats. All rats received aCSF followed by OT (0.3–300 ng). One group (black bars) did not receive another treatment. The 2 other groups received, before aCSF, either an icv injection of 300 ng atosiban (light gray bars) or an intravenous injection of 40 μg/kg atosiban (dark gray bars). Results are expressed as percent change with respect to the value measured in the presence of aCSF alone. Note that the results shown for OT alone (black bars) are those of Fig. 2 converted to percent change. *Significantly different from corresponding aCSF value. See text for statistical significance.
OT and its agonist on uterine motility can be put forward. It is noteworthy that this discrepancy has been observed in other experimental situations. OT, administered intrathecally at the lumbosacral spinal cord level, affects both the frequency and the amplitude of uterine contractions, whereas [Thr⁴,Gly⁷]OT only affects their amplitude (8). In male rats, intrathecal injections of OT significantly increase the frequency of erections and moderately affects their amplitude, whereas the reverse is true for [Thr⁴,Gly⁷]OT (19). Finally, although no simple explanation for the different effects of OT and its agonist on uterine motility can be provided, we have to emphasize that this observation seems to be a constant in different models.

The supraspinal control of uterine motility may rely on neuroendocrine and/or neural routes. The antagonism of the effect of ICV OT by intravenous atosiban suggests that at least part of this effect of OT is linked to the activation of peripheral OT receptors. This activation could be due to injected OT leaking into the bloodstream and reaching the uterus. This possibility is, however, unlikely because the leakage of OT into the bloodstream after its central injection can be considered negligible (51). More probable is the synthesis of OT and its release into the bloodstream after ICV injection of OT. Indeed, the application of OT onto hypothalamic brain slices facilitates the release of OT by magnocellular neurons (28).

Besides the neuroendocrine route, a neural control of uterine motility is also triggered by ICV application of OT. Indeed, the cholineric nicotinic receptor antagonist hexamethonium, which blocks all synapses between pre- and postganglionic neurons, inhibited the OT-induced stimulation of IUP rises. This means that ICV OT also exerts its action through the activation of autonomic efferent pathways innervating the uterus. These pathways include sympathetic noradrenergic and parasympathetic cholinergic ones (50), the latter being, in the female rat, the dominant motor innervation of the uterus (21, 22, 42, 43, 48). We therefore checked for a possible recruitment of this pathway by OT ICV using intravenously administered atropine, a cholineric muscarinic antagonist. Atropine blocked the effects of OT on the frequency of uterine contractions, adding support to our hypothesis that ICV OT controls uterine motility through the activation of neural pathways. In the presence of atropine, ICV OT increased the amplitude of uterine contractions, whereas, in its absence, OT had no effect. A possible explanation is that atropine, injected intravenously, blocks the cholineric input on the uterus and thus contractile noradrenergic sympathetic tone can no longer be antagonized (25). Another explanation is that atropine blocks the acetylcholine-induced synthesis of endothelial NO (14), which has a relaxant effect on the uterus. NO is also a classical transmitter of postganglionic parasympathetic fibers innervating the uterus (32), largely accepted as a relaxant neurotransmitter (29). Nevertheless, uterine effects of NO are complex: they have relaxant effects through the increased production of cGMP (16) and have a contractile effect through the production of prostaglandins (16). The blockade of the effects of ICV OT by the NO synthase inhibitor L-NAME suggests that the central administration of OT activates uterine motility at least in part through the activation of prostaglandin synthesis. It should be mentioned that L-NAME, at 30 mg/kg, elicits a strong hypertensive response, which in turn induces a multitude of neurohormonal responses that may affect the uterus.

The existence of an OT-ergic paraventriculospinal pathway projecting to the parasympathetic nucleus has been demonstrated in the female rat (38). In a previous work (8), we have shown that OT, applied at the site of termination of this
pathway, modulates uterine activity. Here, we show that such is also the case when OT is applied at its origin. The most plausible explanation for the present results is that the ICV application of OT activates this paraventriculospinal pathway. A direct demonstration of this hypothesis is still lacking, in particular because of the technical impossibility to selectively recruit the OT-ergic parvocellular neurons at the origin of the paraventriculospinal pathway and also of the difficulty of delivering at the same time OT ICV and OT antagonists intracereally. Nevertheless, this hypothesis is in agreement with the observation that OT excites the paraventricular OT-ergic neurons of the hypothalamus both in vivo (17) and in brain slice preparations (23, 39) and stimulates a positive feedback mechanism of OT release by these neurons (28). If indeed the motility of the uterus is controlled by neuroendocrine and neural mechanisms, their respective roles and potencies remain to be explored in physiological conditions.

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