Presence of Oxytocin Receptors in the Gonadotrophin-Releasing Hormone (GnRH) Neurones in Female Rats: A Possible Direct Action of Oxytocin on GnRH Neurones

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Gonadotrophin-releasing hormone (GnRH) neurones constitute the final output pathway of a neuronal network that controls the preovulatory luteinising hormone (LH) surge and ovulation. Throughout the reproductive cycle, several neurotransmitters stimulate and inhibit the activity of GnRH neurones, including oxytocin. The central administration of oxytocin antiserum abolishes the pro-oestrous LH surge whereas oxytocin stimulates GnRH secretion from hypothalamic explants suggesting an oxytocin central action. Within the GnRH neuronal population in the rat, GnRH cells in the medial preoptic area (MPOA) are activated at the time of the LH surge. Thus, we hypothesised that GnRH neurones in the MPOA may express oxytocin receptors, and that oxytocin neurones in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) may be differentially activated during the oestrous cycle. Oxytocin receptors mRNA was detected in the MPOA using reverse transcription-polymerase chain reaction. In animals in either metoestrus or pro-oestrus, double-label immunofluorescence indicated that approximately 10% of GnRH neurones in the MPOA coexpressed oxytocin receptors and that a few oxytocin fibres are located in the vicinity of these GnRH neurones. However, other neurones positive for the oxytocin receptors were found near GnRH neurones. At both oestrous stages, double-label immunofluorescence revealed that approximately 30% of oxytocin neurones in the SON were Fos-positive whereas oxytocin neurones in the PVN were consistently Fos-negative. Together, these data suggest that oxytocin may directly control neuronal activity in a subpopulation of GnRH neurones. Moreover, both oxytocin neuronal activity and the oxytocin receptor expression on GnRH cells are not influenced by oestrogen.

Key words: oxytocin, GnRH, oxytocin receptors, oestrous cycle, Fos.

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varies during the oestrous cycle in rats with increased plasma concentration and content in the PVN, SON and pituitary during the pro-oestrous (15–18). This increase may be influenced by oestrogen because oxytocin neurons in the PVN and SON express ERβ (19, 20) and plasma oestrogen is high at this stage (4). In addition, oxytocin antibody administered centrally inhibits the pro-oestrous LH surge (21) and oxytocin induces GnRH release from hypothalamic explants on the afternoon of pro-oestrous (22), suggesting a role of oxytocin in GnRH regulation. Oxytocin receptors have been found in several brain regions, including mediolateral the preoptic area (MPOA) containing the GnRH neurones (23), but it is not known whether GnRH neurones themselves express oxytocin receptors. To verify whether oxytocin may act directly on GnRH cells, we studied the presence of oxytocin receptor mRNA and oxytocin receptors on GnRH cells in the MPOA. These studies were performed in female rats during metoestrus and pro-oestrous to analyse a possible effect of oestrogen (4) on the expression of oxytocin receptors. We also analysed whether oxytocin fibres are present in the MPOA and whether oxytocin neurones are differentially activated in the SON and PVN during the oestrous cycle.

**Materials and methods**

**Animals**

Adult Wistar female rats weighing 180–220 g were maintained on a 12:12 light/dark cycle (lights on 06:00 h) and constant temperature (21 ± 1 °C). Food and water were available ad libitum throughout the experiment. Vaginal smears were taken daily and only rats showing at least three consecutive 4-day regular oestrous cycles were used in the experiments. All protocols were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were previously approved of by the Ethics Committee for Experiment on Animals of the Medical School in Ribeirao Preto-USP.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

RT-PCR was used to detect the presence of oxytocin receptor mRNA in the MPOA. Animals on metoestrus and pro-oestrous (n = 4) were killed by decapitation at 15.00 h. Brains were quickly removed, frozen in dry ice and then stored at −70 °C until processing. For dissection of the MPOA, the micropunch technique was employed (24). Briefly, brain sections (1000 μm) containing MPOA (0.2–1.3 mm posterior to bregma), according to Paxinos and Watson’s atlas (25) were cut into a cryostat, thaw mounted on glass slides, and then frozen at −70 °C until MPOA removal. The MPOA was dissected using blunted needles (diameter of 1.0 mm).

Total RNA was extracted with SV Total RNA Isolation System (Promega, Madison, WI, USA), according to manufacturer’s specifications. Integrity and quality of purified RNA were characterised by formaldehyde denaturing agarose gel electrophoresis and measurement of the A260/A280 ratio. For CDNA synthesis, 1 μg of mRNA was reverse-transcribed using 15 U of ThermoScript (Invitrogen, Carlsbad, CA, USA), 4 μl of 5 × cDNA synthesis buffer, 4 μl of deoxynucleotide triphosphates (dNTPs), 0.1 μl of DTT, 40 μl of RNAse out, 10 pmol forward and reverse primers and DEPC-treated water to a final volume of 20 μl. Reaction mixtures were incubated at 50 °C (60 min) and denatured at 85 °C (5 min). PCR was performed using 3 μl of cDNA template from each RT reaction in 50 μl containing: 5 μl of 10 × PCR buffer, 1.5 μl of MgCl₂ (50 mM), 0.2 μl dNTPs, 10 pmol of sense and antisense primers, 5 U of Taq DNA polymerase (Invitrogen). PCR conditions were: denature step for 5 min at 94 °C followed by 33 cycles of 1 min annealing at 60 °C and 2 min extension at 72 °C (26). A 370-bp oxytocin receptor fragment was amplified: forward primer 5′-GATACTGCCCTAAGGAAG-3′ and reverse primer 5′-GATGCCAAACTAAGACACC-3′ (27). Integrity of total RNA was verified by RT-PCR for the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). A 520-bp GAPDH fragment was amplified: forward primer 5′-GTGAAGCTCTGTCAACGGATTT-3′ and reverse primer 5′-CAGACTCTTTGAGTCGCGTGAACAGTAT-3′ (27). Standard PCR conditions were used: an initial denaturation step (5 min at 94 °C) followed by 30 cycles (1 min at 95 °C; 30 s at 55 °C; 30 s at 72 °C). After PCR amplification, samples were separated on a 1.5% agarose gel with an appropriate molecular weight size marker (Invitrogen), stained with ethidium bromide and visualised under ultraviolet light. The contamination of genomic DNA was excluded by performing amplification without reverse-transcription. Following PCR, the product was subcloned using TOPO TA cloning vector systems (Invitrogen) and sequenced.

**Immunocytochemistry**

**Tissue preparation**

Animals on pro-oestrous and metoestrus were anaesthetised at 15.00 h with a mixture of ketamine [ketamine hydrochloride, Agner, São Paulo, Brazil; 100 mg/kg body weight, i.p.] and xylazine (Coopazine, Coopers, São Paulo, Brazil; 14 mg/kg body weight, i.p.). Approximately 30 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.4) was rapidly perfused through the ascending aorta followed by 300 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Brains were removed immediately after perfusion and immersed in 30% sucrose in PBS (48 h at 4 °C), frozen in cooled isopentane, and stored at −70 °C until sectioning. Serial coronal sections (14 μm) through the lateral septal nucleus (LS), medial portion of the medial preoptic nucleus (MPOM), MPOA, PVN and SON were cut according to Paxinos an Watson’s brain atlas (25) on a cryostat at −20 °C and thaw mounted on slides covered with gelatin. Brain sections were rinsed in PBS, and then placed into 0.1 μm glycine in PBS (20 min) to remove excess aldehydes. After washes in PBS, sections were blocked (1 h) in PBS containing 0.1% Triton X-100 and 1% bovine serum albumin (BSA) and then incubated with primary antibodies.

**Double-label immunofluorescence**

GnRH and oxytocin receptor and GnRH and oxytocin. Sections of MPOA (0.2–1.3 mm posterior to bregma) (25) were incubated with rabbit anti-GnRH, 1:5000 (a kind gift of Dr Gerard Tramu, University of Bordeaux Talence, France) and goat anti-oxytocin receptor 1:400 (sc-8103, Santa Cruz Biotechnology, Santa Cruz, CA, USA), or with goat GnRH antibody (1:5000) and mouse monoclonal anti-oxytocin antibody (PS3B) 1:1000 (a kind gift of Dr Harold Gainer, National Institutes of Health, Bethesda, MD, USA). All antibodies were diluted in PBS containing 0.1% Triton X-100 and 1% BSA and the sections were incubated overnight (20 h) in room temperature. Following PBS washes, sections were incubated (1 h) in a cocktail of fluorescent secondary antibodies. To visualise GnRH and oxytocin receptor, a donkey anti-rabbit IgG labelled with Alexa Fluor 594 (Molecular Probes, Eugene, OR, USA) and donkey anti-goat IgG labelled with Alexa Fluor 488 (Molecular Probes) were used. After a PBS rinse (30 min), the sections where the oxytocin receptor staining appear to be nuclear were placed (10 min) in 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI, 300 μM, Molecular Probes). GnRH and oxytocin were visualised with goat anti-rabbit IgG labelled with Alexa Fluor 594 (Molecular Probes) and goat anti-mouse IgG labelled with Alexa Fluor 488 (Molecular Probes).
Oxytocin receptor gene expression in MPOA

A large population of GnRH neurones is located in the MPOA of the rat and these GnRH neurones have been shown to be associated with the preovulatory surge (29). Thus, we first examined oxytocin receptor expression in the MPOA of female rats during metoestrus and pro-oestrus using RT-PCR. As shown in Fig. 1, the oxytocin receptor transcript was present in the MPOA in rats in either metoestrus or pro-oestrus. Verification of the PCR product as: (i) oxytocin receptor-positive or negative or (ii) closely apposed by oxytocin fibres, depending on the respective experiment. Next, sections scored as containing GnRH-oxytocin receptor positive cells or oxytocin fibres in proximity to GnRH neurones using standard light microscopy were re-examined using confocal microscopy (TCS SP2 AOBS, Leica Microsystems, Manheim, Germany). In the Fos/oxytocin studies, one series of tissue sections through the rostro-caudal extent of the SON and PVN (six sections, 180-μm interval between sections) was used per animal (n = 4, pro-oestrus and n = 4, metoestrus). Sections were visualised on the microscope using a x 20 objective. All GnRH neurones within the section were examined and designated as: (i) oxytocin receptor-positive or negative or (ii) closely apposed by oxytocin fibres, depending on the respective experiment. Next, sections scored as containing GnRH-oxytocin receptor positive cells or oxytocin fibres in proximity to GnRH neurones using standard light microscopy were re-examined using confocal microscopy (TCS SP2 AOBS, Leica Microsystems, Manheim, Germany). In the Fos/oxytocin studies, one series of tissue sections through the rostro-caudal extent of the SON and PVN (six sections, 180-μm interval between sections) was used per animal (n = 4, pro-oestrus and n = 4, metoestrus). Quantitative assessment of double-labelled cells was obtained from captured microscopy images using Image J software (NIH, Bethesda, MD, USA). The mean ± SEM per animal and percent of dual labelled cells were calculated. Student’s paired t-test was used to evaluate the number of double-labelled cells per region in the two animal groups. P < 0.05 was considered statistically significant.

Results

Oxytocin receptor expression in GnRH neurones

Double-label immunofluorescence revealed that GnRH neurones (red) expressed oxytocin receptor (green) in the MPOA, more specifically in the region of the organum vasculosum laminae terminalis (OVLT). There was no difference in percentage of oxytocin receptor-positive GnRH neurones between metoestrus and pro-oestrus rats (11 ± 2.7% and 8 ± 1.4%, respectively). Moreover, there were non-GnRH positive cells that express oxytocin receptor, and these were often located in the proximity of GnRH cells (Fig. 2a–i). In the LS (negative control for OTR), the GnRH cells were present, but no oxytocin receptor was detected (Fig. 2a–i). In the SON and MPOM (positive control), a strong oxytocin receptor staining was detected (Fig. 2a–i). This characteristic localisation of protein is in line with that demonstrated by in situ hybridisation histochemistry (ISHH) and autoradiographic studies (23, 28). In both cases, colocalised with GnRH cells and around them, the oxytocin receptors were found not only in the cell membrane but also in the nucleus, as demonstrated by DAPI staining (blue) of the nucleus and confocal microscopic analysis (Fig. 3a–i). Because the GnRH cells are scattered throughout the brain, they were counted and classified as an oxytocin receptor-positive directly from a fluorescence microscope. Some sections containing OVLT, where the oxytocin receptor staining appeared to be nuclear, were randomly chosen in both metoestrus and pro-oestrus, placed in DAPI and, analysed by confocal microscopy to confirm the presence of oxytocin receptor in the nucleus. Therefore, the percentage of GnRH cells expressing oxytocin receptor includes both, membrane and nucleus staining. Oxytocin receptor immunoreactivity was abolished by omission of the primary antibody and by immunoneutralisation of primary antibody with the corresponding immunising peptide (Fig. 3u–i).

Oxytocin fibre near GnRH neurones

Double-label immunofluorescence was used to determine whether oxytocin was present in the vicinity of GnRH neurones in the MPOA.
In addition to some oxytocin fibres (green) present in the MPOA, we found some oxytocin neurones in the MPOA, in the same section where GnRH cells are present. Although, in our study, we cannot conclude that oxytocin fibres are in contact with the GnRH cells, many of them appear juxtaposed to GnRH neurones (red).

Numerous oxytocin neurones (green) colocalised Fos (red) in sections containing the SON. Double-labelled oxytocin/Fos cells were present throughout the entire extent of the SON and were detected in sections from rats in either pro-oestrus (Fig. 5a–d) or metoestrous (Fig. 5e–h). Fos-positive oxytocin neurones, counted bilaterally, were equally distributed between the rostral and caudal areas of the SON with 32 ± 5% and 30 ± 9% of colocalisations observed in metoestrous and pro-oestrus rats, respectively. In rats on metoestrous and pro-oestrus, no Fos staining was present in sections through the PVN (Fig. 5i–l).

**Discussion**

The results of this work demonstrate that a subpopulation of GnRH neurones in the OVLT region express oxytocin receptor in female rats on the afternoon of metoestrus and pro-oestrus. Some oxytocinergic fibres and oxytocin neurones are present in the MPOA. In addition, we observed that oxytocin neurones are chronically activated on the afternoon of pro-oestrus and metoestrous in SON, indicated by expression of Fos. Taken together, these data suggest that the effect of oxytocin on GnRH neurones described in the
Fig. 3. Gonadotrophin-releasing hormone (GnRH)/oxytocin receptor positive neurones (nucleus). Confocal photomicrographs (14 μm thick section) from medial preoptic area (MPOA) of female rats on pro-oestrus (A–D) and metoestrus (E–H) following double-label immunofluorescence for GnRH (red, Alexa Fluor 594; A,E) and oxytocin receptor (green, Alexa Fluor 488; B,F,J). GnRH neurones (arrows) express oxytocin receptor in the nucleus on pro-oestrus (C) and metoestrus (G). Other non-GnRH/oxytocin receptor-positive cells are also detected close to GnRH neurones (arrowheads). (C,G) Composite images showing double-labelling of GnRH, oxytocin receptor and cell nuclei visualised after DNA staining with DAPI (blue). (D,J) Optical sections (1 μm) through the GnRH/oxytocin receptor cell shown in (C) and (G), respectively, confirming the presence of oxytocin receptor nuclear staining in GnRH neurones. (I) Oxytocin receptor immunoreactivity was abolished by immunoneutralisation of primary antibody with the corresponding immunising peptide. Scale bars = 8 μm (A–D), 20 μm (E–J), 3V, Third ventricle.
literature should be directly mediated by the oxytocin receptor. Also, it appears that oestrogen does not regulate neither activation of the oxytocin neurones, nor the oxytocin receptor expression on GnRH cells, because the results were similar in animals on pro-oestrous and metoestrus, when oestrogen levels are high and low, respectively (4).

The localisation of oxytocin receptor in hypothalamic and extrahypothalamic areas is in agreement with ISHH studies (23, 28). We did not find oxytocin receptor protein in the lateral septal nucleus, but its expression was strong in the SON and MPOM. We also detected oxytocin receptor mRNA in the MPOA in both studied groups. However, one unpredicted result was the presence of the receptor not only in the cellular membrane, but also in the nucleus. In both cases, oxytocin receptor immunoreactivity was abolished by omission of the primary antibody and by immunoneutralisation of primary antibody with the corresponding immunising peptide. Oxytocin receptor is a member of the I class of G-protein coupled receptors (GPCRs) (30) and classically, GPCRs are located in the cellular membrane, triggering signal transduction cascades that alter cellular process. However, some GPCRs, such as type 1 receptors for angiotensin (31), receptors for growth hormones (32), leptin receptors (33) and neurokinin receptors (34), have been found in the cell nucleus, questioning the functional relevance of this phenomenon and also whether there are nuclear targets for membrane receptors. One possible explanation could be that a fragment of these receptors is cleaved and transported to the nucleus. The Notch receptors, after ligand binding, undergo a conformational change and enzymes cleave an intracellular domain, which is transferred to the nucleus where it regulates transcription factors (35).

In our study, we did not know whether some oxytocin receptor internalise and migrate to the nucleus after oxytocin binding, or whether the nuclear localisation occurred because of different parameters used in the immunocytochemistry technique, such as fixation. More studies need to be carried out to clarify this point. However, the oxytocin receptor localisation was specific in determined areas, as shown by ISHH and autoradiographic studies (23, 28) suggesting the specificity of the antibody used in this study.

Previous studies have demonstrated the presence of mRNA and protein for several neurotransmitter and neuropeptide receptors in GnRH neurones, including catecholamine receptors, GABA, glutamate, neuropeptide Y, neuropeptide, vasoactive intestinal polypeptide, angiotensin, and serotonin (36–39). Furthermore, treatment with...
specific antagonists to various neurotransmitters and neuropeptides, including oxytocin, alter LH secretion. Oxytocin induces the secretion of GnRH in the afternoon of pro-oestrus, and it occurs when oxytocin is placed in the basal hypothalamus, where the GnRH cell bodies and fibres are located, and not when oxytocin is applied at the level the medial eminence, where the GnRH terminals are located (22). Our study suggests that the effects, or part of the described effects of oxytocin on GnRH neurones may be mediated directly by the oxytocin receptor. In addition to GnRH neurones, other oxytocin receptor-positive cells were identified near GnRH neurones. The phenotype of these non-GnRH-oxytocin receptor positive cells remains to be determined. However, the presence of these cells raises the possibility that the effect of oxytocin on GnRH neurones may be either direct and/or indirect. A study performed in vitro demonstrated that oxytocin elicits GnRH release from pro-oestrous explants, stimulating nitric oxide production via oxytocin receptors, which in turn acts on GnRH cells (40). Either directly or indirectly, the presence of oxytocin receptors in the OVLT suggests that oxytocin might be one more factor important for GnRH regulation. A subpopulation (40%) of all GnRH neurones was activated on the proestrous afternoon (29) and the larger number of activated neurones appears in the OVLT region (29), where we found the colocalisation of oxytocin receptor and GnRH. However, the question remains as to whether the oxytocin receptors present on GnRH cells are important for GnRH regulation during some point of the oestrous cycle.

Because we found oxytocin receptors on GnRH cells in the MPOA, we investigated whether oxytocin fibres were present in that region. In both groups studied, we found a few fibres and also some oxytocin neurones close to GnRH cells. This is not the first time that oxytocin neurones have been found in the MPOA (41). In the present study, the presence of the fibres in the MPOA should suggest a possible site for oxytocin release. However, data from neuronal activity, based on the presence of Fos on oxytocin neurons in the PVN and SON, do not support this idea. We investigated whether the activation of oxytocin neurones varies during the metoestrus and pro-oestrus phases of the oestrous cycle because oestrogen appears to modulate oxytocin neurones in basal and stressful conditions (15–18). The antibody used in this study recognises not only Fos, but also FRA-1 and FRA-2 (fos-related antigens), which are expressed chronically in basal neurone metabolism (42, 43). This study was carried out at 15.00 h to

Fig. 5. A subpopulation of oxytocin neurones express Fos during pro-oestrous and metoestrous. Photomicrographs from the middle portion of the supraoptic nucleus (SON) of rats on pro-oestrus (A–D) and metoestrous (E,F) and the paraventricular nucleus (PVN) of rat on pro-oestrus (G–I), following double-label immunofluorescence for Fos (red, Alexa Fluor 594: A,G) and oxytocin (green, Alexa Fluor 488: B,H). (C,E,I,) Composite images; (D,F) high magnification image of (C) and (E), respectively. Oxytocin/Fos-positives neurones (arrows, Gö) and oxytocin/Fos-negative neurones (arrowheads) are present in the SON whereas Fos staining was not detected in sections through the PVN (G). Scale bars = 50 μm.

investigate the mechanism controlling GnRH secretion and LH surge occurring between 17.00 h and 18.00 h. The time point of 15.00 h is when plasma oestrogen levels are high (4). Thus, temporally, the oxytocin neurones could be activated before the LH surge. The analysis of Fos expression revealed that similar numbers of activated oxytocin neurones were present in SON during both phases, whereas no expression was observed in the PVN. The absence of Fos in the PVN does not mean that oxytocin neurones are completely inactive. It is possible that other immediate-early genes, and not c-fos, may be activated in the basal metabolism of the PVN. Alternatively, the metabolism of oxytocin neurones in the PVN, expressed by Fos, may differ from SON and other brain sites and, for this reason, our methods are not sensitive enough to detect very low levels of Fos activity. It is not the first time that the PVN and SON have demonstrated different regulatory mechanisms. Previous work has shown that ovarian hormone treatment increased oxytocin receptor binding in the SON, but not the PVN (44). Furthermore, the expression of GABA<sub>A</sub> receptor subunit mRNA is up-regulated by oestrogen and progesterone in the SON, but not the PVN (45). SON is composed almost or exclusively of magnocellular cells that project to the posterior pituitary gland. Because oxytocin is released from centrally projecting parvocellular neurones of the PVN that contain much less peptide than do magnocellular neurones, and because magnocellular neurones do not project centrally, it is often assumed that centrally measured oxytocin originates from parvocellular neurones. Retrograde tract tracing from the region of GnRH cell body was used to identify and compare the sources of inputs to these cells in female rats, and revealed only a low incidence of retrograde labelling within the PVN. In that study, there was no retrograde labelling in the SON (46), suggesting that the few oxytocin fibres found in the MPOA probably originate from the parvocellular neurones of the PVN. However, studies have shown that magnocellular neurones release a large amount of peptide from their dendrites that diffuses through the brain to reach the cerebrospinal fluid and areas far from the SON (47). This may explain the disparity between the projection fields of oxytocin neurones and sites where oxytocin receptor are densely expressed. More studies are required to address whether oxytocin reaches the MPOA by diffusion from SON and then regulates GnRH cells there. In the rat, there is a postpartum preovulatory surge of gonadotrophin, which is abolished in animals submitted to caesarean section (48). It is known that parturition provides physiological stimuli that strongly activate oxytocin neurones in the SON. Oxytocin is released not only from their terminals in the posterior pituitary gland into the blood, but also from their dendrites (49, 50). These data suggest that oxytocin released from dendrites may reach the MPOA and be an important factor for this postpartum preovulatory surge of gonadotrophin, and that this may occur via oxytocin receptor expressed in the GnRH cells.

In the present study, the expression of Fos in the oxytocin neurones in the SON was the same at metoestrus and pro-oestrus, suggesting that there is no effect of oestrogen on the oxytocin neurones and no oestrous cycle relationship. Previous studies have shown that short- or long-term oestrogen treatments do not change the immunomorphological appearance of oxytocin in the PVN and SON (41). Alternatively, oestrogen may modulate oxytocin receptors instead of the peptide. In our study, there was no statistical difference in the number of GnRH cells that expressed oxytocin receptors in high and low plasma oestrogen levels. However, studies have shown that oestrogen increases the oxytocin receptor binding affinity in the MPOA in rats (51), suggesting a possible mechanism where oestrogen may regulate the action of oxytocin on GnRH neurones during the oestrous cycle.

In addition to the effects of oxytocin demonstrated in adult rodents, studies have shown that oxytocin also exerts a powerful action on foetal neurones. GABA is one of the most important neurotransmitters regulating GnRH neurones across development (52). In immature neurones, GABA is the primary excitatory neurotransmitter and oxytocin from the mother triggers an excitatory-to-inhibitory switch of GABA actions (53). Another study demonstrated the importance of oxytocin from the mother on GnRH neurones. In explants obtained from both neonatal rats born by caesarean and foetal rats before labour, there were no spontaneous GnRH pulses (54) whereas, in explants obtained 2 h after completed parturition and vaginal delivery, spontaneous GnRH secretory pulses were consistently observed. The administration of oxytocin and oxytocin antagonist suggests a facilitatory effect of endogenous oxytocin on pulsatile GnRH secretion, which was effective perinatally and persisted in postnatal life (54). These results show the importance of oxytocin on mechanisms that regulate GnRH neurones not only during the oestrous cycle, but also during the development.

In conclusion, the presence of oxytocin receptor on GnRH cells in the OVLT area suggests that the effect of oxytocin on those neurones, either during development or during adult life, should act directly, via oxytocin receptors present in GnRH neurones, and/or indirectly through non-GnRH cells in the MPOA that express oxytocin receptor. Fos analysis revealed that a subpopulation of oxytocin neurones, only in the SON, is chronically activated during the oestrous cycle. Because there is no projection from oxytocin neurones present in the SON to OVLT, one possibility is that oxytocin released from SON dendrites reaches the MPOA by diffusion. Our data suggest that oestrogen does not up-regulate the neuronal activity in the oxytocin neurones, nor the percentage of the GnRH neurones expressing the oxytocin receptors. However, oestrogen may regulate the action of oxytocin on GnRH neurones during the oestrous cycle by increasing oxytocin receptor binding affinity in the MPOA in rats. Further investigations should be conducted to clarify the interaction of oxytocin in the multifactor neural network that controls GnRH neurones during the oestrous cycle.

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