J Mol Neurosci (2017) 62:356–362
DOI 10.1007/s12031-017-0943-8

Oxytocin Intranasal Administration Affects Neural Networks Upstream of GNRH Neurons

Mohammad Saied Salehi[1] · **Homayoun Khazali**[1] · **Fariba Mahmoudi**[2] · **Mahyar Janahmadi**[3]

Received: 8 May 2017 / Accepted: 20 June 2017 / Published online: 29 June 2017

Abstract The last decade has witnessed a surge in studies on the clinical applications of intranasal oxytocin as a method of enhancing social interaction. However, the molecular and cellular mechanisms underlying its function are not completely understood. Since oxytocin is involved in the regulation of hypothalamic-pituitary-gonadal axis by affecting the gonadotropin-releasing hormone (GNRH) system, the present study addressed whether intranasal application of oxytocin has a role in affecting GNRH expression in the male rat hypothalamus. In addition, we assessed expression of two excitatory (kisspeptin and neurokinin B) and two inhibitory (dynorphin and RFamide-related peptide-3) neuropeptides upstream of GNRH neurons as a possible route to relay oxytocin information. Here, adult male rats received 20, 40, or 80 μg oxytocin intranasally once a day for 10 consecutive days, and then, the posterior (PH) and anterior hypothalamus (AH) dissected for evaluation of target genes. Using qRT-PCR, we found that oxytocin treatment increased *Gnrh* mRNA levels in both the PH and AH. In addition, oxytocin at its highest dose increased kisspeptin expression in the AH by around 400%, whereas treatments, dose dependently decreased kisspeptin mRNA in the PH. The expression of neurokinin B was increased from the basal levels following the intervention. Furthermore, although intranasal-applied oxytocin decreased hypothalamic RFamide-related peptide-3 mRNA level, the dynorphin mRNA was not affected. These observations are consistent with the hypothesis that applications of intranasal oxytocin can affect the GNRH system.

Keywords Intranasal-applied oxytocin · Gonadotropin-releasing hormone · Kisspeptin · Neurokinin B · RFRP-3

Introduction

Over recent years, considerable effort has focused on understanding the molecular and cellular mechanisms through which intranasal administration of oxytocin enhances social interaction in humans with social anxiety, autism, schizophrenia, and borderline disorder to improve sociability and communication (Veening and Olivier 2013). Since intranasal-applied oxytocin reaches the brain (Neumann et al. 2013), it may influence various pathways in the central nervous system; however, the neural targets ultimately responsible for its biological functions are not well understood.

Gonadotropin-releasing hormone (GNRH) is the hypothalamic decapeptide that stimulates gonadotropin secretion from the pituitary gland. GNRH neurons are considered as a final output of the central nervous system driving fertility in all mammals. Up until now, several lines of evidence have shown that oxytocin is involved in the regulation of hypothalamic-pituitary-gonadal axis by affecting the GNRH system. In this regard, central injection of oxytocin antisera completely abolished the preovulatory luteinizing hormone (LH) surge in the rat (Johnston et al. 1990) and oxytocin, stimulated

✉ Homayoun Khazali
h_khazali@sbu.ac.ir

[1] Department of Physiology, Faculty of Biological Sciences and Technology, Shahid Beheshti University, Tehran, Iran

[2] Faculty of Basic Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

[3] Neuroscience Research Center and Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

GNRH release from the rat hypothalamic explants (Selvage and Johnston 2001). It is worth noting that all abovementioned impacts of oxytocin on GNRH neurons occur in a condition which only 10% of GNRH neurons in the rat medial preoptic area (mPOA) express oxytocin receptor and a few oxytocin fibers are located in the vicinity of these neurons (Caligioni et al. 2007).

Therefore, the effects of oxytocin on GNRH neurons may be mediated either directly by the oxytocin receptor at the level of GNRH cells or indirectly by interneurons that impinge on GNRH neurons as GNRH secretion is controlled by a complex network of inhibitory and excitatory inputs alter GNRH neuronal activity (Herbison 2015). Among these inputs, RFamide peptides along with tachykinins and endogenous opioid peptides are located in the center of attention during the last 15 years.

Kisspeptins are a family of RFamide peptides which are encoded by the *Kiss1* gene. Kisspeptin is the most potent stimulator of GNRH neurons so far discovered (Rønnekleiv and Kelly 2013). Another factor which has recently been introduced as a fundamental player in the central control of reproductive function is neurokinin B (NKB). NKB is a member of tachykinin peptides which are encoded by preprotachykinin B (Rance et al. 2010; Navarro 2012) and interactions between kisspeptin and NKB in the control of GNRH secretion are reported in the rat (Navarro et al. 2011) and sheep (Goodman et al. 2013).

Another element that has been long known as an inhibitor of gonadotropin secretion is dynorphin (DYN) (Schulz et al. 1981; Kinoshita et al. 1982) which belongs to the endogenous opioids peptides family and is considered to mediate the negative feedback effects of progesterone on GNRH secretion (Goodman et al. 2004). It has been reported that DYN presumably exerts its inhibitory effects through kisspeptin neurons (Wakabayashi et al. 2010). Therefore, DYN plays a pivotal role in the regulation of the GNRH system. In contrast to kisspeptin, RFamide-related peptide 3 (RFRP-3) is another member of the RFamide peptides family which hyperpolarizes GNRH neurons and inhibits the decapeptide release (Kriegsfeld et al. 2010; Salehi et al. 2015).

Since oxytocin reaches the brain after intranasal administration, we hypothesized that the GNRH system is affected following intervention as it was reported in previous in vitro experiment (Selvage and Johnston 2001). Accordingly, we evaluated the effects of different levels of oxytocin after its intranasal administration on GNRH expression in the male rat hypothalamus. In addition, since oxytocin receptors are widely distributed in the hypothalamus especially in the nuclei in which kisspeptin, NKB, DYN, and RFRP-3 are expressed (Gimpl and Fahrenholz 2001), we tested whether expression of these GNRH regulators is affected in consequence of the treatment as possible routes to relay oxytocin information.

Material and Methods

Animals

All experiments were conducted in the Laboratory Animal Center and in compliance with the recommendations of the Animal Care Committee of the Shahid Beheshti University, Tehran, Iran. Twenty-four randomly selected adult male Sprague-Dawley rats (200–250 g body weight) were housed under controlled temperature (22 °C), humidity (~40%), and lighting (12:12 h light-dark cycle) with free access to food and water.

Nasal Application

Conscious rats received either oxytocin (1, 2, or 4 μg/μl; rats 2 × 10 μl; AnaSpec, USA, $n = 6$) or vehicle (sterile normal saline, 2 × 10 μl, $n = 6$) as described previously (Lukas and Neumann 2012; Neumann et al. 2013) once a day for 10 consecutive days. Briefly, oxytocin solution was applied bilaterally on the rhinarium which was shown to be highly innervated by free nerve endings (Silverman et al. 1986) using a 10-μl pipette. Applications to each rhinarium lasted about 1 min.

Tissue Collection

One hour after the last administration, the rats were anesthetized, the brains were immediately removed, and the hypothalamus was dissected to the boundaries as the followings: rostral to the anterior border of the optic chiasm, caudal to the posterior border of the mammillary body, dorsal to the ventral border of the thalamus, and 2 mm lateral to the third ventricle. Since kisspeptin neurons localized in two distinct hypothalamic nuclei, the third coronal cut was made in the middle of the optic tract, just rostral to the infundibulum (Salehi et al. 2012). Then, the anterior and posterior hypothalamus were snap-freeze and stored at −80 °C until use.

RNA Extraction, cDNA Synthesis, and Quantitative Polymerase Chain Reaction

Total RNAs were extracted from the anterior and posterior hypothalamus samples using the YTzol Pure RNA buffer (Yekta Tajhiz, Iran). After treatment of RNAs with DNase I (Thermo Scientific, USA), the DNase-treated RNAs were used to synthesize cDNA using Easy cDNA Synthesis kit (Pars Tous, Iran). All procedures were performed according to the manufacturer's instructions.

Triplicate reactions for measurement of *Gnrh*, *Kiss1*, *Nkb, Dyn*, and *Rfrp-3* mRNA levels were carried out on cDNA samples using the gene-specific primers presented in Table 1. Relative expression of *Gnrh*, *Kiss1*, *Nkb*, *Dyn*, *and*

Table 1 Primer sequences (5′–3′) used in quantitative polymerase chain reaction (qPCR)

Gene	Accession number	Sequence	Amplicon (bp)
Gnrh	NM_012767	F-GCCGCTGTTGTTCTGTTGACTG	133
		R-CCTCCTCCTTGCCCATCTCTTG	
Kiss1	NM_181692	F-TGATCTCGCTGGCTTCTTGGC	98
		R-GGGTTCAGGGTTCACCACAGG	
Rfrp-3	NM_023952	F-GAGTCCTGGTCAAGAGCAAC	93
		R-ACTGGCTGGAGGTTTCCTAT	
Nkb	NM_019162	F-GGAAGGATTGCTGAAAGTGCTGAG	130
		R-GGGAGTGTCTGGTTGGCTGTTC	
Dyn	NM_019374	F-CGGCGTCAGTTCAAGGTGGTG	150
		R-AGCAAGCGAAGGAATAAGCAGAGG	
Actin, Beta	NM_031144	F-TCTATCCTGGCCTCACTGTC	122
		R-AACGCAGCTCAGTAACAGTCC	

Rfrp-3 genes was evaluated on both the anterior and posterior hypothalamic samples. The copy number of each target transcript was normalized to the copy number of the beta-actin transcript for each sample.

Twenty-microliter reactions were prepared to contain SYBR Green PCR Master Mix (Ampliqon, Denmark) and using an ABI StepOne Real-Time PCR system (Applied Biosystems, USA). Samples were heated to 95 °C for 10 min before 40 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. At the end of the PCR reaction, each amplicon yielded a single peak and did not show any peak when the template was not included. In addition, random amplified products (5 μl) were subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide which showed a single band of the expected size. Quantitative real-time PCR data were analyzed using the comparative Ct method and relative expression of the target mRNAs over reference values was calculated by the arithmetic formula $2^{-\Delta\Delta CT}$ (Livak and Schmittgen 2001).

Hormone Assay

Serum LH concentration was measured 1 h after the tenth intranasal administration of oxytocin or normal saline using a rLH [I_{125}] RIA kit (Izotop, Hungary) according to the manufacturer's instructions.

Statistical Analysis

Data on the relative expression of target genes were subjected to the test of normality. Comparisons between groups were made by one-way analysis of variance plus the Tukey post-hoc test using SPSS statistical program (version 16). The mean ± SEM are reported in the text and $P < 0.05$ was considered statistically significant.

Results

Gnrh Expression in Response to Different Levels of Intranasal-Applied Oxytocin

Previous reports have shown oxytocin stimulates GNRH secretion in the rat hypothalamic explant. In order to further investigate, effects of intranasal administration of oxytocin were accessed. Here, quantitative reverse transcription-PCR was undertaken in the posterior (PH) and anterior hypothalamus (AH) following ten continuous days of the intervention. Oxytocin treatment increased the *Gnrh* mRNA levels in both the PH and AH; however, only the highest dose of the treatment was effective to induce a significant elevation in the gene expression (Fig. 1).

Effects of Oxytocin Treatments on *Kiss1* and *Nkb* Expression

Next, we investigated the impacts of intranasal-applied oxytocin on two excitatory neuropeptides upstream of GNRH

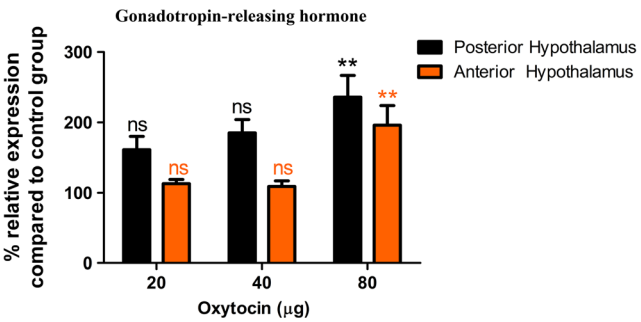

Fig. 1 Relative expression of *Gnrh* mRNA in the rat posterior and anterior hypothalamus after intranasal administration of oxytocin compared to control group ($n = 6$ in each group). Data are represented as mean ± SEM. $^{**}P < 0.01$. *ns* non-significant

neurons as possible messengers to relay oxytocin effects. To address this issue, kisspeptin expression was examined which is known as the main stimulator of GNRH neurons. We found that oxytocin treatment at its highest dose increased the level of *Kiss1* mRNA in the AH by around 400%. *Kiss1* expression in the AH of rats was unaffected by the lower doses of the treatment (Fig. 2a). On the other hand, oxytocin intervention dose dependently decreased *Kiss1* mRNA levels in the PH (Fig. 2b). Neurokinin B is another hypothalamic neuropeptide that has a fundamental role in the regulation of the GNRH system. Here, the basal level of *Nkb* mRNA was increased following oxytocin treatments. Again, only the highest dose of the treatment could significantly upregulate *Nkb* expression in both the AH and PH (Fig. 3).

Rfrp-3 and *DYN* Expression in Response to Intranasal Administration of Oxytocin

Next, the effects of oxytocin administration on two inhibitory neuropeptides—RFRP-3 and DYN—that can control GNRH neurons were evaluated. In this regard, application of 80 μg oxytocin for 10 days decreased the *Rfrp-3* mRNA level in the PH (Fig. 4). Expression of *Rfrp-3* was virtually undetectable in the AH. Additionally, as shown in Fig. 5, although *Dyn* expression slightly increased by the oxytocin treatments, the mRNA levels of *Dyn* neither in the PH nor the AH were statistically affected.

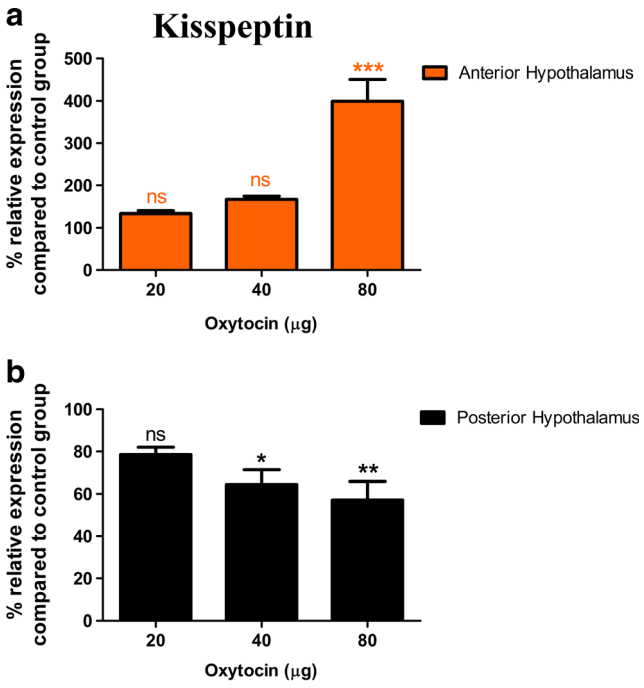

Fig. 2 Relative expression of *Kiss1* mRNA in the rat posterior (**a**) and anterior (**b**) hypothalamus after intranasal administration of oxytocin compared to control group (n = 6 in each group). Data are represented as mean ± SEM. *P < 0.05, *P < 0.01, ***P < 0.001. *ns* non-significant

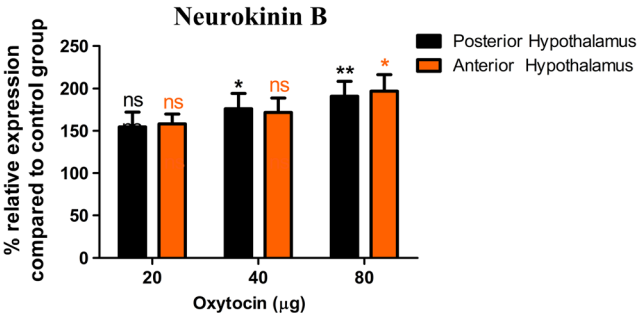

Fig. 3 Relative expression of *Nkb* mRNA in the rat posterior and anterior hypothalamus after intranasal administration of oxytocin compared to control group (n = 6 in each group). Data are represented as mean ± SEM. *P < 0.05, **P < 0.01. *ns* non-significant

Finally, we sought to determine whether oxytocin intranasal administration leads to elevation of LH release. To do so, the LH concentration was measured in the serum of experimental animals, 1 h after the last intranasal administration of oxytocin. Here, no significant difference was detected between various experimental groups. The mean serum LH concentrations in experimental groups are represented in Table 2.

Discussion

In the present study, we employed intranasal application as a possible route of delivery to the brain. It has been reported earlier that intranasal-applied oxytocin through several feasible pathways such as vascular pathway, neuronal access via olfactory sensory neurons and perineuronal spaces, and also paravascular routes reaches the brain (Neumann et al. 2013; Veening and Olivier 2013). It has been proposed that intranasal-applied oxytocin through mitral cell layer of the olfactory bulb activates oxytocin neurons in the paraventricular and supraoptic hypothalamic nuclei which subsequently can impose long-term effects of oxytocin

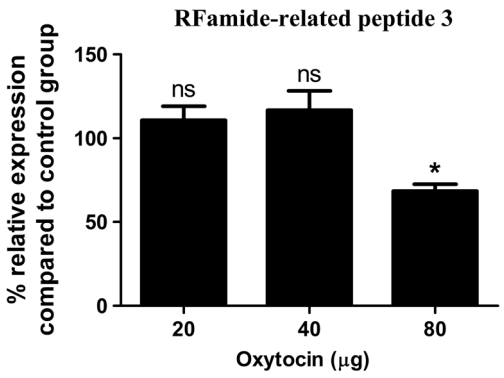

Fig. 4 Relative expression of *Rfrp-3* mRNA in the rat posterior hypothalamus after intranasal administration of oxytocin compared to control group (n = 6 in each group). Data are represented as mean ± SEM. *P < 0.05. *ns* non-significant

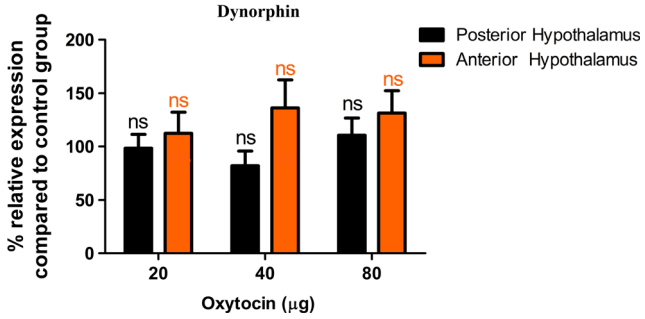

Fig. 5 Relative expression of *Dyn* mRNA in the rat posterior and anterior hypothalamus after intranasal administration of oxytocin compared to control group (n = 6 in each group). Data are represented as mean ± SEM. *ns* non-significant

(Veening and Olivier 2013). As the efficiency of uptake via this approach is limited, we employed various doses (20, 40, and 80 μg/rat) that were usually used in human (Born et al. 2002). In addition, since acute intranasal oxytocin administration did not have direct impacts on the hypothalamic-pituitary-adrenal or hypothalamic-pituitary-gonadal axes in humans (Wirth et al. 2015), here, rats were treated for ten continuous days which is approximately the minimum time required for chronic oxytocin treatment (Macdonald and Feifel 2013). As oxytocin was found to be increased in microdialysates from several brain regions with peak levels occurring 30–60 min after nasal administration (Neumann et al. 2013), in the present study, tissue and blood were collected 1 h after the last oxytocin administration. Due to hormonal cycles decrease the homogeneity of the study population and confound effects of experimental manipulation (Beery and Zucker 2011), only male rats were included in the study.

In the present study, we have shown that *Gnrh* expression elevates following intranasal administration of oxytocin. It has been reported formerly that oxytocin exerts its stimulatory effects on GNRH secretion at interconnections located in the basal hypothalamus which contains GNRH cell bodies and axons rather than GNRH terminals within the median eminence (Rettori et al. 1997; Selvage and Johnston 2001). It has been suggested that oxytocin stimulates GNRH release from basal hypothalamus explant in vitro by releasing nitric oxide through activation of oxytocin receptor and nitric oxide synthase-mediated mechanisms (Rettori et al. 1997; Selvage and Johnston 2004). Beside a few number of GNRH neurons in the mPOA that co-expressed oxytocin receptor, other oxytocin receptor-positive neurons have been found in the proximity of GNRH neurons (Caligioni et al. 2007). This issue raises the possibility that the effects of oxytocin on GNRH neurons can be either direct and⁄or indirect. Given the fact that several neuropeptides and neurotransmitters are involved in the neuroendocrine control of GNRH, the results shown here may indicate the existence of a multi neurotransmitter mechanism that ultimately causes the upregulation of *Gnrh*.

In the rat hypothalamus, kisspeptin neuronal cell bodies are restricted to the arcuate (ARC) and anteroventral periventricular (AVPV) nuclei (Mikkelsen and Simonneaux 2009) which based on the method of the hypothalamus dissection here, the anterior and posterior part of the hypothalamus represents the AVPV and ARC nuclei, respectively. Our results revealed that oxytocin had dual effects on kisspeptin neural populations in the AH and PH. It is worth noting that it is not the first time that the ARC and AVPV have demonstrated different regulatory mechanisms and previous works have shown that sexual hormones increase kisspeptin expression in the AVPA while downregulate Kiss1 expression in the ARC. In male and female rodents, ARC kisspeptin neurons mediate negative feedback action of sex steroids on GNRH release and kisspeptin cells of the AVPV mediate estrogen positive feedback effect on GNRH surge. Although kisspeptin neurons in the male AVPV have the same response similar to the female AVPV, its physiological role is not well defined as males normally do not generate sex steroid positive feedback (Smith 2013).

To the best of our knowledge, there is no report to investigate whether kisspeptin neurons express oxytocin receptor. Therefore, the direct impact of oxytocin to exert its dual effects on two subpopulations of kisspeptin neurons has yet to be precisely detailed. Moreover, according to obtained data, elevation of *Gnrh* expression following oxytocin administration may stimulate synthesis and secretion of gonadotropin as well as gonadal testosterone, creating a negative feedback loop that results in the inhibition of kisspeptin expression in the ARC meanwhile elevates *Kiss1* expression in the AVPV. In the present study, we did not observe a statistical change in serum LH concentration 1 h after the tenth intranasal administration of oxytocin, as it might have been altered at different time points.

In the rat hypothalamus, the majority of *Nkb* mRNA-expressing neurons resides in the ARC and lateral hypothalamic area (Navarro et al. 2011). Considering the

Table 2 Mean ± SEM serum LH concentration 1 h after the tenth intranasal administration of oxytocin

	Experimental groups			
	Control	20 μg oxytocin	40 μg oxytocin	80 μg oxytocin
Mean LH concentration (ng/ml)	1.97 ± 0.24	2.13 ± 0.19	2.23 ± 0.15	2.24 ± 0.41

impacts of inactivating mutations in the genes encoding NKB or its receptor which resulted in hypogonadotropic hypogonadism, the NKB system plays essential roles in the reproductive function. In contrast to the clear impact of the NKB system on GNRH/LH, there is no agreement on its effects when pharmacological approaches are employed. These contradictory findings can be due to selected animal model and gonadal status (Grachev et al. 2013). Our findings showed that, oxytocin dose dependently stimulates hypothalamic *Nkb* expression. Since arcuate NKB-expressing cells directly interact with GNRH neurons especially at median eminence (Krajewski et al. 2005) and stimulatory impacts of NKB on GNRH/LH depend upon kisspeptin/GPR54 signaling (Ramaswamy et al. 2011), therefore, oxytocin may modulate GNRH secretion at least in part by regulating NKB expression.

DYN has widely distributed throughout the hypothalamus (Lin et al. 2006) and increasing number of evidence have shown that DYN plays a fundamental role in the regulation of reproduction. In this regard, Wakabayashi et al. (2010) have shown that DYN along with NKB is involved in the process of generating the rhythmic discharge of kisspeptin, hence it can be taken into account as a modulator of the GNRH system. However, in the present study, no significant change was detected in the level of *Dyn* mRNA following oxytocin treatment.

RFRP-3 is the mammalian ortholog of the avian gonadotropin-inhibitory hormone. Rodent RFRP-3-expressing neurons are exclusively located in the dorsomedial hypothalamic nucleus and by sending projections to GNRH cells, suppress their activity (Kriegsfeld et al. 2010; Salehi et al. 2015). Our findings demonstrated the highest dose of oxytocin-reduced *Rfrp-3* expression and interestingly at this level, *Gnrh* expression increased in the hypothalamus. Given the fact that RFRP fibers are located near the GNRH neurons in all mammalian species, so far evaluated and subpopulations of GNRH neurons express RFRP receptor (Herbison 2015), results shown here serve to reinforce the notion that RFRP neurons may be involved to relay oxytocin information into the GNRH system. Further elucidation is required to define whether this effect of oxytocin on RFRP neurons is direct or not.

In conclusion, our results showed that intranasal administration of oxytocin for 10 continues days elevates relative expression of *Gnrh* and *Nkb*, whereas decreases *Rfrp-3* mRNAs in the male rat hypothalamus. This treatment has dual opposite effects on *Kiss1* expression in the AH and PH. Therefore, it can be hypothesized that at least part of described effects of oxytocin on the GNRH system might be mediated by neural networks upstream of GNRH neurons.

Acknowledgements This study was financially supported by Shahid Beheshti University Grant No. 60092. The authors are thankful to the Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, for the technical support of this work.

Compliance with Ethical Standards All experiments were conducted in the Laboratory Animal Center and in compliance with the recommendations of the Animal Care Committee of the Shahid Beheshti University, Tehran, Iran.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Beery AK, Zucker I (2011) Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 35:565–572

Born J et al (2002) Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 5:514–516

Caligioni C, Oliver C, Jamur M, Franci C (2007) Presence of oxytocin receptors in the gonadotrophin-releasing hormone (GnRH) neurones in female rats: a possible direct action of oxytocin on GnRH neurones. J Neuroendocrinol 19:439–448

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81:629–683

Goodman RL et al (2004) Evidence that dynorphin plays a major role in mediating progesterone negative feedback on gonadotropin-releasing hormone neurons in sheep. Endocrinology 145:2959–2967

Goodman RL, Coolen LM, Lehman MN (2013) A role for neurokinin B in pulsatile GnRH secretion in the ewe. Neuroendocrinology 99:18–32

Grachev P, Millar R, O'byrne K (2013) The role of neurokinin B signalling in reproductive neuroendocrinology. Neuroendocrinology 99:7–17

Herbison AE (2015) Physiology of the adult gonadotropin-releasing hormone neuronal network. In: Plant TM, Zeleznik AJ (eds) Knobil and Neill's physiology of reproduction (fourth edition). Academic Press, San Diego, pp 399–467

Johnston CA, Lopez F, Samson WK, Negro-Vilar A (1990) Physiologically important role for central oxytocin in the preovulatory release of luteinizing hormone. Neurosci Lett 120:256–258

Kinoshita F, Nakai Y, Katakami H, Imura H (1982) Suppressive effect of dynorphin-(1–13) on luteinizing hormone release in conscious castrated rats. Life Sci 30:1915–1919

Krajewski SJ et al (2005) Morphologic evidence that neurokinin B modulates gonadotropin-releasing hormone secretion via neurokinin 3 receptors in the rat median eminence. J Comp Neurol 489:372–386

Kriegsfeld LJ et al (2010) The roles of RFamide-related peptide-3 in mammalian reproductive function and behaviour. J Neuroendocrinol 22:692–700

Lin S et al (2006) Distribution of prodynorphin mRNA and its interaction with the NPY system in the mouse brain. Neuropeptides 40:115–123

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25:402–408

Lukas M, Neumann ID (2012) Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: social versus non-social effects. Neuropharmacology 62:398–405

Macdonald K, Feifel D (2013) Helping oxytocin deliver: considerations in the development of oxytocin-based therapeutics for brain disorders. Front Neurosci:7

Mikkelsen JD, Simonneaux V (2009) The neuroanatomy of the kisspeptin system in the mammalian brain. Peptides 30:26–33

Navarro VM (2012) New insights into the control of pulsatile GnRH release: the role of Kiss1/neurokinin B neurons. Front Endocrinol (Lausanne) 3:48

Navarro VM et al (2011) Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. Am J Physiol Endocrinol Metab 300:E202–E210

Neumann ID, Maloumby R, Beiderbeck DI, Lukas M, Landgraf R (2013) Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. Psychoneuroendocrinology 38:1985–1993

Ramaswamy S, Seminara SB, Plant TM (2011) Evidence from the agonadal juvenile male rhesus monkey (*Macaca mulatta*) for the view that the action of neurokinin B to trigger gonadotropin-releasing hormone release is upstream from the kisspeptin receptor. Neuroendocrinology 94:237–245

Rance NE, Krajewski SJ, Smith MA, Cholanian M, Dacks PA (2010) Neurokinin B and the hypothalamic regulation of reproduction. Brain Res 1364:116–128

Rettori V, Canteros G, Renoso R, Gimeno M, McCann S (1997) Oxytocin stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamic explants by releasing nitric oxide. Proc Natl Acad Sci U S A 94:2741–2744

Rønnekleiv OK, Kelly MJ (2013) Kisspeptin excitation of GnRH neurons. In: Kauffman AS, Smith JT (eds) Kisspeptin signaling in reproductive biology. Springer, New York, pp 113–131

Salehi MS, Namavar MR, Jafarzadeh Shirazi M, Rahmanifar F, Tamadon A (2012) A simple method for isolation of the anteroventral periventricular and arcuate nuclei of the rat hypothalamus. Int J Exp Clin Anat 6-7:48–51

Salehi MS, Tamadon A, Shirazi MRJ, Namavar MR, Zamiri MJ (2015) The role of arginine-phenylalanine-amide-related peptides in mammalian reproduction. Int J Fertil Steril 9:268–276

Schulz R, Wilhelm A, Pirke KM, Gramsch C, Herz A (1981) β-Endorphin and dynorphin control serum luteinizing hormone level in immature female rats. Nature 294:757–759

Selvage D, Johnston C (2004) Interaction between norepinephrine, oxytocin, and nitric oxide in the stimulation of gonadotropin-releasing hormone release from proestrous rat basal hypothalamus explants. J Neuroendocrinol 16:819–824

Selvage D, Johnston CA (2001) Central stimulatory influence of oxytocin on preovulatory gonadotropin-releasing hormone requires more than the median eminence. Neuroendocrinology 74:129–134

Silverman RT, Munger BL, Halata Z (1986) The sensory innervation of the rat rhinarium. Anat Rec 214:210–225

Smith JT (2013) Sex steroid regulation of kisspeptin circuits. In: Kauffman AS, Smith JT (eds) Kisspeptin signaling in reproductive biology. Springer, New York, pp 275–295

Veening JG, Olivier B (2013) Intranasal administration of oxytocin: behavioral and clinical effects, a review. Neurosci Biobehav Rev 37:1445–1465

Wakabayashi Y et al (2010) Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci 30:3124–3132

Wirth MM, Gaffey AE, Martinez BS (2015) Effects of intranasal oxytocin on steroid hormones in men and women. Neuropsychobiology 71:202–211