Excitatory and inhibitory influence of exogenous neurotransmitters on reproduction in female rats

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ABSTRACT

Neurotransmitters are mediators inside the nervous system responsible for transmitting neural-neural or neural-organs signals. Several neural studies have tried to unveil the role of such mediators whose action extended outside the nervous system such as immune, digestive, circulatory and reproductive systems. The present study aimed to investigate the role of the excitatory-glutamate and inhibitory-GABA transmitters in female rats reproduction including their effects on gonadotropins; luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the sex steroids; estrogen (E2) and progesterone (P4) during the estrous cycle as well. Furthermore, the responsive changes on the ovarian tissue were also studied. Synthetic glutamate and GABA were injected intraperitonentially (ip) in those animals throughout four successive estrous cycles. Interestingly, the ip injections of glutamate increased the levels of LH, E2 and P4 but decreased those of FSH significantly. However, the ip injections of GABA significantly decreased the levels of LH in the 4th cycles and FSH throughout treatment period while it increased the levels of E2 and P4. All changes occurred in those reproductive hormones caused by glutamate has been recovered after cessation of glutamate injection, including; the 5th, 6th and 7th cycles, while the changes caused by GABA were not recovered except in FSH level. Regarding to histopathological examination, ovaries of treated rats showed deleterious changes. The glutamate-treated rats ovaries showed atrophy of the primary follicles with degenerative changes in those secondary and tertiary follicles with obvious degeneration in the granulosa cell layer with vacuolated cytoplasm. On the other hand, those received GABA showed degeneration of the oocytes with congestion of blood vessels supplying the corpora lutea (CL) associated with endothelial changes. The histopathological changes in CL have been improved after glutamate cessation while not changed after GABA cessation.

Key words: Glutamate, GABA, Neurotransmitters, FSH, LH, Ovarian steroids
INTRODUCTION

The development and maintenance of reproductive systems are necessary for the propagation of all vertebrates and the efforts of scientists are directed toward this object to improve reproductive performance. Female reproduction is under control of gonadotropin releasing hormone (GnRH) which is released from the hypothalamic GnRH-secreting neurons into the hypothalamic-hypophyseal portal circulation (Marshall et al., 1991) which reaches the anterior pituitary gland stimulating the release of gonadotropins which known as follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Millar, 2005) which act on gonads to stimulate the release of steroid hormones (estrogens & progestins). Those sex steroid hormones are transported through the general circulatory system and feedback to the pituitary and hypothalamus, inhibiting/stimulating the hypothalamic release of GnRH either directly or via the adjacent kisspeptin-secreting neurons (Ezzat et al., 2015). Glutamate and gamma amino-butyric acid (GABA) are regulators of development, adulthood and age-related control of GnRH function as they are the primary excitatory and inhibitory amino acid neurotransmitters, respectively (Lovingier, 2011). Their receptors are widely expressed in the hypothalamic nuclei and their neuronal cells (Van den Pol, 1991 and Zhang et al., 2009). They have several classes of receptors, which in turn made up of subunits whose expression varies by developmental age, sex, and region of reproductive neuroendocrine activity (Clarkson and Herbison, 2006; Henderson, 2007). Moreover, GnRH neurons themselves express glutamate (Bourguignon et al., 1995; Kiss et al., 2003) and GABA receptors isoforms GABAA and GABAB (Todman et al., 2005; Zhang et al., 2009) and this co-expression varies across the life cycle (Miller and Gore, 2002; Bailey et al., 2006). In addition, the hypothalamic regions that mediate the effects of steroid hormones negative and positive feedback are abundant in glutamatergic and GABAergic receptors. Numerous other neuroactive substances are also critically involved in integrating the function of GnRH neurosecretion (Malyala et al., 2005; de la Iglesia and Schwartz, 2006). To bridge communication gap between these neurotransmitters and female reproduction, glutamate and GABA were intraperitoneally injected for four successive estrous cycles in adult female rats followed by treatment cessation (recovery) period. Female reproduction evaluated by monitoring reproductive hormones including; FSH, LH, E₂ and P₄ in addition to ovarian histopathological changes in both follicular and luteal phases of estrous cycle two days after injection as well as during recovery period.

MATERIALS AND METHOD

Animals

Eighty adult female albino rats Sprague-Dawley strain with an average weight of 150-170 g obtained from the private farm for lab animals, Helwan province, Egypt to Physiology Department, Faculty of Veterinary Medicine, South Valley University. Animals were kept in plastic cages in complete healthy condition in lab animal house and maintained on a 12 h light/ dark cycle and at temperature of 26 ± 2 °C; all rats were allowed to free access drinking of water and basal diet ad-libitum two weeks to be acclimatized to the lab environment and adjusting the regularity of the estrous cycle.

Chemicals and reagents

Exogenous neurotransmitters used in this study are glutamate (From LOBA CHEMIE, Mumbai, India) and GABA (From
Merck – Schuchardt, Germany). Moreover, Serum FSH and LH concentrations were measured by using a commercially available Enzyme Immunoassay (EIA) Kit (Monobind inc, lake forest Ca 29630, USA) Also, Serum P_4 and E_2 concentrations were measured by using a commercially available EIA Kit (DSI S.R.L. Sarono (VA), Italy).

**Experimental design**

Female rats were randomly distributed into three groups, control (CTL) one, includes 20 animals injected by saline (0.9% NaCl) while the 2nd and 3rd groups contain 30 animals each and injected by glutamate (4 mg/kg body weight (b.w)) (Ortiz et al., 2006) and GABA (10 mg/kg b.w) (Borycz et al., 1992) respectively. For all groups, the chemicals were freshly prepared in saline then injected by i.p rout once per cycle beginning from the proestrus (first injection) for four successive estrous cycles. Injection was ceased for three successful cycles for recovery. Estrus cycle in all rats were determined according to McLean et al., (2012) through vaginal smear cytology (Fig. 1) for differentiation of different phases of cycle cycles. Each phase can be recognized depending on type of cells found in vaginal smear which contain three types of cells: epithelial cells, cornified cells and leukocytes. By using plastic pipette, 10 µl saline (0.9% NaCl) introduced into vagina then collected back on glass slides. One drop was collected with a clean tip from each rat and examined under a light microscope with 10 and 40 x objective lenses. Three types of cells were observed; circular and nucleated ones are epithelial cells; irregular and non-nucleated ones are the cornified cells; and the little round ones are the leukocytes. A proestrus smear consists of a predominance of nucleated epithelial cells; an estrous smear primarily consists of nucleated cornified cells; a metestrus smear consists of the same proportion among leukocytes, cornified, and nucleated epithelial cells, and a diestrus smear primarily consists of a predominance of leukocytes.

**Samples collection**

Individual blood samples were collected from retro-orbital venous plexus two days after each injection during treatment period (four estrus cycles) without anticoagulant then centrifuged at 3000 rpm for 15 min. sera were separated; collected in eppendorf tube then kept at -20 °C until immunological assay of the studied hormones (FSH, LH, P_4 and E_2). After the end of four estrus cycles period, half number of each group were sacrificed, and ovaries were collected, washed by saline and kept in 10 % formalin for histopathological examination. The other half of animals in each group (recovery) kept for 12 days (three estrus cycles) without any treatment with continuous blood samples collection every two after proestrus. After 12 days, rats were sacrificed, and ovaries were collected and kept in 10 % formalin for histopathological examination.

**Statistical analysis**

Results were analyzed statistically by Graph pad prism software (Graph Pad Prism, San Diego, USA, Co.). Data were expressed as mean ± standard error of mean (SEM) and differences between groups were analyzed by using Two-way analysis of variance (ANOVA). Differences compared to control rats were considered significant at P<0.05.

**RESULTS**

Figure 2 showed serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to ip injection of glutamate and GABA. Serum LH increased significantly
Figure 1. Cytological assessment of vaginal smears can be used to identify estrous stage. Three main cell types are detected in vaginal smear samples: (A) nucleated epithelial cells, (B) cornified squamous epithelial cells, and (C) leukocytes. The ratio of these cell types present in the smear can be used to identify mice in (D) proestrus, (E) estrus, (F) metestrus, or (G) diestrus as described in representative results. Black arrowheads in E, F and G point to representative cornified squamous epithelial cells. Black arrows in C, F and G point to representative leukocytes. White arrows in D and G highlight representative nucleated epithelial cells (McLean et al., 2012).

(P<0.05) after glutamate injection during treatment period compared with corresponding control however, it declined near to control value at the end of recovery period (Fig. 2A). On the other side, serum LH decreased significantly (P<0.05) after GABA injection at the end of treatment and beginning of recovery periods while, it elevated again at the end of recovery period (Fig. 2B). Unexpectedly, glutamate and GABA showed an inhibitory effect on FSH by significant decrease (P<0.05) its serum level in both treatment and recovery periods (Fig. 2C)
and D, respectively) compared with control group.

Fig. 3 demonstrated the serum estradiol (E$_2$) and progesterone (P$_4$) in response to ip injection of glutamate and GABA. Both glutamate and GABA increased serum E$_2$ level significantly (P<0.05) during treatment and beginning of recovery period compared with control rats. While, serum level of E$_2$ declined toward control level at the end of recovery period (Fig. 5A and B respectively). Likewise, serum P$_4$ increased significantly (P<0.05) in response to glutamate and GABA injection and declined at the end of recovery period compared with control group (Fig. 5C and D respectively).

Histological examination of ovaries in the control group showed that the primary follicle lined by a thin single layer of squamous follicular epithelium and around the periphery appeared cuboidal in shape with developed thick, translucent an amorphous, non-cellular glycoprotein covering the zona pellucida (Zp) (Fig. 4A). In addition, the secondary follicles in CTL group lined by stratified follicular epithelium enclosing oocytes with a distinct Zp, the secondary follicle cavity is small, but it grows when more fluid pumped into antrum (Fig. 4B). Moreover, tertiary follicle in CTL group has oocyte isolated into number of follicular cells (F). A sub-population of cells in the corona radiata arranged forming a single layer immediately adjacent to the oocyte and present against the Zp, this layer termed the corona radiata which go with the oocyte when it is ovulated, leaving the membrana granulosa (G) on the membrane of the follicles around which zona granulosa is well developed (Fig. 4C). In the Glutamate treated-rats, the Primary follicle was atrophied with no oocyte, in addition to condensed follicular cells surrounded with fibrocytes. The surrounding ovarian tissue free of primordial follicles replaced by fibrous stroma (Fig. 4D). However, Secondary ovarian follicle showed degeneration in the zona pellucida and oocyte with thick vacuolated follicular epithelium (Fig.4E). While, tertiary follicle in the exhibited degenerated Zp, CO and Oocyte, beside hypertrophy and degeneration in the granulosa layer with vacuolated cytoplasm (Fig. 4F). Likewise, GABA treated-group showed follicular cyst with oocyte degeneration in the primary follicles (Fig. 4G). Similar findings also, found in secondary follicles which exhibited oocyte without nucleus, intact zona pellucida and hypertrophy of thecal cells (Fig. 4H). Moreover, deleterious effect of GABA on tertiary follicle represented by absence of corona radiata with vacuolation of granulosa and hypertrophy of thecal cells (Fig. 4I). The histopathological findings of ovaries after cessation of glutamate administration showing that tertiary follicle revealing degenerated ZP, CO and Oocyte, with hypertrophy and vacuolation in the granulosal cell layer (Fig. 4J) compared with CTL rats. Also, the deleterious histopathological findings still appear in rat’s ovaries after cessation of treating by GABA, tertiary follicle exhibit absence of ovum and corona radiata (Fig. 4K).

Corpus luteum showed hypertrophied lutein cells with cytoplasmic vacuolation and congestion of blood vessels after glutamate injection (Fig. 5A) in addition to hypertrophy, congestion of blood supply after GABA injection (Fig. 5B). On the other side, corpus luteum recovered from all deteriorated effects after cessation of glutamate injection and appeared normal (Fig. 5C). However, CL of GABA-injected rats still showed lutein cells with dilated and congested blood vessels (Fig. 5D) after cessation of GABA injection compared with CL in control group (Fig. 5E).
Figure 2: Serum luteinizing hormone (LH) in response to intraperitoneal (ip) injection of glutamate (4 mg/kg b.w) and GABA (10 mg/kg b.w) is shown in fig. 2A and B, respectively. Serum follicle-stimulating hormone (FSH) in response to ip injection of glutamate and GABA is shown in fig. 2C and D, respectively. The number (n) of rats used in control (CTL), treatment, and recovery groups were; n= 20, 30 and 15, respectively. During the treatment period, animals were treated once per cycle beginning from proestrus throughout four successful estrus cycles. Animals in the CTL group were injected with saline (1 ml/rat). During the recovery period throughout three successive cycles after treatment, both LH and FSH were measured in assigned time as 2 days from proestrus. Different letters (a, b, c, d) on bars denote the significance at P < 0.05.
Figure 3: Serum estradiol ($E_2$) in response to ip injection of glutamate and GABA is shown in fig. 3A and B, respectively. Serum progesterone ($P_4$) in response to ip injection of glutamate and GABA is shown in fig. 3C and D, respectively. Other explanations were given in Fig. 2.
Figure 4: The histopathology of ovarian primary (A, D, G), secondary (B, E, H) and tertiary (C, F, I) follicles in response to saline (CTL), glutamate and GABA ip injections in rats are shown respectively. The tertiary follicles in the recovered animals after the cessation of glutamate and GABA treatments are shown in J and K images. The tissue sections were fixed and stained with haematoxylin and eosin (H&E) with magnification of 40X. (F: follicular tissue, O: oocyte, ZP: zona pellucida, PR: primary follicle, AC: antral cavity, CO: cumulus oophorous, Se: secondary follicle, CR: Corona radiata, T: thecal cells, G: granuloza cells)
Figure 5: The histopathology of corpus luteum (CL) in response to ip injection of glutamate and GABA (congestion of blood supply represented by arrows) are shown in Fig. 5 A and B, respectively. The histopathology of CL in the recovered animals after cessation of glutamate (lutein cells: arrow) and GABA (Vacuolation: arrow) treatments are shown in Fig. 5 C and D, respectively. The Fig. 5E shows the histopathology of CL response to saline in CTL group with normal lutein cells (arrow). Other explanations are given in Fig. 4.
DISCUSSION

Glutamate and GABA are the predominant excitatory and inhibitory neurotransmitters in CNS respectively. They are involved in synaptic transmission, learning and memory (Lovingier, 2011). Previous recorded studies proved the remote-effect of these neurotransmitters extended to different body systems. Reproductive system is one of these systems influenced by external excitatory and inhibitory neurotransmitters. This remote-effect proved by expression of glutamate receptors as NMDA and AMPA in hypothalamic GnRH neurons (Van den Pol, 1991; Van den Pol et al., 1990). In addition, GnRH neurons received synaptic input from GABA afferent (Jansen et al., 2003) besides, GABAA and GABAB receptors localized in GnRH neurons (Temple and Wray, 2005; Zhang et al., 2009). In spite of previous studies, glutamergic and GABAergic cross-action with GnRH neurons and ovarian tissue still elusive. So, the current study is to investigate systemic (on GnRH neurons) and localized (on ovarian tissue) effect of exogenous glutamate and GABA on female reproduction.

Although glutamate is the main excitatory neurotransmitter, it has heterogenous effect outside CNS. Here, glutamate has stimulatory effect on LH while, its effect on FSH was inhibitory. Despite, LH and FSH controlled by the same regulatory secretory cells (GnRH), there are many factors making pattern of secretion in diverge manner as occur in different estrous cycle stages (Niswender et al., 1975). One of these factors is intracellular control; increase intracellular Ca$^{2+}$ stimulates LH secretion from hypophyseal cells not FSH by calcium calmodulin-dependent protein kinase II (Rebers et al., 2003). However, stimulation of FSH secretion In vitro from hypophyseal cells required activation of different second messenger protein kinase C (Kile ane Netl, 1994). Depending on all previous recorded results, it is easily explained the dual effect of glutamate on LH and FSH in the current study. Glutamate increases intracellular Ca$^{2+}$ in immortalized hypothalamic cells (Spergel et al., 1994) therefor, we suggest glutamergic stimulatory and inhibitory effect on LH and FSH respectively owing to the increasing intracellular Ca$^{2+}$ and activation of different second messenger. Likewise, glutamate, GABA has heterogenous influence on GnRH neurons although it is dominant inhibitory neurotransmitter. It is proved that GABA has stimulatory effect on GnRH (Donoso et al., 1992; Bilger et al., 2001) however, injection of GABAA receptor agonist has inhibitory effect on GnRH especially LH (Herbison and Dyer, 1991). Here, GABA injection has inhibitory effect on LH and decreases FSH secretion may be through direct or indirect mechanisms. Direct action on GnRH neurons through activation of GABAA receptors or indirect through activation of GABAA receptors which activate B-endorphinergic and dopaminergic neurons (Tomaszewksa-Zaremba et al., 2001).

Regarding to the effect of glutamate on ovarian steroid hormones, glutamate stimulate secretion both E$_2$ and P$_4$ although degenerative changes in ovarian tissues after glutamate injection. Degeneration process associated with inflammatory reaction, cell swelling rupture of cell membrane and leakage of cellular content including stored hormones (Raffray and Cohen, 1997; Wyllie et al., 1980). So, we suggest elevation level of both hormones during degenerative changes due to cell swelling and rupture and these hormones so, the levels of both hormones may be declined if they monitored after longer time from glutamate injection. GABA and GABA receptors were localized in the ovarian and fallopian tube cells (Martin del Rio and Caballero, 1980; Erdo and Lapis, 1982).
therefore, GABA has localized effect on ovarian tissue and function. In the present study, GABA concomitant in ovarian stimulatory effect as serum E$_2$ and P$_4$ elevated after GABA injection. However, previous studies proved GABA stimulatory effect on E$_2$ and inhibitory on P$_4$ (Erdõ et al., 1985). Histopathological findings run parallel with ovarian hormone level, there is hypertrophy and of granulosa cells reflected by high level of E$_2$ and dilatation of blood vessels that increase blood supply to CL indicated by high level of P$_4$.

CONCLUSION

Generally, glutamate and GABA are main excitatory and inhibitory neurotransmitters in CNS. While, their action is different outside CNS as exogenous glutamate injection increased serum level of LH but reduced FSH and exogenous GABA injection decreased serum level of both LH and FSH. Unexpectedly, both exogenous glutamate and GABA injection increased serum levels of LH and FSH. Moreover, ovarian histological architecture was altered after glutamate and GABA injection. Although cessation of glutamate and GABA injection, the deleterious effect only recovered in glutamate-treated rats.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

Bailey, J.D, Centers, A., Jennes, L. (2006) Expression of AMPA receptor subunits (GluR1-GluR4) in gonadotrophin-releasing hormone neurons of young and middle-aged persistently oestrous rats during the steroid-induced luteinizing hormone surge. J Neuroendocrinol. 18:1–12.

Bilger, M., Heger, S., Brann, D.W., Paredes, A., Ojeda, S.R. (2001) A conditional tetracycline-regulated increase in Gamma amino butyric acid production near luteinizing hormone-releasing hormone nerve terminals disrupts estrous Cyclicity in the rat. Endocrinology. 142: 2102–2114.

Borycz, J., Borycz, J.A., Bugajski J. (1992) Effect of gamma-aminobutyric acid and muscimol on corticosterone secretion in rats. J Physiol Pharmacol. 43(3):259-269.

Bourguignon, J.P., Gerard, A., Alvarez Gonzalez, M.L., Purnelle, G., Franchimont, P. (1995) Endogenous glutamate involvement in pulsatile secretion of gonadotropin-releasing hormone: evidence from effect of glutamine and developmental changes. Endocrinology. 136:911–916.

Clarkson, J., Herbison, A.E. (2006) Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty, Mol. Cell. Endocrinology. 254-255:32-38.

de la Iglesia, H.O., Schwartz, W.J. (2006) Minireview timely ovulation: circadian regulation of the female hypothalamo-pituitary-gonadal axis. Endocrinology. 147:1148–1153.

Donoso, A.O., Lopez, F.J., Negro-Vilar, A. (1992) Cross-talk between Excitatory and inhibitory amino acids in the regulation of luteinizing hormone-Releasing hormone secretion. Endocrinology. 31: 1559–1561.

Erdö, S., Varga, B., Horváth, E. (1985) Effect of local GABA administration on rat ovarian blood flow, and on progesterone and estradiol secretion. Eur J Pharmacol. 20;111(3):397-400.

Erdö, S.L., Lapis, E. (1982) Presence of GABA receptors in rat oviduct. Neurosci. Lett. 33, 275–279.

Ezzat, A., Pereira, A., Clarke, I.J. (2015) Kisspeptin is a component of the pulse generator for GnRH secretion in female sheep but not
the pulse generator. Endocrinology. (5):1828-1837.

Henderson, L.P. (2007) Steroid modulation of GABAA receptor-mediated transmission in the hypothalamus: effects on reproductive function. Neuropharmacology. 52:1439–1453.

Herbison, A.E., Dyer, R.G. (1991) Effect on luteinizing hormone secretion of GABA receptor modulation in the medial preoptic area at the time of proestrous luteinizing hormone surge. Neuroendocrinology. 53: 317–320.

Jansen, H.T., Cutter, C., Hardy, S., Lehman, M.N., Goodman, R.L. (2003) Seasonal plasticity within the GnRH system of the ewe: changes in identified GnRH inputs and in glial association. Endocrinology. 144:3663–3676.

Kile, J.P., Nett, T.M. (1994) Differential secretion of follicle-stimulating hormone and luteinizing hormone from ovine pituitary cells following activation of protein kinase A, protein kinase C, or increased intracellular calcium. Biol Reprod.; 50(1):49-54.

Kiss, J., Kocsis, K., Csa´ki, A., Hala´sz, B. (2003) Evidence for vesicular glutamate transporter synapses onto gonadotropin-releasing hormone and other neurons in the rat medial preoptic area. Eur J Neurosci; 18:3267–3278.

LoVinger, D.M. (2011) Neurotransmitter Roles in Synaptic Modulation, Plasticity and Learning in the Dorsal Striatum. Neuropharmacology. 58(7): 951–961.

Malyala, A., Kelly, M.J., Ronneklev, O.K. (2005) Estrogen modulation of hypothalamic neurons: activation of multiple signaling pathways and gene expression changes. Steroids. 70:397–406.

Marshall, J.C., Dalkin, A.C., Haisenleder, D.J., Paul, S.L., Ortolano, G.A., Kelch, R.P. (1991) Gonadotropin-releasing hormone pulses: regulators of gonadotropin synthesis and ovulatory cycles. Recent Prog Horm Res; 47(47): 155-187.

Martin del Rio, R., Caballero, A.L. (1980) Presence of γ-aminobutyric acid in rat ovary. J. Neurochem; 34, 1584–1586.

McLean, A.C., Valenzuela, N., Fai, S., Bennett, S. A. (2012) Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification. Journal of visualized experiments: JoVE.

Millar, R.P. (2005) GnRHs and GnRH receptors. Anim Reprod Sci; 88(88): 5-28.

Miller, B.H., Gore, A.C. (2002) N-Methyl-D-aspartate receptor subunit expression in GnRH neurons changes during reproductive senescence in the female rat. Endocrinology. 143:3568–3574.

Niswender, G.D., Moore, R.T., Akbar, A.M., Nett, T.M., Diekman, M.A. (1975) Flow of blood to the ovaries of ewes throughout the estrous cycle. Biol Reprod.; 13:381-388.

Ortiz, G., Bitzer-Quintero, O., Zárate, C., Rodríguez-Reynoso, S., Larios-Arceo, F., Velázquez-Brizuela, I., Pacheco-Moisés, F., Rosales-Corrall, S.A. (2006) Monosodium glutamate-induced damage in liver and kidney: a morphological and biochemical approach. Biomedicine and Pharmacotherapy. 60 (2): 86-91.

Raffray, M., Cohen, G.M. (1997) Apoptosis and necrosis in toxicology – a continuum or distinct modes of cell death. Pharmacol Ther; 75:153–177.

Rebers, F.E., Bosma, P.T., van Dijk, W., Goos, H.J., Schulz, R.W. (2003) GnRH stimulates LH release directly via inositol phosphate and indirectly via cAMP in African catfish. Am J Physiol Regul Integr Comp Physiol; 278(6): R1572-8.
Spergel, D., Krsmanovic, L., Stojkovic, S., Catt, K. (1994) Glutamate modulates [Ca2+]i and gonadotropin releasing hormone secretion in immortalized hypothalamic GT1-7 neurons. Neuroendocrinology. 59: 309-317.

Temple, J.L., Wray, S. (2005) Developmental changes in GABA receptor subunit composition within the gonadotrophin-releasing hormone-1 neuronal system. J. Neuroendocrinol. 17: 591–599.

Todman, M.G., Han, S.K., Herbison, A.E. (2005) Profiling neurotransmitter receptor expression in mouse gonadotropin-releasing hormone neurons using green fluorescent protein-promoter transgenics and microarrays. Neuroscience. 132:703–712.

Tomaszewska-Zaremba, D., Przekop, F., Mateusiak, K. (2001) The involvement of GABA(A) receptors in the control of GnRH and beta-endorphin release, and catecholaminergic activity in the ventromedial-infundibular region of hypothalamus in anestrous ewes. J Physiol Pharmacol; 52(3):489-500.

Van den Pol, A. (1991) Glutamate and aspartate immunoreactivity in hypothalamic pre-synaptic axons. J. Neurosci. 11: 2087-2101.

Van den Pol, A., Waurin, J., Dudek, F. (1990) Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science. 250:1276-1278.

Wyllie, A.H., Kerr, J.F., Currie, A.R. (1980) Cell death: the significance of apoptosis. Int Rev Cytol; 68:251–306.

Zhang, C., Bosch, M., Ronnekleiv, O.K., Kelly, M.J. (2009) GABA_B receptor mediated inhibition of GnRH neurons is suppressed by Kisspeptin-GPR54 signaling. Endocrinology. 150, 2388–2394.

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#Erratum2018.001: Affiliation (No 3) is added.