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ALTERATION OF RODENT ESTROUS CYCLE LENGTH FOLLOWING INTRAPERITONEAL ADMINISTRATION OF EXOGENOUS OXYTOCIN TO NEONATES

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ABSTRACT

Oxytocin (OT) possesses uterotonic and galactopoietic properties. To the best of the knowledge, the relevant data regarding to the estrous cyclicity was scared. The present study investigates the chronic effect of low dose OT on estrous cyclicity in detail by using rodent model albino mice. Twelve immature female mice, *Mus musculus* weight 8±2g were divided into 2 groups as control (n=6) and treated (n=6). The treated and control groups were received intraperitoneal (i.p.) dose of synthetic oxytocin (50mIU/5µl/g body weight) and physiological saline (5µl/g body weight) respectively for 90 days from postnatal day 10 (PND 10) to PND 100. Day of vaginal opening and first onset of estrus as well as estrous cycle study were done. The major observations during the research study were that, OT was showed significant decrease day of vaginal opening and first onset of estrus as well as significant shorten the length of estrous cycle as compared to control group. Finally the research study was concluded that, synthetic OT altered sexual maturity as well as estrous cyclicity of mice at selected dose and duration; therefore a care must be taken for long term use.

KEYWORDS: **Synthetic oxytocin (OT); Neonates; Estrous cycle; *Mus musculus.***

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INTRODUCTION

Steroid and peptide hormones play important role in reproductive processes in which oxytocin (OT) is one of them. In mammals nonapeptide OT is synthesized by hypothalamic neurosecretory cells (magnocellular neurons) present in paraventricular (PVN) and supraoptic nuclei (SON) region, then it is stored and released into blood stream by posterior pituitary.[1] OT specifically binds with neurophysin I which is carrier protein and transport OT from hypothalamus to pituitary gland and this associate form further released into blood stream.[2] It also known as a paracrine hormone due to it also synthesized by peripheral tissues such as, ovary (granulosa cells, corpus luteum), uterus, placenta, amnion, testis (Leydig cells).[3,4] OT exerts many different biological functions through G-protein-coupled transmembrane receptors (OTR) present in ovarian follicles, corpus luteum, oviduct, uterus and other reproductive tissues.[3] Moreover, cross reactivity of OT also noted with vasopressin receptors *viz.,* V1 subtype (V1a and V1b) and V2 type.[5] V1b also known as V3 type which is present on myometrium during gestation period.[4] OTR expression on uterine epithelium can be affected by uterine contraction, ovarian steroids, tissue prostaglandins and cholesterol content of plasma membrane.[4] In human, oxytocin promotes a number of reproductive behaviors (pair bonding, grooming, arousal, lordosis, orgasm, birthing, parturition and lactation), social behaviors (social memories, social bonds and friendships) and maternal behaviors (infant caregiving, retrieval, grouping of pups, licking of pups, nest building, and crouching).[6] Almost all synthetic oxytocin are same, only pharmacology is different in which synthetic oxytocin Pitocin is one of them which used same endogenous oxytocin receptors for binding and effective action. Oxytocin is effective after administration by any parenteral route such as i.p., s.c., i.m., i.v., intranasal and orally. It's safe at low dose and short term administration, but it has cause adverse effects on high dose and long term administration by any mode of administration. Nowadays, synthetic OT used in different way other than clinical purpose as unmonitored use in postpartum period due to its uterotonic property and enhancing milk synthesis due to its galactogenic property.[7] In this regard, several research study were showed that, OT residues has also found in milk approximately 138.53 ng/L when analyzed by ELISA in different branded and non branded milk samples.[8] The cells of gastrointestinal tract are equipped with OT receptors which facilitates the absorption of orally administered OT.[9,10] OT is involved in sexual maturation, if synthetic OT given to female neonates which can be disturbed sexual maturation and may be leads to precocious puberty as well as ovarian anomalies including increased ovarian weight, number of follicles, corpus luteum and ovulation via alteration of expression of proteins related to ovarian maturation and changing of hypothalamo-hypophyseal-gonadal axis and altering receptor expression of steroid in reproductive organ and brain region.[11] Neonatal manipulation by OT altering gonadal steroid systems via increasing estrogen receptor α (ERα) expression in the different regions of brain.[12, 13] Administration of OT to neonates from PND 0 to PND 7 caused increasing of ERα- immunoreactive cells at PND 75 in rat. OT administration during peripubertal period was increased OTR mRNA expression in the hypothalamus and plasma OT levels in adult rat. A repeated administration of an OT antagonist (OTA) to neonates from PND 0 to PND 7 decreases the expression of ERα in brain of rat.[14] Apart from these, exogenous OT was co-administrated with gonadotrophins to adult female rabbit increases proportion of atretic or degenerated follicles with luteinization.[15] Moreover, single dose of synthetic oxytocin to adult mice can cause impairment in folliculogenesis, ovulation and endometrial growth via alteration of pituitary gonadotrophins (LH and FSH).[16] On the basis of above background detail related to effects of OT on sexual maturation and sex steroids; therefore the present study tried to explore the chronic effect of OT on the puberty and estrous cyclicity by using a immature female mice model.

MATERIALS AND METHODS

Experimental chemical

The experimental chemical was synthetic oxytocin (OT) which commercially available and purchased from the registered medical shop of Bhopal (India), trade name Pitocin IP injection (10USP equivalent to 10IU/ml) manufactured by Pfizer Ltd., Nani Daman- 396210.

Animals and treatment protocol for intraperitoneal administration

Twelve Swiss albino mice female pups (10 days and body weight 8±2g) were obtained from the breeding colony of animal house of the Bioscience Department, Barkatullah University, Bhopal, MP, India. The animals were housed into polypropylene cages and acclimatized to standard laboratory condition i.e. temperature 23-25˚C with 10:14h, light: dark cycle and had free access to mice for food and water ad libitum. Maintenance and experimentations for care and use of laboratory animals were carried out as per the guidelines of Institutional Animal Ethical Committee (IAEC), Bhopal, MP, India. Twelve pups (along with their mother till weaning) were randomly divided into 2 groups as control and treated of 6 each. The treated and control groups were administered with an intraperitoneal (i.p.) dose of synthetic OT (50mIU/5µl/g body weight) and physiological saline (5µl/g body weight) respectively via insulin syringe (100U of 1 ml) up to 30, 60 and 90 days to avoid any skin damage. The day of birth of female pups was considered as postnatal day 1 (PND 1). This research was done according to the protocol of experimental bioassay on small animals from the Institutional Animals Ethics Committee (IAEC) of Barkatullah University, Bhopal, India with Registration No. 1885/GO/Re/S/16/CPCSEA and under the guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Vaginal opening determination

For this, female was hold in palm and examined the genitalia. When female mice mature or attain puberty, the vestibular skin covering of genitalia become thin, crease, finally open and exposed the vaginal pore.[17,18]

This was done from PND 10 till vaginal opening of treated and control group.

Estrous cycle study

Once vaginal opening determination was done then estrous cycle study were performed via vaginal smear analysis test.[17,19] For this about 10 µl of physiological saline (0.9 % NaCl) was inserted into vaginal cavity up to 2-5 mm by using tip of micropipette without any harm and flush the vagina cavity three to five times with same tip of micropipette. Later on vaginal fluid 10 µl was recovered, spread on the glass slide and kept for air drying. Then fixed specimens were stained with leishman stain and examined under light microscope. The each stage of estrous cycle was confirmed by the presence of mainly three type of cells such as anucleated cornified cells, epithelial cells and neutrophils. Then, photomicrography was done at 40x magnification via photomicrography unit (Motic-DMB1-B microscope fitted with digital camera). Estrous cycle study was performed daily from the day of vaginal opening to the ending of experiment and stage of estrous cycle of each group/day was decided on the basis of mean ± SEM of six animals of both.

Statistical analyses

One way analysis of variance by ANOVA software and standard error of mean (SEM) were done to compare and assess any/at all differences at P-values *P<0.05, **P<0.01 and ***P<0.001 as less significant, more significant and highly significant between the numerical data which were collected from the control and experimental groups by the method of Tukey's test.[20]

RESULTS

Effects of oxytocin on day of vaginal opening (VO)

OT treatment to female mice pups were showed significant decrease the day of vaginal opening (24.16 ± 0.30***) as compared to control (26.33 ± 0.21) [Figure 1].

Effects of oxytocin on day of first onset of estrus (I estrus)

In the research study, treatment of OT to immature mice pups were caused significant decrease the day of first onset of estrus (29.0 ± 0.36 ***) as compared to control (32.33 ± 0.42) [Figure 1].

The stages of estrous cycle of OT treated as well as control mice (Leishman 40X)

Proestrus stage was identified by mainly epithelial cells; estrus stage was identified by chiefly anucleated cornified cells; Metestrus stage was identified by many neutrophils and epithelial cells and very few cornified cells; Diestrus stage was identified by few cells, cellular debris and mucus strands [Figure 2].

Effects of oxytocin on length of estrous cycle

In control as well as treated mice estrous cycle repeated at intervals of 4-5 days in a cyclic manner as proestrus (P), estrus (E), metestrus (M) and diestrus (D) [Figure 2]. OT administration did not alter the length of normal estrous cycle up to PND 59 or 49th day of treatment. after then the cyclicity henceforth chanced in a way that the diestrus stage become short and this was sustained till PND 100 or 90th day of treatment whereas control group was not show any dramatic change as OT treated group [Figure 3].

Figure 1

Effect of synthetic oxytocin was showed significant decrease (P<0.001) day of vaginal opening and first estrus in female neonates. Mean ± standard error of mean of n = 6 animals; ***= highly significant (p<0.001) difference from the control with treated group by one way ANOVA.***

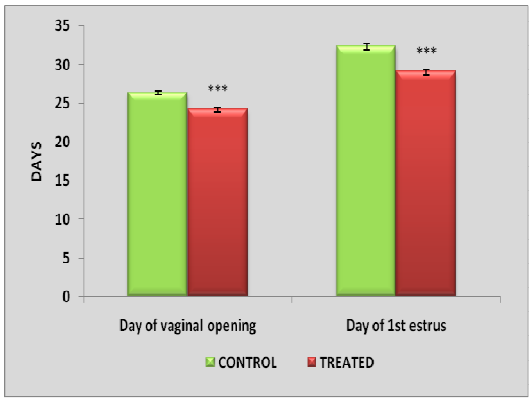


Figure 2

The stages of estrous cycle of OT treated as well as control mice were: proestrus (epithelial cells and few neutrophils); estrus (mainly cornified cells); metestrus (many neutrophils, epithelial cells and few cornified cells); diestrus (few cells and cellular debris) (Leishman 40X).

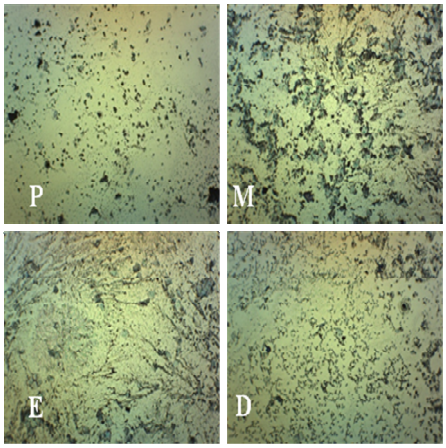
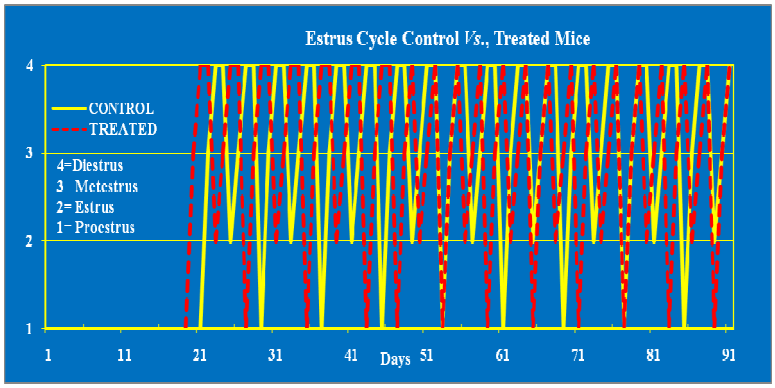


Figure 3

Estrous cycle of female albino mice Mus musculus (P), from 1st to 90th day of experiment of OT treated and control group. Treatment of OT shorts estrous cycle length from 49th day which was continued till 90th day. Whereas, control group shows normal estrous cycle.



DISCUSSION

Age of vaginal opening and onset of 1st estrus both are concurrent process which signifies puberty or maturation in rodents. In the present study, synthetic OT treated mice were showed significant decrease the day of vaginal opening and first onset of estrus as compared to control group. The research study of Parent and colleagues were showed that, OT advances the onset of female puberty and first estrus in the immature female rat via accelerate pulsatile gonadotrophin releasing hormone (GnRH) synthesis in hypothalamus and this was achieved by glial production of Prostaglandin E2. PGE2 mimicked the stimulatory effect of oxytocin on GnRH pulse frequency. They also proved that, administration of oxytocin antagonist (OTA) or inhibition of PG synthesis in immature female rat which can be decreased GnRH pulse frequency and delayed the age at vaginal opening and first estrus.[21] Further research study of Mishra and her coworkers next to proven that, production of PGE2 is increased via the elevation of carboxygenase-2 (COX-2) enzyme in OT treated neonates.[11] Apart from this, GnRH of hypothalamus trigger to the pituitary for releasing pituitary gonadotrophins (LH and FSH). When exogenous OT given to immature rat at proestrus caused advanced gonadotrophins secretion.[22] The increase level of LH and FSH further enhances preovulatory follicular development, advances ovulation via releasing ovarian steroid hormones secretion such as estrogen and progesterone.[10,16,22] As well as, exogenous OT alters the several proteins expression such as AKT and ERK which are related to first onset of estrus i.e. oocyte maturation, cumulus expansion and ovulation in immature rat.[11] Moreover, authors present a research study in which exogenous OT can dilates the cervix of adult nonpregnant ewes during treatment but mechanism was not be discussed.[23] Female mice are polyestrous, breeding throughout the year and they exhibit regular estrous cycles on attaining the age of puberty. Estrus the period of sexual receptivity of the female usually occurs spontaneously at night and lasts for 12 to 14 hours. Ovulation occurs spontaneously within 8 to 12 hours after the onset of estrus.[24] The present study suggest that, OT treated group was showed significant shorten the estrous cycle as compared to control group. The synthetic oxytocin administration caused premature luteolysis which reduced the length of the estrous cycle in ewes.[25,27] Although reports were available that, OT regulates estrous cycle length via follicle luteinization and ovarian

steroidogenesis.[3,27] Only one or two day's treatment of OT on adult mice caused abnormal ovarian development such as significant increase volume of ovaries, corpus luteum and ovulation than control due to enhance ovarian steroidogenesis.[16] Then ovarian steroid give positive feedback to pituitary and hypothalamus for gonadotrophins and GnRH secretion for further releasing of more ovarian steroids (estrogen and progesterone).[16,28,29] One more study also proved that, exogenous OT significant (3 fold) increase serum estrogen levels than controls mice.[10]

CONCLUSION

Exogenous OT significant decreases the day of vaginal opening and first estrus of immature mice at a test dose 50mIU/g body weight and selected experimental duration. There was also found that, synthetic OT altered the estrous cyclicity of mice. Therefore, synthetic oxytocin may cause unwanted consequences on reproductive process that need to be explored further.

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CONFLICTS OF INTEREST

Conflicts of interest declared as none.

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