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Research Paper

Effects of exogenous oxytocin and atosiban antagonist on GABA in different region of brain

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ABSTRACT

Gamma aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the vertebral central nervous system. It functions by altering the membrane conductance of Cl− ions, maintaining the membrane potential close to the resting potential. The hormone oxytocin (OT) has a central action where it acts as a neuromodulatory peptide and exerts its action depending upon the distribution of OT receptors (OTR) in the target site. OTRs are G-protein-coupled receptors (GPCRs) comprising different subunits (Gq, Gi, and Gs). The G-protein isoforms have the ability to activate different pathways, but specific agonists and antagonists may show different affinities to OTRs, depending on the specific G-protein isoform to which they are coupled. It is well documented that OTR distribution varies with age and species and in regions of the brain. In this study, we attempted to observe the impact of OT and atosiban (OTA), an OT antagonist, on GABA levels in different regions of the brain. Study animals were exposed intraperitoneally (I.p.) to normal saline (0.89%), OT 0.0116 mg/kg, and OTA 1 mg/kg in different combinations, for 30 days. It was observed that OT and OTA administration modulated GABA levels in different regions of brain, while normal saline had no effect. It may be due to OTR receptor expression in different regions of the brain.

This is significant because region-specific expression of different receptors could be important in the development of new drugs targeting specific neuropsychiatric disorders.

1. Introduction

Oxytocin (OT) is a neuropeptide synthesized in the hypothalamus by neurosecretory cells (magnocellular neurons) of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON), and secreted by the posterior pituitary lobe into the blood (Bargmann, 1949). OT neurons are also present in the parvocellular neurons of the PVN, suprachiasmatic nucleus, bed nucleus of stria terminalis (BST), medial amygdalae, dorsomedial hypothalamicus, vertical diagonal band of Broca, and olfactory bulb nuclei in rats (Buijs, 1978; Caffé and Leeuwen, 1983). However, OT neurons are absent from the dorsomedial hypothalamus, vertical diagonal band of Broca, and olfactory bulb of mice (Caffé and Leeuwen, 1983; Tobin et al., 2010). It is possible that this is related to species-dependent differences in social behavior. OT is a hormone involved in different physiological and pathological functions like sexual activity, penile erection, ejaculation, pregnancy, uterus contraction, milk ejection, maternal behavior, and social bonding among others (Stoop, 2012). In addition, OT acts centrally as a neurotransmitter and the release of OT within the brain occurs from dendrites, axons, and somata of magnocellular neurons of the PVN in different regions of the brain (Dumais and Veenema, 2016; Moghadam et al., 2018). Furthermore, OT plays an important role in the brain by interacting with specific receptors in different regions of the brain and helps in neuromodulations. It has been shown that receptor distribution varies with age (Elizabeth and Hammock, 2015) and species of animal (Dumais and Veenema, 2016).

In a recent study, it was also shown that OT protects against inflammation and oxidative stress, which is due to OT and GABA receptor interaction in the CNS (Kaneko et al., 2016). Gamma amino butyric acid (GABA) is the principal inhibitory neurotransmitter synthesized by decarboxylation of glutamate through the action of glutamic acid decarboxylase (GAD) and binds to three receptors namely GABA_A, GABA_B, and GABA_C (Kaneko et al., 2016; Roberts, 1960).

GABA_A receptors are ionotropic Cl− channels gated by the major inhibitory neurotransmitter γ-aminobutyric acid and are widely expressed throughout CNS. They play a major role in synaptic inhibition in the CNS (Kaneko et al., 2016; Wisden and Seeberg, 1992). OT modulates GABA_A receptor subunit expression, which mediates the
Numerous studies in humans and animals have established that OT affects the social life of mammals and reduces anxiety (Sabihi et al., 2017). Various brain regions have been identified as the site of action for the anxiolytic effects of OT, including the hypothalamic PVN (Blume et al., 2008; Smith et al., 2016), amygdala (Bale et al., 2001; Neumann, 2002), raphe nucleus (Yoshida et al., 2009), and prelimbic (PL) region of the medial prefrontal cortex (mPFC) (Sabihi et al., 2014a,b). Apart from this, evidence also suggests that OT interacts with GABA to reduce anxiety (Nuss, 2015; Smith et al., 2016). Therefore, interest is growing towards the study of neuropeptides and their region-specific receptors that may be important when designing new drugs targeting specific neuropsychiatric diseases (Busnelli et al., 2013). The mouse, which is widely used in neuroscience research, has a rich social life. Like other rodents, their social communication and behavior depends upon their sex, age, and hormonal status, among other factors. The important of OT on different regions of the mouse brain is less well-documented. Therefore, in the present investigation we have tried to evaluate the impact of exogenous OT and atosiban (OTA) on GABAergic transmission in different regions of the brain.

2. Results

OT and OTA treated animals showed significant changes in GABA contents in different regions of the brain relative to the control group. After 30 days exposure to OT and OTA, GABA levels were significantly higher (p < 0.01, one-way analysis of variance [ANOVA]) in the hypothalamus and cerebellum of the brain of Mus musculus than those of the control group. However, these values were significantly lower (p < 0.01, one-way analysis of variance [ANOVA]) when OTA was administered along with OT for 30 days than in the exclusively OT-or OTA-treated groups (Fig. 1). OT reduced the GABA levels in the hippocampus, cerebral cortex, medulla oblongata, and striatum of the brain of Mus musculus relative to the control groups. However, when OTA was administered along with OT, out of the seven regions of the brain considered, only four regions of the brain i.e., the hippocampus, cerebral cortex, medulla oblongata, and striatum, showed an increase in the GABA level in comparison to those in OT exposed animals. Three regions, i.e., the hypothalamus, cerebellum, and midbrain, showed greater lowering of the GABA levels when we administered OT along with OTA for up to 30 days.

3. Discussion

Numerous studies on humans as well as animals have shown that OT reduces anxiety (Ayers et al., 2011; Bale et al., 2001; Blume et al., 2008; Oliveira et al., 2012), and leads to expressions of social behavior (Stoop, 2012) by interacting with GABA in the CNS, effects which vary with species and sex (Elizabeth and Hammock, 2015). The mouse, which is widely used in neuroscience research, has a rich social life. Similar to other rodents, their social communication and behavior depends upon their sex, age, and hormonal status, among other factors. The present study revealed a novel mechanism underlying the effect of inter-epitrochinal (i.p.) administration of OT and OTA on GABAergic transmission in different regions of the brain. Intraperitoneal administration of OT crosses blood brain barrier (Mizuno et al., 2015; Neumann et al., 2013; Peñagarikano et al., 2015) and modulate social behavior buffer anxiety in autism, schizophrenia as well as recovery of neurodegenerative disorder. In present study OT significantly increased (p < 0.01) GABAergic neuronal activity in the hypothalamus, midbrain, and cerebellum in the experimental group.

OT activates GABAergic neurons by interacting with the GABA_A receptor in the hypothalamo-pituitary-adrenal (HPA) axis in the hypothalamic PVN, and reduces anxiety, increases social buffering, and calmness in females (Blume et al., 2008; Smith et al., 2016). Apart from this, exogenous OT increases GABA in the midbrain. Central OT plays an important role in the reward system through its effects on social behavior like social reward, social learning, pair bonding, parenting, and mating (Choe et al., 2011; Dolen et al., 2013; Gimpl and Fahrenholz, 2001; Love, 2014; Marlin et al., 2015). A number of studies have demonstrated the modulatory action of OT and dopamine (DA) in the CNS and characterized axonal projection from OT neurons of the hypothalamic PVN to midbrain DA regions (Charlet and Grinevich, 2017; Xiao et al., 2017). OT neurons are exclusively present in the hypothalamus, but DA neurons are present in the ventral tegmental area (VTA) and substantia nigra (SN). However OTR are present in the VTA as well as the SN. OT activates two pathways; it directly activates VTA neurons and indirectly inhibits SN neurons through local GABAergic interneurons (Charlet and Grinevich, 2017; Xiao et al., 2017). In this experiment, it was also found that GABA activity was higher in the cerebellum. The cerebellum is known to play an important role in classical conditioned reflex responses, mental imagery, affective behavior, and control of sensory data acquisition (Fatemi et al., 2012). Many of these functions are disturbed in autism. GABA is the principal inhibitory neurotransmitter in several brain regions including the cerebellum and is synthesized by decarboxylation of glutamate through the action of GAD. GABA binds to three receptors, namely, GABA_A, GABA_B, and GABA_C (Kaneko et al., 2016; Roberts, 1960). GABA_A receptors help in the opening of chloride ion channels in the cell membrane that are gated by GABA, causing hyperpolarization and inhibition of neuronal excitation (Bing et al., 2018).

The GABAergic neuromodulatory mechanism of OT depends upon the distribution of OTRs, which varies in different regions of the brain. OTRs are members of the G-protein-coupled receptor (GPCR) superfamily. The structure of GPCRs is characterized by seven
transmembrane (7-TM) α-helices connected by three intracellular (IL-1 to IL-3) and three extracellular loops (EL-1 to EL-3). These receptors can be coupled to different G-proteins and exhibit different functions. An OTR coupled with the heterotrimeric Gq/11 protein activates the phospholipase Cβ pathway (PLCβ), causing the release of Ca²⁺ from intracellular stores, increasing neuronal excitation (Gimpl and Fahrenholz, 2001) and thereby enhancing GABA release from interneurons (Breton et al., 2008; Lara et al., 2009; Piloni et al., 2006). The Gq/11 family of proteins consists of four members, two of which (Gq and G11) are almost solely expressed in the CNS (Tanaka et al., 2000). These appear responsible for maternal behavior after parturition in females (Wetschureck et al., 2004). Furthermore, OT can also activate the inward rectifying current through the Gi/o protein, which is also responsible for its antiproliferative effect (Gravati et al., 2010). In addition, the Gs protein of the OTR can also increase cAMP production by activating adenylyl cyclase, which opens sodium channels (Stoop, 2012).

In fetal rats, OT increased the intracellular chloride concentration in GABA neurons, thereby reducing neuronal excitability; this process was thought to protect the neonate from anoxic injury (Tyzio et al., 2006, 2014).

Apart from this OTA activates different signaling pathways by coupling with different OTR subunits. For example, OTA is used in the treatment of preterm labor due to its antagonistic ability to block Gs/PLC/calcium signaling pathway in myometrial cells (Stoiber et al., 2018). However, OTA shows biased agonist properties by coupling with OTR Gs, inhibiting the proliferation of some cancer cells (Reversi et al., 2005), therefore its use limited to only 48 h in patients with a high risk of preterm delivery (Kim et al., 2017). Recent evidence indicates the antagonistic properties of OTA in the CNS (Abdullahi et al., 2018), OT is well known for its action on social memory and memory consolidation when identifying novel objects in the nucleus basalis of Meynert; OTA also impaired memory consolidation in the CNS (Gard et al., 2012). Our finding also supported the antagonistic properties of OTA in the hypothalamus, midbrain, and cerebellum of the experimental group. However, in certain regions, i.e., the medulla oblongata, cerebral cortex, striatum, and hippocampus, OTA does not show an inhibitory action on OTRs, indicating that OTA activates different signaling pathways in the CNS by binding with different receptor subunits.

Apart from this OT and vasopressin (VP) are two important neuropeptide in CNS and plays an important role in the control of social, cognitive, and neuroendocrine function (Sala et al., 2011). OT and VP have high degree of similarity in their structure and structure of receptor i.e. OTR and V1a receptor (Gimpl and Fahrenholz, 2001; Mynbauer et al., 2008; Zhimin et al., 2016). Evidences from previous study support that OT and VP control social behavior and physiological responses by activating each other's receptor i.e. OTR and V1a, in CNS (Zhimin et al., 2016). In summary, the findings of the current study demonstrated that long term exposure to exogenous OT and OTA activate different pathways in different region by binding with different receptor subunits in female mice. This represents a very valuable tool for investigating the molecular basis of neuropeptide action in the CNS.

3.1. Conclusion

From the study we can conclude that G protein isoforms have the ability to activate different pathways, but specific agonists and antagonists may show different affinities to OTRs because, their action depends on the specific G protein (Gq, Gi or Gs) to which they are coupled. For example, the OTR antagonist, OTA does not affect receptor internalization, possibly due to the selective activation of only those OTRs that are coupled to a Gi protein (Busnelli et al., 2013). We can also conclude that region-specific expression of different receptors could be important for the development of new drugs targeting specific neuropsychiatric disorders.

4. Experimental procedure

4.1. Animals

The present experiment was performed on mature female mice, Mus musculus, weighing 25 ± 5 g. All animals were acclimatized to laboratory conditions, i.e., 25 ± 3°C, and light and dark photoperiod (12 h light:12 h dark) in the animal house of the Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal, India. Hygienic conditions were maintained with rice husk bedding in separate polypropylene cages. Animals were provided with standard feed and water ad libitum. The study was performed with the approval of the ethical committee, i.e., Institutional Animal Ethics Committee (IAEC) of CPCSEA (Ref No.1885/GO/S/16/CPCSEA/IAEC/B.U./04).

4.2. Chemical

OT (brand name Pitocin) was purchased from a local medical shop in Bhopal, Madhya Pradesh, and the OT antagonist i.e., OTA, was obtained from Sigma –Aldrich, for this experimental study.

4.3. Preparation of dose

OT 0.0116 mg/kg and OTA 1 mg/kg was prepared in 0.89% normal saline. The dose of OT and OTA was finalized after reviewing various previous studies and confirmed through experimental investigation (Han et al., 2016; Mizuno et al., 2015; Teng et al., 2016).

4.4. Experimental design

The mice were divided into four groups of six each. The control group received a balanced diet, water ad libitum, and normal saline (0.89%) for 30 days. Group 2 received a balanced diet, water ad libitum and was treated daily with OT 0.0116 mg/kg i.p. for 30-days. Group 3 received a balanced diet, water ad libitum, and was treated daily with OT 0.0116 mg/kg and OTA 1 mg/kg i.p. for 30-days. Group 4 received a balanced diet, water ad libitum, and was treated daily only with OTA 1 mg/kg i.p. for 30-days.

4.5. Dissection

After 30 days of treatment, all animals from the different groups were killed by cervical dislocation. The brains were carefully removed, washed in normal saline, and kept at –20°C. The dissection was performed on an ice-cooled glass plate. The frozen tissue was divided into seven regions, the medulla oblongata, midbrain, cerebellum, cerebral cortex, striatum, hippocampus, and hypothalamus, adopting the methodology of (Glowinski and Iversen, 1966) as follows.

First, the rhombencephalon (A) was separated by a transverse section from the rest of the brain and dissected into two parts, the cerebellum and medulla oblongata. A transverse section was made at the level of the optic chiasma, which delimits the anterior part of the hypotalamus, and through the anterior commissure. This section separated the cerebrum into two parts, B and C. Part B was divided into five fractions. First, the hypothalamus was dissected by taking the anterior commissure as a horizontal reference and the line between the posterior hypothalamus and mammillary bodies as the caudal limit. The striatum was dissected with the external walls of the lateral ventricles as the internal limits and corpus callosum as the external limits. The frontal parts of the striatum, which are in section, were dissected separately from the remaining parts of the brain. The hippocampus was then dissected. The remainder of part B was combined with the remainder of part C to form the cortex. An example of the reproducibility of this procedure is given in Table 1.
4.6. Assay

Different regions of tissue were blotted, weighed, and placed in different tubes containing 5 ml ice cold TCA (10%W/V), homogenized, and centrifuged at 10,000 rpm for 10 min at 0 °C. Next, 0.1 ml of supernatant was dissolved in 0.2 ml of ninhydrin solution (0.15 M) in 0.5 M carbonate-bicarbonate buffer (pH 9.95). This mixture was kept in a water bath maintained at 60°C for 30 min and then cooled. The cooled mixture was treated with 5 ml of copper tartarate reagent. After 10 min, florescence was observed at 377–455 nm by using a spectrofluorometer (Lowe et al., 1958).

4.7. Data analysis

Results are expressed as the mean and standard error of mean of different groups. The intergroup variation was measured using a one way analysis of variance (ANOVA) followed by Tukey's test (Tukey, 1949). Statistical analysis was performed using the Sigma Stat Statistical Software version 3.5. p < 0.05 was considered statistically significant.

Conflict of interest

The authors declare no conflict of interest.

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