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Evaluation of Antianxiety Effect of Oxytocin in Rats

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Abstract: ***Aim: To evaluate the anti-anxiety effect of oxytocin in male rats using elevated plus maze. Materials and Methods:18 male rats were randomly allocated into three groups of six animals each. Rats in group 1 served as control and received distilled water intraperitonealy. Group 2 served as standard and received Diazepam 2 mg/kg ip, the 3 rd group received oxytocin 4mcg/kg ip. Each of the eighteen rats is placed individually at the center of elevated plus maze and observed for five minutes. Preference for first entry, number of entries into the open and closed arms and time spent in each arm of the maze will be recorded. One hour after drug administration the experiment is repeated and results tabulated and analyzed using suitable statistical method. Results: The time spent in open arm is increased in oxytocin treated group when compared with control and is comparable to diazepam.Conclusion: The findings in this study suggest that oxytocin possess Antianxiety activity and various studies has shown that oxytocin acts by inhibiting the amygdala through GABA receptors like diazepam. Further studies and researches are needed to optimize the dose and use it as a novel antianxiety agent.***

Keywords: Anxiety, oxytocin, open arm, elevated plusmaze, GABA, Amygdala

1. Introduction

Oxytocin is a medication and hormone.[1] It was discovered in 1952[2], It is on the WHO's list of essential medicines the most important medication needed in basic health system[3]. Oxytocin is normally produced in the hypothalamus and stored in the posterior pituitary gland.[4,5]. It is released due to stretching of cervix and uterus during labour and with stimulation of nipples from breast feeding.This help with birth, bonding with the baby, and milk production[5,6]. It plays a role in social bonding, sexual reproduction in both sexes, and during and after childbirth.

Studies have looked at oxytocin's role In various behaviors including orgasm, social recognition, pair bonding, anxiety, and maternal behaviours.[7] Behavioral effects is due to release from centrally projecting oxytocin neurons and is different from those that project from pituitary.[8]

Oxytocin receptors are expressed in neurons of many parts of brain and spinal cord including the amygdala ventromedial hypothalamus, septum, nucleus accumbens and brain stem. It evokes feelings of contentment, reduction in anxiety and feelings of calmness and security. This shows Oxytocin may be important for inhibition of brain regions associated with behavioral effects, stress, fear and anxiety. Research demonstrates that Oxytocin can decrease anxiety and protects against stress. Nasally administered oxytocin is reported to reduce fear possibly by inhibiting amygdala (which is thought to be responsible for fear response).Studies in rodents has shown oxytocin can efficiently inhibit fear responses by activating an inhibitory circuit with in amygdala [9]

Anxiety and fear are normal emotions with great adaptive value that have been selected along the evolutionary process. While fear occurs in response to specific threats, the source of anxious behavior is usually undefined or unknown. In contrast to normal/adaptive anxiety, anxiety disorders affect the individual performance of daily life tasks,[10] representing a high cost for public health care all over the world. [11] Hence there is a continuous search for an ideal anxiolytic.

The present study was proposed to evaluate anxiolytic effects of oxytocin in comparison with diazepam using elevated plus maze.

2. Materials and Methods

Oxytocin: 2mcg /unit. Each ampoule containing 1ml/ 5u/ 10 mcg
Diazepam: Each ampoule contains 5mg / ml.
Animals:
Albinorats were obtained from the animal house of the Institute of Pharmacology, Madurai Medical College Madurai. Animals were preconditioned in the lab for one week before the experiment to get accustomed to the laboratory condition. They were allowed free access to food and water under a 12 hour light dark cycle. The animal procedures have been approved and prior permission from Institutional ethical committee was obtained as per prescribed guidelines.

3. Procedures

Elevated plus maze
The elevated plus maze (EPM), perhaps the most employed animal model of anxiety in current practice, was first proposed by Handley & Mithani [12] and further validated by File et al. [13] The apparatus is raised 50cm above floor level, and is composed of two enclosed arms (50cm x10cm x 40cm) opposed perpendicularly by two open arms (50cm x 10 cm), (picture 1)

The test is based on the natural tendency of rodents to explore novel environments and their innate avoidance of unprotected, bright, and elevated places (represented by the open arms).

Physiological signs of stress are confinement to the closed arms, increased defecation and increased cortisol level, [13] whereas exposure to classical anxiolytic drugs, such as benzodiazepines, increases exploration of these arms. [13]

Picture 1
ELEVATED PLUS MAZE - STANDARD FOR RAT

The animals were divided into three groups each group containing six animals (n=6)

Table 1

S. NO	*Group*	*Treatment*
1	Control	Normal food and water + distilled water ip
2	Standard	Normal food and water + Diazepam 2mg/Kg- ip
3	Test	Normal food and water + Oxytocin 4mcg/Kg ip

Animals are treated accordingly and after one hour of drug administration the rats are placed at the centre of plus maze facing towards open arm. The animals were observed for five minutes and experiment was video recorded. The preference of the animal for the first entry, number of entries into the open and closed arm and time spent in both arms are recorded and tabulated.

4. Results

The effects of oxytocin (4 mcg/kg-ip) and diazepam (2mg/Kg- ip) resulted in significant increases in the total number of entries into the open arms as in Figure 1.

Significant increase in the duration of time spent in the open arm was observed in oxytocin (4 mcg/kg) treated rats compared with control ($p < 0.001$) and is comparable to that of Diazepam as in figure 2..Diazepam produce a lowest number of entries in the closed arm($p<.001$) while control had the highest closed arm entry value of (Figure 3) Number of entries in closed arm produced by Oxytocin has also decreased when compared with control($p < 0.001$) as in figure. The time spent in closed arm did not have significant change in oxytocin treated group compared with diazepam.

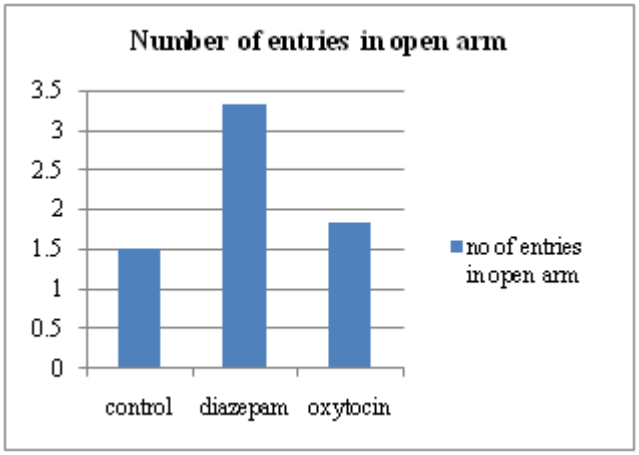

Figure 1

Administration of oxytocin (4 mcg/kg) to the rats has significant ($p <. 003$) increase in the frequency of the open arm entries when compared with control group as seen in picture 2.

Rat entering open arm of elevated plus maze:

Picture: 2

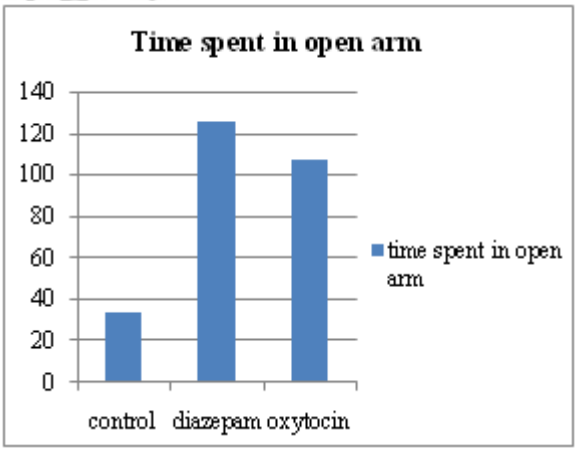

Figure 2

Significant increase in the duration of time spent in the open arm was observed in oxytocin (4 mcg/kg) treated rats compared with control ($p < 0.001$) and is comparable to that of Diazepam.

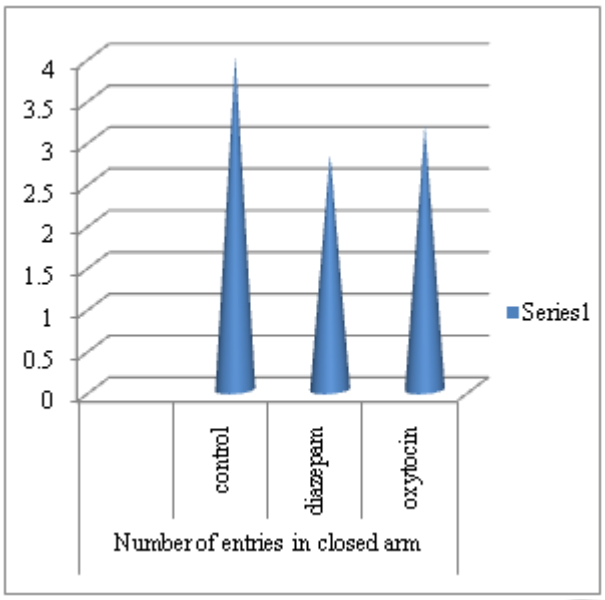

Figure 3: Number of entries in closed arm

Diazepam produce a lowest number of entries in the closed arm($p<.001$),while control had the highest closed arm entry value of (Figure 3) Number of entries produced by Oxytocin has also decreased when compared with control($p<0.001$)

5. Statistical Analysis

All analysis was performed using spss software. Datas are analysed using one way ANOVA whenever ANOVA was significant further comparison between vehicle and drug treatment groups were performed by post hock method. The level of significance adopted was $p < 0.05$.

6. Discussion and Conclusion

Oytocin has produced increase in time spent in open arm in elevated plus maze. Number of entries into closed arm is also decreased. This shows that there is decreased anxious behavior in the rats compared to control group. Oxytocin is a neuropeptide that can reduce neophobia and improve social affiliation. In vitro, oxytocin induces a massive release of GABA from neurons in the lateral division of the central amygdala which results in inhibition of a subpopulation of peripherally projecting neurons in the medial division of the central amygdala. Common anxiolytics, such as diazepam, act as allosteric modulators of GABA (A) receptors. Because oxytocin and diazepam act on GABAergic transmission, it is possible that oxytocin can potentiate the inhibitory effects of diazepam if both exert their pre, -respectively postsynaptic effects on the same inhibitory circuit in the central amygdala. These findings show that oxytocin and diazepam act on different components of the same GABAergic circuit in the central amygdala and that oxytocin can facilitate diazepam effects when used in combination. This raises the possibility that neuropeptides could be clinically used in combination with currently used anxiolytic treatments to improve their therapeutic efficacy. The trust inducing property of oxytocin might help those with social anxiety and depression. Evidence shows it has a novel treatment in autism and schizophrenia[16]

Animal models of anxiety do not intend to replicate all features and symptoms of a specific anxiety disorder but rather generate a state of anxiety that could be related to these disorders.[17]Hence further evaluation and studies are needed to use oxytocin as an anxiolytic drug.

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8. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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