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Does Orexin B Infused into Nucleus Accumbens Affect Open Field Activity In Wistar Rats?

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ABSTRACT

Orexins, which have been located in several parts of brain particularly in the hypothalamus and parts of diencephalon were implicated role in ingestive behaviour, role in sleep, wakefulness and other modalities of activities in animals. Nucleus accumbens is a large nuclear mass involved in appetitive behaviour and addiction. In order to evaluate if the Orexinergic system in open field activity, we carried out studies by infusing Orexin B and its antagonist into nucleus accumbens, in Wistar albino rats weighing 200±20 g at the time of selection. Orexin B was infused bilaterally into Nucleus accumbens and tested in open field for time spent in the peripheral squares, central squares, defecation score, rearing, grooming. Similar recording was done following infusion of Orexin B antagonist (TCS-OX2-29) into Nucleus accumbens in the next experiment. The infusion of orexin B into nucleus accumbens rats made significantly more ($p<0.001$) central square entries compared to controls. There was also a significant increase ($p<0.001$) in time spent in central squares (28±0.73) compared to controls (20± 1.15). But treatment didn't show any alteration in the number of rearing, grooming and fecal excreta. The infusion of orexin B antagonist (TCS-OX2-29) into nucleus accumbens made significantly fewer ($p<0.01$) central squares entries compared to controls. There was also a significant decrease ($p<0.001$) in time spent in central squares compared to controls but didn't show any alteration in the number of rearing, grooming and fecal excreta. The results of this study reveal that the Orexin infusion decreased anxiety and increased exploratory behaviour in the rats.

Keywords: Orexin B, Orexin B Antagonist TCS-OX2-29, Open field, Anxiety behaviour.

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INTRODUCTION

Orexins are couple of peptides found in dorsal and lateral hypothalamic areas [1]) and were of interest due to their varied localisations. Other than brain areas, they are also found in GIT, testis and pancreas. [2] Sakurai and others have suggested role for orexins in feeding behaviour [3]. Orexins have their respective G protein linked receptors, OX1R, OX2R, [2] and they were identified in the hypothalamus [3]. Particularly Orexin 2R found in paraventricular nucleus. [4] Orexins reportedly lead to release of Noradrenalin in the central nervous system, which is Ca++ dependant. [5] Studies by intracerebroventricular infusion studies of orexins revealed increased cFos expression in centres like raphae nucleus, supraoptic and paraventricular nucleus, [6] suggesting that it may affect the water consumption etc. Orexinergic system has been implicated in the depression related neurophysiological physiological processes and cognition [7,8] and learning and memory [9,10]. An increased interest has arisen as for the function of orexinergic system in neuropsychiatry. [11] Orexin B antagonist has been used to block the orexinergic activity to study the effects. Focal injections of TCS OX2 29, specific antagonist for orexin B into nucleus accumbens to study the conditioned place preference, showed slight effect, but Orexin A antagonist showed marked effect on parameters studied [12]

In the present study, we intended to infuse Orexin B into Nucleus accumbens and evaluate the effect on the exploratory and anxiety behaviours in the Wistar rats in open field. Since the reports suggest role for orexins in alertness, sleep and wakefulness and cognitive parameters, we predicted a role for it in the anxiety behaviour. We tested the rats by focal infusion of orexin B and subesequently its receptor antagonist using stereotaxic technique and tested the animals in open field. It is first study to our knowledge using the microinjection technique into nucleus accumbens and the results are discussed.

MATERIALS AND METHODS

Wistar albino male rats (n=18), weighing about 200±20 g at the time of selection were procured from the central animal house and housed individually in plexi glass cages with ad.lib. food and water, in the standard animal house. All procedures and animal care was done according the highest ethical standards. Before the beginning of experiments, clearance was obtained from institutional ethical committee for animals experiments. Cannula were implanted in the nucleus accumbens bilaterally by method developed in our laboratory explained in our previous paper.[13] They were aneasthetised by injecting a cocktail of Ketamine (60 mg) and xylazine (6 mg) per kg body weight. Stereotaxic coordinates were selected using Paxinos and Watson stereotaxic atlas for rat brain. [14] The guide cannula was was implanted and secured by screws and dental cement. They were left for a week for healing and hardening of the implant. Subsequently the infusion procedure was carried out in awake free moving animals in their home cage after fasting for 24 hours before the trials.

Orexin B was infused at a dosage one microliter solution of 30 nM/microliter over a period, to infuse 0.6 microlitre per minute. The internal cannula was left in place for additional 30 sec for complete transfer of fluid. Orexin B antagonist was infused at a rate of one microliter of solution containing 10 microgram/microliter.

Three trials were carried out and the average scores were tabulated. Separate groups of rats were infused with Orexin or its receptor antagonist into nucleus accumbens. Following the infusion, they were tested in the open field apparatus. The control rats did not receive orexin, but they were infused with normal saline and rest of procedures followed were same as experimental animals.

Confirmation of accuracy of site of infusion was done by histological sectioning of the brain collected following study. The brain carefully collected, was preserved in formalin, processed to prepare wax blocks for sectioning. They were sectioned into 5 micron sections and stained by cresyl blue stain and observed under light microscope at 10X magnification for the location of cannula. Only the data from rats receiving proper cannula implantation position are tabulated and statistically analysed.

Open field exploration test (Procedure)

This is one of the most widely used methods to assess the motor, exploratory activities and emotional reactivity of rats.[15] The open field apparatus (Techno, Lucknow, India) consists of a rectangular box (100x100x40 cms); the floor area marked into 25 squares – 16 peripheral and nine central squares (5x5 cms). A uniform illumination was provided with 60 watts bulb fixed 60 cm above the centre of field.

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Procedure: Rat was placed in one corner of the open field test apparatus and its behaviour was observed for 5 minutes. The number of peripheral (close to wall) and central crossings were observed for individual rat. In addition to this, rearing (frequency with which rats stood on their hind limb) and grooming (use of head, tongue and fore limb for the process of cleaning various part of the body) behaviours were quantified. After exploration the rat was returned to home cage. The number of faecal boli were counted. Those animals which ambulate less and defecate more are considered more anxious than the animals with high ambulation and less defecation score. [15] Between tests, the apparatus was thoroughly cleaned with 70% Ethyl alcohol and allowed to dry. All testing was carried out in a temperature, noise and light controlled room.

RESULTS

Effect of bilateral micro infusion of orexin B & antagonist (TCS-OX2-29) into nucleus accumbens on exploratory and anxiety behaviour of rats (Table 1)

The infusion of orexin B into nucleus accumbens made significantly more (p<0.001) central square entries (17.83±1.24) compared to controls (11.3±0.49) .There was also a significant increase (p<0.001) in time spent in central squares (28±0.73) compared to controls (20± 1.15). Treated rats did not show any alteration in the number of rearings, grooming and fecal excreta. The infusion of orexin B antagonist (TCS-OX2-29) into nucleus accumbens led to significantly fewer (p<0.01) central squares entries (6.5±0.76) compared to controls (11.3±0.49). There was also a significant decrease (p<0.001) in time spent in central squares (10.33±0.42) compared to controls (20± 1.15). Orexin antagonist infusion in nucleus accumbens also didn't show any alteration in the number of rearing, grooming and number of fecal excreta.

Table 1: Open field in orexin B and antagonist infused rats in nucleus accumbens. Data are expressed as mean±SEM. (n=6 for each group) (One way ANOVA, Student -Newman- Keuls. multiple comparison test)

Groups	Open field test-Nucleus accumbens						
	No. of central squares moved	No.of peripheral squares moved	Time spent in central squares (seconds)	Time spent in peripheral squares (seconds)	Grooming	Rearing	Defecation
Control	11.3± 0.49	23.33± 1.38	20± 1.15	280± 1.15	3.5± 0.43	1.33± 0.33	0.83 ± 0.40
Orexin B	17.83± 1.24***	24.5± 2.4	28± 0.73***	272± 0.73***	4.5± 0.43	1.17± 0.30	1.67 ± 0.30
TCS-OX2-29	6.5± 0.76$$	19± 2.25	10.33± 0.42$$$	289± 0.74$$$	4.17± 0.30	0.83± 0.3	0.83± 0.30

****p<0.001 control vs. orexin B. $$p<0.01, $$$<0.001 control vs. TCS-OX2-29*

DISCUSSION

Apart from role of orexin B in feeding and addiction, it has been also implicated in sleep and wakefulness, [16] and dysregulation of orexin neuropeptide system affected sleep and motor activities. [17] Orexin deficiency caused narcolepsy in humans and animals. [18] In the present study, we investigated the open field activity of rats following micro injection of Orexin B into nucleus accumbens. We found an increased activity and reduced anxiety behavior in such rats. They occupied central squares more time and peripheral squares less. Reduced anxiety behaviour was evident and supports the view that orexins are active in producing wakeful state. However there was no sign of anxiety as evidenced by unchanged effect on fecal excretion, grooming and rearing. It supports the earlier findings reporting that orexin neurons in the PFH and DMH regulate arousal. [19] Studies on transgenic animals had demonstrated that orexin could be involved in connecting information about nutritional and metabolic states and promotion of arousal. Rats responded to reduced food availability by becoming more wakeful and active. [20] Also, orexin neurons receive abundant projections from the limbic system which might be important for increasing arousal during emotional stimuli. It was reported that projections from limbic system regulate orexin neurons during active period which could involve emotional inputs [21] also support the findings of our experiments.

Previous studies have reported that effect of orexin on wakefulness is largely mediated by activation of histaminergic system through OX_2R. [21, 22] However, to date there are no specific studies of infusion of orexin

B into limbic areas such as NAc to assess the motor, exploratory activities and anxiety behaviour of rats. In the present study, orexin B and it's antagonist - TCS-OX2-29 were infused bilaterally into NAc of Wistar rats and open field exploration test was conducted to assess the motor, exploratory activities and anxiety behaviour of rats. Infusion of orexin B into NAc induced significantly more central square entries in injected rats when compared to controls (Table 1). There was also a significant increase in time spent in central squares compared to controls (Table 1). Micro infusion of orexin B antagonist (TCS-OX2-29) into NAc induced significantly fewer central squares entries and significantly less time spent when compared to controls (Table. 1). These observations indicate the effect of orexin B on exploratory behaviour of rats. Noradrenergic transmission may be associated with exploratory behaviour and novelty. [23, 24] The observed exploratory behaviour in the present study could be due to increased noradrenergic activity in NAc following micro infusion of orexin B. It was reported that ICV injection of orexin A increased locomotor activity in rats [25]. Earlier it was reported that ICV injection of orexin during light period potently increased the duration of wakefulness. [22] It was also reported that intra VTA infusions of orexin A increased the time that rats spent awake and grooming and these behaviours were correlated to elevated dopamine release in PFC. [26] Intra nuclear injection of orexin to accumbens has been attempted for the first time in our experiments. Increased grooming was also previously reported when orexin A was administered ICV, chronically for 7 days. [27,28,29] This is a characteristic behavioural response to some hypothalamic neuropeptides, including corticotrophin-releasing factor and corticotrophin. [27] But in our experiements with infusion of Orexin B we did not see any changes in grooming, rearing and defecation. It was also confirmed by the infusion of Orexin B antagonist infusion. Since orexinergic neurons densely innervate the cerebral cortex and limbic system, probably it might have also roles in cognitive, emotional and motivational aspects of feeding behavior. [30]

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