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Full Length Research Paper

Cholinergic Modulation of Restraint Stress Induced Neurobehavioral Alterations in Mice

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ABSTRACT

The involvement of the cholinergic system in restraint stress induced neurobehavioral alterations was investigated in rodents using the hole board, elevated plus maze, the open field and the light and dark box tests. Restraint stress (3h) reduced significantly ($p<0.05$) the number of entries and time spent in the open arm, number of head dips in the hole board test , grooming and rearing frequencies and locomotion activity compared with the control group. The time spent in the lit chamber of the light/dark board test was also reduced. Pre-treatment with atropine (2mg/kg) before the restraint procedure significantly ($p<0.05$) reversed all these changes induced by restraint stress. These results suggest that a cholinergic mechanism may be involved in restraint stress induced neurobehavioral alterations.

Key words: Restraint Stress, neurobehavior, atropine.

INTRODUCTION

Stress is any aversive stimulus, which can disturb physiological homeostasis and ability to cope with such stressful stimuli is a crucial determinant of health and disease (Gold *et al*., 2002) and exposure to stress provides widespread physiological and behavioural effects in animals including man (Mashood et al.,2010) Stress has been shown to alter cognition, learning, memory and emotional responses, causing mental changes like depression and anxiety (Ambareesha *et al*., 2013). Stress affects CNS leading indirectly to the modulation of the activity of steroid, catecholamine, peptides and the opioid system (Ray, 1990; McEwen, 2006).

There exists sex and age dependent variations among individuals in response to various stressors (Walker, 1992). Age is known to influence CNS functions (Chen *et al*., 2006, Doresmus *et al*., 2004).

The mechanisms involved in stress response are not clearly defined. Recent studies demonstrated the involvement of the endocannabinoid system (Mathew and Bruce, 2011) and nitric oxide (Ayanasha *et al*., 2011, Abrin *et al*., 2002).

The involvement of the cholinergic system has not been given much attention; hence this study investigated its role in stress induced neurobehavioral alterations in rats to further broaden the available knowledge on the subject.

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MATERIALS AND METHODS

Animals

Male Swiss Albino mice (25-35g) obtained from the pre-clinical animal house, College of Medicine, University of Ibadan, Nigeria were used for the study. They were kept at room temperature under standard laboratory conditions with a 12-h light-dark cycle and fed with mouse cubes (Ladokun feeds Nig Ltd., Ibadan) and water *ad libitum.*

Drugs and Chemicals

Atropine (Research Biochemical Inc., Natick, M.A.).

Restraint procedure

The animals were restrained for three hours in a perplex tube measuring18cm long and 5cm radius with a wire mesh at both ends for adequate ventilation (Ayesha and Naheed, 2009). The animals were divided into three groups. Group I served as the control (non- restraint), group II was restrained and group III was given 2mg/kg atropine before being subjected to the restraint procedure. Behavioural assays were carried out on all the three groups.

Behavioural assays

Elevated Plus-Maze Test: The anxiety status of the animal was assessed using the above named test (Pellow *et al*., 1986). The elevated plus-maze consisted of two open arms (30×5cm) and two closed arms (30×5×15cm) that extended from central platform (5×5cm). The entire maze was elevated 40cm above the floor.

During the first 5min of the free exploration, the number of entries into and the time spent in the open and closed arms were recorded. An entry was defined as the point the animal places all the four paws onto the arm. The maze floor was constructed from black Plexiglas and walls from clear Plexiglas.

Hole – board test: Anxiety levels were also evaluated in male mice by using a hole- board apparatus (35cm × 35cm × 15cm). Its walls were made of clear Plexiglas and the arena floor was constructed from black Plexiglas and divided into 16 equal squares with 16 holes (diameter 3.5cm). The equipment was elevated 56cm above the floor. Each animal was placed on the central square of the arena and the number of head dips was recorded for 5min. An increase in the number of head dips reveals a positive anxiolytic-like effect (File and Pellow, 1985).

Light –dark exploration test: The apparatus consisted of a Plexiglas box with two compartments (20cm × 20cm each). One of which was illuminated with white light, while the other remained dark. Each animal was placed at the junction of the light/dark areas, facing the illuminated compartment.

The time spent as well as the number of entries into the light and dark spaces were recorded for 5min (Young, 1991).

Open- field test: The open –field box is a rectangular area composed of a hard floor measuring 36cm × 36cm × 26cm and made of a white painted wood. The floor was divided by permanent read markings into 16 equal squares at the bottom.

Each mouse was introduced singly in one corner of the field and the total locomotion (number of units entered with all paws), rearing frequency (number of times the animal stood on its hind limbs or with its four limbs against the walls of the observation box or free in the air) and frequency of grooming (number of body clearing with paws, picking of the body and pubs with mouth and face washing activities) within each 10min interval were recorded.

After each of these assays the arena was cleaned with 70% alcohol to eliminate olfactory bias and the area allowed to dry before introducing a fresh animal.

Statistical analysis

Results were expressed as mean± SEM. The behavioural data were analysed using the student's t-test. A value of $p < 0.05$ was regarded as significant.

RESULTS

Elevated plus Maze Test

The results showed that there was a significant ($p<0.05$) reduction in the time spent in the open arms (Fig 1a) and a significant increase in the time spent in the close arms of the elevated plus maize in the restraint rats(fig 1b). However, pre-treatment with atropine resulted in a reversal of these observations as there was an increase in the time spent in the open and a decrease in the time spent in the closed arms compared with the stressed group.

Hole –Board Test

As shown in figure 2, restraint animals exhibited decrease in exploratory activity as shown by a fall in the number of head dips. Pre –treatment with atropine led to a significant ($p<0.05$) increase in the number of head dips compared with the stressed group.

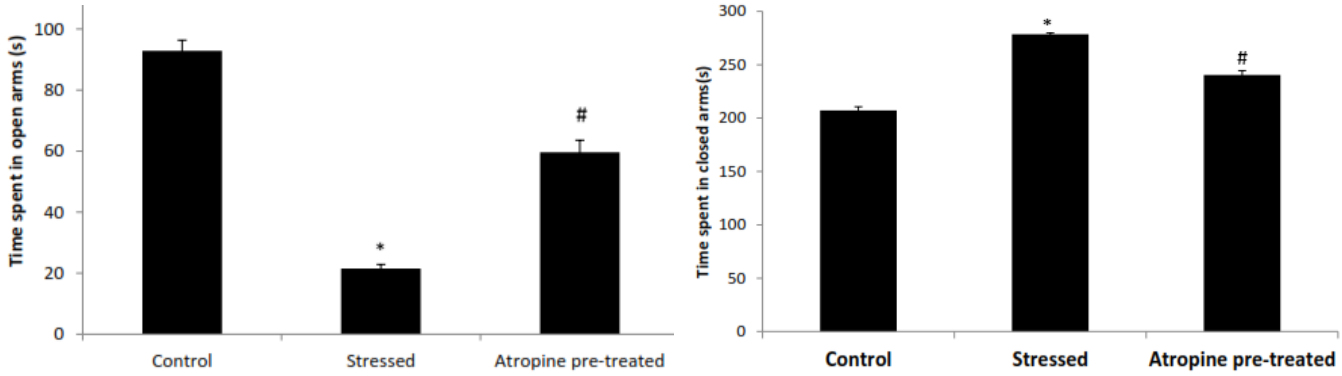

Figure 1:
Effect of atropine pre-treatment on time spent in (a) open arms and (b) closed arms of the elevated plus maze in stressed mice
Values are mean ± standard error of mean (S.E.M.), n =6.
*P<0.05, (Control vs. stressed); #p<0.05, (stressed vs. atropine pre-treated)

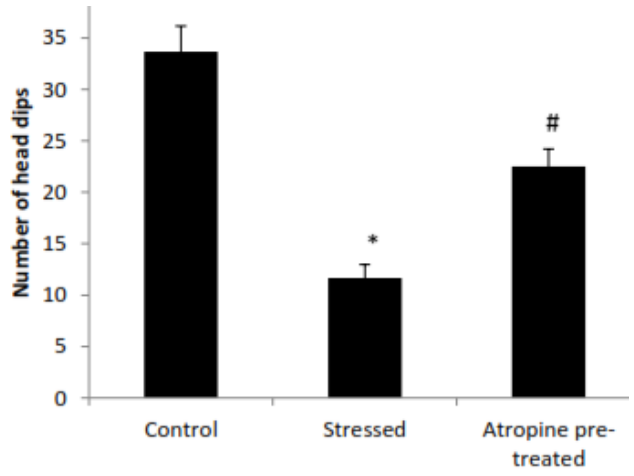

Figure 2:
Effect of atropine pre-treatment on the number of head dips in stressed mice in the hole board test. Values are mean ± standard error of mean (S.E.M.), n=6
*P<0.05, (Control vs. stressed) #p<0.05,
(stressed vs. atropine pre-treated)

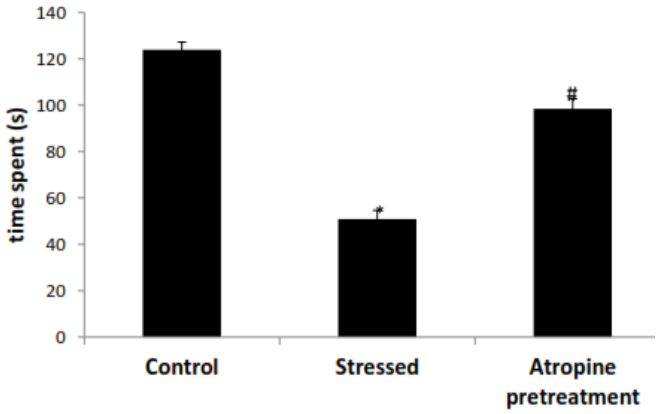

Figure 3:
Effect of atropine pre-treatment on time spent in the lit chamber of the light/dark box test in stressed mice.
Values are mean ± standard error of mean (S.E.M.), n=6
*P<0.05, (Control vs. stressed)
#p<0.05, (stressed vs. atropine pre-treated)

Light / Dark Exploration Test

There was a significant (P<0.05) decrease in the time spent in the lit compartment of the box in stressed rats, application of atropine before the procedure reversed this trend as more time was now spent in the lit compartment compared with the stressed group (Fig. 3).

Open Field Test

The rearing (Fig. 4a), grooming (Fig 4b) and locomotion activities (Fig 4c) were all reduced significantly (p<0.05) in the stressed animal compared with the control. Pre-treatment with atropine significantly (p<0.05) reversed these trends when compared with the stressed animals.

DISCUSSION

Stress-induced effects are an outcome of altered activity of different systems such as central neurotransmitters, neurohormonal factors particularly those linked with the pituitary- adrenal axis, and free radical generation. These responses are believed to be developed during the first exposure to stress and /or novel environment.

Acute immobilization (restraint) procedure was used to investigate the changes induced by short acting severe stressor. Among the various models, immobilization has been used extensively and accepted widely for studying stress- induced alterations.

The elevated plus maze represents one of the most widely used animal models for screening anxiolytic agents (Lister, 1987). It is a widely used test based on the natural aversion of rodents to height and open spaces and is sensitive to both anxiolytics and anxiogenics (Lister, 1987). Results defined in the test showed a significant decrease in the time spent and number of entries into the open arm in stressed rats compared with the control, pre-administration of atropine significantly reversed the reduction obtained in stressed rats.

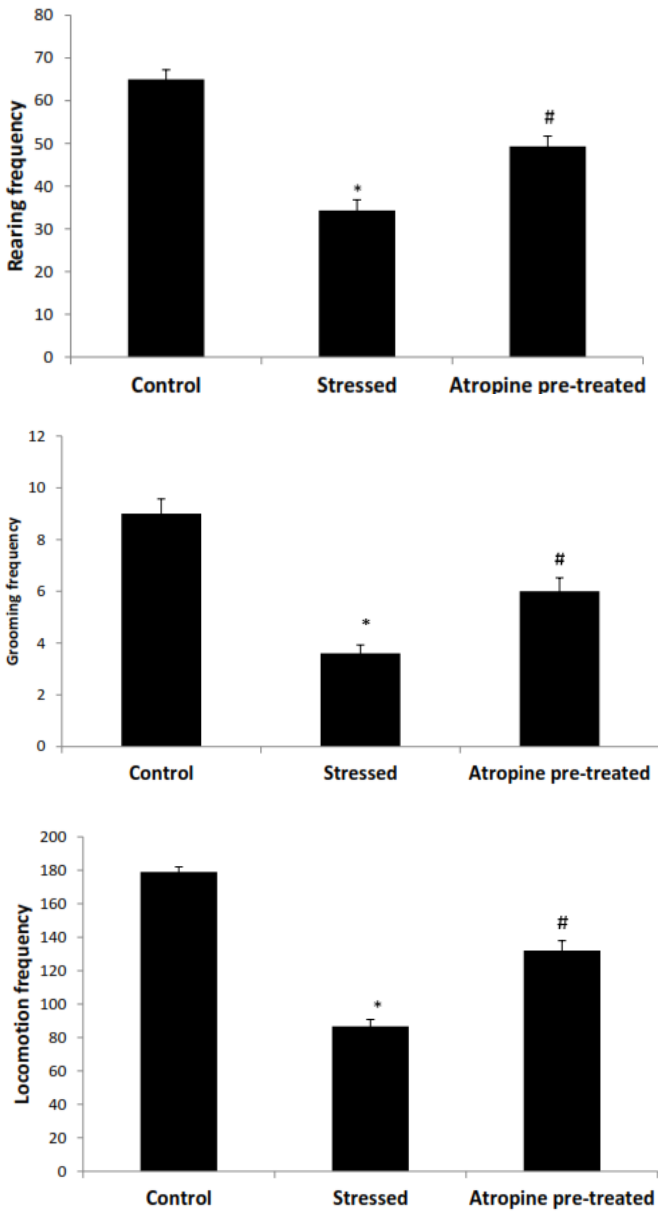

FIGURE 4: Effect of atropine pre-treatment on (a) rearing frequency, (b) grooming frequency and (c) locomotion frequency in open field test in stressed mice.
Values are mean ± standard error of mean (S.E.M.), n =6
*P<0.05, (Control vs. stressed).
#p<0.05, (stressed vs. atropine pre-treated)

In the hole –board test, restraint resulted in a significant decrease in the number of head dips compared with the control. Atropine pre- administration annulled this decrease as there was a significant increase in the number of head dips. It has been reported that an increase in the number of head dips is a measure CNS anti-depressant activity (Adzu *et al*., 2002).

The results of the open field test showed a significant reduction in the frequency of rearing, grooming and locomotion activities, which were all reversed by atropine pre- treatment. Locomotion, rearing and grooming are widely accepted as indicators of exploratory activity (Kelly, 1993).

In the light/dark exploration test, there was also a significant decrease in the time spent in the lit compartment of the apparatus. This was also an anxiety-like behaviour which was reversed by pre –treatment with atropine.

In conclusion, the study highlighted the modulatory effects of the cholinergic system in restraint-stress induced neurobehavioral alterations.

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