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Research Article

RATS EXPOSED TO REPEATED IMMOBILIZATION STRESS TREATED WITH HALOPERIDOL REVERTED THEIR MOTOR DEFICITS.

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ABSTRACT

Any physiological or psychological demand causes the body to respond in a non-specific manner to stress. Stress-induced neurochemical and behavioral deficits have been shown in preclinical studies. Haloperidol was used to induce acute parkinsonian-like effects in rats in the present study, which examined the hypothesis that stress adaptation would reduce the responsiveness of somatodendritic 5-hydroxytryptamine (5-HT)-1A receptors. After just a single immobilization stressful events of 120 minutes, there have been negative effects on food consumption, overall growth, and motor performance; however, subsequent immobilization stress exposure did not result in these stress-induced behavioural abnormalities (120 minutes per day for 6 days).This suggests that behavioral tolerance to similar stress occurs. Compared to their respective control animals, animals exposed to repeat immobilization stress showed reversal of the haloperidol-induced motor deficits. Dopamine system could be released from inhibitory effects of serotonin induced by stress by desensitizing somatodendritic 5-HT-1A and 5-HT-2C receptors. Increasing 5-HT-1A receptor activity modulates dopaminergic neuron activity directly and may reverse haloperidol-induced parkinsonian symptoms in repeated-immobilized rats with haloperidol.

Keywords: Dopamine, dopamine, haloperidol, 5-HT-1A receptors, repeated stress, parkinsonian symptoms, and 5-HT-2C receptors.

INTRODUCTION

Various neuropsychiatric disorders are treated with neuroleptic drugs. Typical neuroleptics, such as haloperidol, apply antagonistic effects to dopamine (DA) receptors [1]. They treat positive symptoms of schizophrenia. Mesolimbic DA is blocked by this drug. In addition to regulating arousal, memory, stimuli processing, locomotion, and motivation, the mesolimbic pathway may also regulate arousal [2]. As a result, hyperactivity in this area causes over excitation of different processes and may also contribute to schizophrenia-like symptoms. It has been shown that acute parkinsonism occurs due to blockage of DA neurotransmission, which results in super sensitivity of DA receptors if these conditions persist for a long period of time [3].

The modulation of dopaminergic neurons appears to be controlled by serotonergic systems. These mechanisms play an important role in controlling mood, cognition, and motor behavior, both by modulating dopaminergic transmission and by controlling mood, cognition, and motor behavior [4]. Several pathological and psychophysiological conditions are associated with multiple serotonin (5-HT) receptors. The etiology of schizophrenia can be attributed to 5-HT1A receptors [5].

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Animal models of depression had been produced by exposing them to stressful stimuli. A repeated exposure to uncontrollable stress can lead to adaptive changes [6].

This mechanism also includes serotonergic responses to stress. Accordingly, adaptation to stress may involve changes in receptor responsiveness [7]. It has been shown in later studies that there is an association between adaptation to repeated restraint stress and diminished 5-HT-1A receptor activity at dendritic terminals [8]. Alternatively, stimulation of 5-HT1A receptors in somatodendritic cells reduces 5-HT availability at 5-HT-2C receptors in the striatum, where 5-HT inhibits DA neurotransmission [9].

An acute parkinsonian like effect induced by haloperidol could in theory be reduced or reversed by a possible reduction in 5-HT-1A receptor function in the soma following adaptation to stress. Haloperidol-induced EPS is attenuated through a mechanism [10].

PROCESSES AND MATERIALS

Animals

34 locally raised male Albino-Wistar rats weighing 155-250g were housed in individually built cages with a sawdust paper bottom, full access to water, and a rodent food for at least 3 - 4 days prior to the research.

Administration of drugs

During the study, all drugs and chemicals were supplied by Indian Chemical Company. Control rats received 0.001 l/kg of saline and 0.001 g/kg of haloperidol respectively intraperitoneally.

Protocol for experimentation

After being evaluated, the twenty-four animals were randomly split into two groups of seventeen: (1) those held without constraint, and (2) those held with restriction. The everyday bodyweight and meal consumption of the mice were recorded. Each animal's food pail was weighed and filled with the appropriate quantities of food before injection. In order to immobilize the animals in the stressed groups, a wire grid was wrapped across their legs.

When agitated animals were discharged from the grids, they were remained in their home cages and also had accessibility to food and drink. Following the first and sixth days of stress, alterations in cumulative food intake and body weight were observed between 7 and 8 hours later. During the period between the first and sixth day following stress, exploratory activity was monitored in familiar and novel environments (home cage and open field) and impairment of motor activity was monitored on Rota-Rod. We divided animals into two groups on day 7 of stress (i) with saline (0.9%NaCl) injections (0.001l/Kg) and (ii) with haloperidol injections (0.001g/kg). In a balanced design, saline and haloperidol injections were followed up between 8 and 11 hours following injection of a familiar (home cage activity) and a novel environment (open field) for behavioral activity and impairment of motor function.

Restrictive measures

Using wire grids of 11"x9" and Perspex plates measuring 9"x6.0", the animals were restrained. With Zinc Oxide plaster tape, we immobilized the rats' forelegs by forcing them through the gaps in the metal grids. In addition to the hind limbs, the head of the animal was placed on the Perspex plate and the hind limbs were taped to them. The animals were restrained for 120 minutes, after which they were freed & put back in their cages.

METHODOLOGY BASED ON BEHAVIOR

Test of open field activities

We assessed locomotors activity and exploration in an open field. It involves measuring motor activity in an open novel space containing an obstacle that prevents rats from escaping. In the present study, a square area measuring 75x75 cm with walls measuring 43 cm high was used as an open field apparatus. 26 equal squares were drawn on the floor. Rats were placed in the open field in the center square to determine activity. Scores were taken for the number of squares crossed by all 4 paws for a period of six minutes.

Coordination of motor functions

Rota-rods were used to test motor coordination. For training, a Rota-rod with a 8 cm radius drum rotates at 3 to 25 revolutions per minute, while for testing, it rotates at 25 revolutions per minute. Until they achieved 160 seconds on Rota-rod in a single session the day before treatment, rats were trained in a single session. To measure motor coordination, 160 seconds of latency to fall were taken in a test session.

Test of Home Cage Activity

Home cage activity tests assessed locomotors activity and exploration in an environment familiar to the animal in order to determine whether drug administration affected it. A rectangular Perspex activity cage was used in this study, consisting of a small square area of 27x27x27 cm on a sawdust-covered floor. An environment of white light and silence was used during testing. A 20-minute habituation period was conducted before monitoring the activity. In order to determine how many crossed over the box, a 15-minute monitoring was conducted.

ANALYSIS OF STATISTICAL DATA

These results are presented as means ± standard deviations. ANOVA was used to analyze behavioral data.

The Newman-Kuels test was used to compare individuals.

RESULTS

Based on a two-way ANOVA, stress was significantly correlated with days ($F=179.05$ $p<0.01$) and days were significantly associated with stress ($F=64.82$ $p<0.02$). Stress and days interact significantly ($F = 4.08$ $p<0.02$). Following their initial bout of 120 min of immobilization stress on day one, saline-injected rats showed a significant reduction in 1 day cumulative food intake, according to a post hoc analysis by Newman Kuels test.After three and four days of 120 minute /day immobilization, saline-injected animals adapted to stress and died by day five ($p<0.02$) and days ($F=64.85$ $p<0.02$) $T<0.02$). There was also a significant interaction between stress and days ($F=5.99$, P0.01). The Newman Kuels test showed that animals injected with saline were significantly less likely to consume food on day one after being exposed to 120 minutes of immobilization stress.
The declines were lessened after the second and third days of immobility (120 minutes each day), but they did not appear after the fifth and sixth days, indicating that the saline-injected animals had become accustomed to stressors. Significant effects of anxiety ($F=22.90$ p0.01 df 1,120) and days ($F=79.60$ p0.01 df) were found by two-way ANOVA. Stress and day interactions were also significant ($F=80.09$, p0.01). The Newman Kuels tests indicated that the animals' bodyweight significantly decreased during their first 120 minute immobility episode on day 2. The declines reduced and didn't happen after five and six days of immobilisation, indicating adaptation, after three and four days of 120-minute immobilisation.

DISCUSSION

As a result of adaptation to stress, the soma's responsiveness to dendritic 5-HT1A receptors decreased, attenuating haloperidol-induced acute parkinsonian effects [11]. When repeated (30min/day for 6 days) exposure to immobilization stress was performed, no behavioral deficits were observed in food intake, growth rate or locomotors activity [12]. The findings of the current investigation show that rats subjected to haloperidol experience reversible motor impairments following multiple immobilization stress [13].

Every life is filled with stress, which can lead to depression, nervous breakdowns, and even cardiac arrest. Especially depression, it acts as a precipitating and predisposing factor. Pre - clinical research has demonstrated that experiences of unmanageable stress are associated with neurochemical changes and behavioral impairments [14]. There are a variety of triggers which can induce stress in the body, such as high blood pressure, pain, tumors, and unpleasant thoughts, whether they arise from external causes, such as heat, cold, loudness, or from within, such as high blood pressure, pain, and unpleasant thoughts.

According to previous studies, stress induces behavioral deficits such as decreased food consumption, growth rate, and locomotion. Behavioral deficits were not observed following repeated exposure to extreme stress (120 minutes/day for 6 days) but following single exposure (120 minutes/day for 120 min) to immobilization stress [15].

Animals tend to adapt to a stress schedule when they are repeatedly exposed to the same stressor, as demonstrated in the current data on the effects of immobilization on food intake and body weight changes [16].

Control of locomotor behavior is the main function of the DA system. Controlling motor activity was known to be a function of the striatum, a brain region. The inhibition of spontaneous locomotor activity by haloperidol is well established. Due to its antagonistic activity against D-2 receptors, haloperidol produces this effect [17]. Additionally, these findings support the previous finding that haloperidol (0.001g/kg) decreased exploratory locomotor activity in a novel environment. It was found that, in rats adapted to repeated immobilization stress than in rats not exposed to stress, haloperidol reversed parkinsonian-like effects [18].

Five-HT was clearly implicated in stress responses according to several lines of evidence. In order to cope with stress, several 5-HT receptors are involved in the central 5-HT system. Exercise and foot shock have been reported to enhance 5-HT turnover, even though the level of 5-HT in the brain doesn't always alter after exposure to these stressors [19]. Research has also demonstrated that 5-HT is produced more rapidly when tryptophan, the precursor of 5-HT, or tryptophan hydroxylase, an enzyme important for 5-HT synthesis, are made more available during stress. After exposure to different types of stressors, microdialysis studies showed increases in extracellular serotonin levels in different brain areas [20].

There was preclinical evidence that 5-HT-1A receptors are particularly important for the regulation of serotonergic neurotransmission in the raphe-hippocampal region. HT availability at postsynaptic and terminal sites could be controlled by a feedback mechanism. Serotonergic neurons have 5-HT-1A receptors, whereas regulatory mechanisms are mediated by 5-HT-1B receptors at the nerve terminal end. An increase in 5-HT availability in the terminal region may be caused by a decrease in the efficiency of both 5-HT and 2-HT receptors.

Conversely, ethanol treatment impaired adaptation in repeatedly restrained rats by increasing the effectiveness of these receptors. The reversal of haloperidol-induced tardive dyskinesia was also associated with a decrease in somatodendritic 5-HT-1A

receptor responsiveness. However, it is difficult to conclude that decreased responsiveness of somatodendritic 5-HT-1A receptors is responsible for attenuating the haloperidol-induced parkinsonian effects as observed in the present study. Increasing the expression of 5-HT-2C receptors at dopaminergic neurons may increase its inhibitory effects if the somatodendritic 5-HT-1A receptor effectiveness decreases [21].

Dopaminergic neurons are thought to be activated by the serotonin system. Both the somatodendritic region and the terminal region of the DA system are thought to be regulated by inhibitory signals [22]. By releasing DA neurotransmission from 5-HT's inhibitory influence, 5-HT-2C receptor antagonists, such as mianserin or mesulergine, could attenuate typical neuroleptics' acute parkinsonian effects. Adaptation to repeated stress and the long-term use of selective serotonin reuptake inhibitors have been shown to change the responsiveness of 5-HT-2C receptors.

Rats who have become accustomed to prolonged immobilisation stress have less effective 5-HT-2C receptors, which further reduces the inhibitory effect of 5-HT on DA transmission. Desensitizing dendritic 5-HT-2C receptors in the soma allowed rats that had been continuously immobilised to recover from parkinsonism which had been brought on by haloperidol [23].

Dopaminergic neurons may be activated by post-synaptic 5-HT-1A receptor stimulation rather than 5-HT-2C receptor stimulation in neurochemical studies. A supersensitive post-synaptic 5-HT-1A receptor may also contribute to antidepressant and adaptive effects following an uncontrollable stressor. As a result of inhibiting haloperidol-induced blocking of DA D2 receptors, the present results were also explained in terms of the reversal of haloperidol's parkinsonian effects.

CONCLUSION

The present study demonstrated that repeated exposure to immobilization stress (120 minutes/day for 6 days) did not result in behavioral deficits following a single exposure (120 minutes/day for 120 minutes). These findings suggest behavioral tolerance occurs to similar stress. The present study shows that repeated immobilization stress reverses haloperidol-induced motor deficits in animals. The dopamine system may be stimulated and inhibited by serotonin.

Stress may have an impact on the engagement of 5-HT-1A and 5-HT-2C receptors in the soma, which could lead to desensitisation of the 5-HT-1A and 5-HT-2C receptors. In contrast, a rise in 5-HT-1A receptor activity has direct stimulatory effects on the activity of dopaminergic neurons and it may help counteract the effects of haloperidol on parkinsonian-symptomatic rats that have been continuously immobilized.

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