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Ameliorative potential of sodium cromoglycate and diethyldithiocarbamic acid in restraint stress-induced behavioral alterations in rats

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Abstract:
The present study was designed to investigate the ameliorative effects of sodium cromoglycate and diethyldithiocarbamic acid in acute stress-induced behavioral alterations in rats subjected to restraint stress. The rats were placed in the restrainer (5.5 cm in diameter and 18 cm in length) for 3.5 h. Restraint stress-induced behavioral alterations were assessed using the hole-board, social interactions and open field tests. Restraint stress resulted in a decrease in the frequency of head dips, rearing in the hole board, line crossings and rearings in the open field, and an increase in avoidance behaviors in the social interaction tests. Sodium cromoglycate (25 mg/kg and 50 mg/kg, *ip*), a mast cell stabilizer, and diethyldithiocarbamic acid (75 mg/kg and 150 mg/kg, *ip*), a selective NF-κB inhibitor, were employed to modulate restraint stress-induced behavioral changes. The administration of sodium cromoglycate and diethyldithiocarbamic acid significantly attenuated the restraint stress-induced behavioral changes. The noted beneficial effects of sodium cromoglycate and diethyldithiocarbamic acid may possibly be attributed to mast cell stabilization and inhibition of NF-κB activity, respectively.

Key words:
mast cells, nuclear factor-κB, restraint stress, behavioral alterations

Introduction

Stress is described as a sum total of all the reactions of the body that disturb the normal physiological equilibrium and threaten homeostasis. Stressful events trigger changes in the different organ systems, including the cardiovascular system [40], gastro-intestinal system [8] and central nervous system (CNS) [9, 59]. Stress is associated with post-traumatic stress disorders, major depression, schizophrenia and neurodegenerative diseases [9, 40]. Exposure to stress stimuli induces various changes in the body including alteration in behavior, autonomic function, and hyperactivation of hypothalamus-pituitary adrenal (HPA) axis followed by subsequent secretion of hormones such as adrenocorticotropin hormone (ACTH) and corticosterone [21, 62].

Mast cells are multi-effector cells with wide tissue distribution in the body [5], including the brain, where these cells are perivascularly localized and scattered in parenchyma. The median eminence of the hypothalamus is also rich in mast cells [20, 25, 44, 47]. Mast cells are also found in abundance in the pituitary gland [15]. Histamine released from the mast cells has been documented to regulate the functioning of the hypothalamus [52]. Furthermore, hypothalamic mast cell activation has been shown to stimulate HPA axis

[10, 35]. There have been a number of studies demonstrating mast cell activation during acute stressful conditions, [11, 20, 22] suggesting the critical role of mast cells in stress-induced changes.

Sodium cromoglycate is a mast cell stabilizer and has been employed clinically for the management of bronchial asthma, allergic rhinitis and allergic conjunctivitis [2, 45, 46]. It has also been reported that sodium cromoglycate produces gastro protection against water immersion restraint stress (WIR)-induced gastric ulceration [60]. NF-κB, a transcription factor, is widely expressed in the CNS, including the frontal cortex, hippocampus, hypothalamus and pituitary [28, 40, 67]. Acute stress has been documented to trigger signal transduction pathways involving activation and potentiation of NF-κB in different brain parts of rats [9, 40]. The stress-induced activation of NF-κB has also been documented in humans and transgenic mice subjected to psychological or immobilization stress, respectively [3]. Diethyldithiocarbamic acid is a widely employed selective NF-κB inhibitor [18, 64] for pharmacological modulation of NF-κB.

Though mast cell activation and potentiation of NF-κB activity have been reported in stress conditions, studies have not been conducted on the pharmacological modulation of these targets in restraint stress-induced behavioral changes in rats. Therefore, the present study was designed to explore the ameliorative effects of sodium cromoglycate and diethyl dithiocarbamic acid in restraint stress-induced behavioral alterations in rats.

Materials and Methods

Animals

Female albino Wistar rats (Punjabi Agriculture University, Ludhaina, Punjab, India) weighing 150–200 g were employed in the present study. The activity of the pituitary-adrenal axis is gender-dependent and lower in male rats. Furthermore, it has been documented that females show high emotionality compared to males when exposed to chronic stress situations [50]. Animals were fed a standard laboratory diet and water *ad libitum*. They were housed in the departmental animal house and exposed to natural cycles of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee, and care of the animals was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, government of India (Reg. No. 107/1999/CPCSEA).

Drugs and chemicals

Sodium cromoglycate employed in the study was available as cromolyn sodium manufactured by FDC Ltd., Dist., Aurangabad, India. Diethyldithiocarbamic acid was procured from Sigma Aldrich, USA. Both drugs were dissolved in physiological saline and freshly prepared before use.

Induction of restraint stress

Restraint stress was induced by placing the rats individually in the semi-cylindrical, acrylic restrainer (5.5 cm in diameter and 18 cm in length) for 3.5 h; the restrainer had holes for air circulation [34]. Different research groups have employed different time intervals of immobilization, such as 1 h [34, 56], 2 h [65], 2.5 h [19], 3 h [66], 4 h [29] and 6 h [17, 31], for the induction of variable degrees of acute stress. In the present study, rats were subjected to restraint for 3.5 h to induce acute stress, as this time was found to create reproducible and optimum stress in rats during the pilot studies.

Behavioral assessment

Before being subjected to restraint stress, the rats were acclimatized for 5 min on each piece of behavioral test equipment for 3 days. After subjecting the rats to restraint stress, the battery of behavioral tests was performed in animals with the sequence of the hole board, the open field, and the social interactions with a time gap of 5 min between the successive tests. All behavioral test equipment was cleaned after each test with alcohol and water. Corticosterone levels are at the highest levels in the morning; therefore, all the experiments were started in the morning at 7:00 am. To counter the variation in the reproductive cycles of the experimental groups, female rats in the same estrous cycle (frank-estrous) were employed.

Hole board test

The hole board test was employed to assess the exploratory behavior of animals. The hole board consisted of a wooden box measuring 68 cm × 68 cm. The walls were 40 cm high, and the box was raised 28 cm above the ground on a stand. Four holes (4 cm in diameter) were cut into the floor of the apparatus; each hole was 28 cm from a corner of the box along the diagonal from the corner to the center. The floor of the box was marked out into four outer areas and one central area using black masking tape. The central area was delineated by four lines of tape, each 20 cm from one of the walls, while the four outer areas were marked out by diagonal lines of tape running from the corners of the floor to the corners of the central square. The four holes were thus located at the corners of the central area. The animals were assessed for 10 min during which the following behavioral patterns were recorded [7]: 1) head dips into one of the holes; 2) rearing, i.e., standing on its back paws and raising its forepaws off the ground.

Open field test

The open field test was employed to assess the spontaneous activity, general exploration and ambulation of the rodents [6, 53]. The open field consisted of a wooden box with dimensions of 90 cm × 90 cm × 38 cm positioned in a dimly lighted room. The walls were painted black, while the floor was painted white and divided by 1 cm wide black lines into 25 squares of 17 cm × 17 cm (16 peripheral squares and 9 central squares). The rats were placed in the center of the open field for 10 min. The number of line crossings and the time spent in the peripheral and central areas were recorded.

Social interaction test

The social interaction test was carried out according to the method described previously [51, 61]. After performing the open field test, the social interaction test was performed in the same box. The animals were placed in the center of the open field test box with dimensions of 90 cm × 90 cm × 38 cm positioned in a dimly lighted room. The animals were assessed for the behaviors of following other rats (an indication of social behavior) and avoiding other rats (indicative of non-social behavior). During the 10-min test, these behaviors were assessed and expressed in seconds. The boxing, biting or threatening of the partner rat was also considered as aggressive/non-social behavior and self-grooming/ignoring the partner was considered non-social behavior.

Experimental protocol

Eight groups, each comprised of 6 Wistar albino rats, were employed in the present study. The dose selection of diethyldithiocarbamic acid and sodium cromoglycate was based on previous reports [18, 24].

Group I: Normal control group

The rats were not subjected to restraint stress, and the hole board, the open field and the social interaction tests were performed.

Group II: Stress control group

The rats were subjected to restraint stress for 3.5 h, and the 3 different behavioral tests were subsequently performed as described in group I.

Group III and Group IV: Sodium cromoglycate 25; 50 mg/kg in stress control group

Rats were subjected to the restraint stress as described in group II, but sodium cromoglycate (25; 50 mg/kg, *ip*) was administered 30 min prior to the stress protocol. After 3.5 h of restraint stress, the 3 different behavioral tests were performed as described in group I.

Group V: Sodium cromoglycate 50 mg/kg, *per se*

Sodium cromoglycate (50 mg/kg, *ip*) was administered 4 h prior to behavioral assessment in normal rats, which were not subjected to restraint stress, and the different behavioral tests were performed as described in group I.

Group VI and Group VII: Diethyldithiocarbamic acid 75; 150 mg/kg in stress control group

Rats were subjected to restraint stress as described in group II. Diethyldithiocarbamic acid (75; 150 mg/kg, *ip*) was administered 30 min prior to the stress protocol. After 3.5 h, the behavioral tests were performed as described in group I.

Group VIII: Diethyl dithiocarbamic acid 150 mg/kg, *per se*

Diethyldithiocarbamic acid (150 mg/kg, *ip*) was administered 4 h prior to the behavioral assessment in normal rats not subjected to restraint stress, and the behavioral tests were performed as described in group I.

Statistical analysis

The results were expressed as the mean ± standard error of means (SEM). The results were analyzed using one-way ANOVA followed by *post-hoc* analysis using Tukey's Multiple Comparison Test. The p value < 0.05 was considered to be statistically significant.

Results

Effect of sodium cromoglycate and diethyldithiocarbamic acid on head dips and rearing in the hole-board test

The head dips are considered to be an index of curiosity or exploration, while the frequency of rearing reflects the exploration of novel surroundings. In restraint subjected rats, the frequency of head dips and rearing decreased significantly compared to the normal control group. Treatment with sodium cromoglycate (50 mg/kg, *ip*) and diethyldithiocarbamic acid (150 mg/kg, *ip*) significantly attenuated this restraint stress-induced decrease in the frequency of head dips and rearing. However, treatment with sodium cromoglycate (25 mg/kg, *ip*) and diethyldithiocarbamic acid (75 mg/kg, *ip*) did not modulate stress-induced changes in the frequency of head dips and rearing in a significant manner. Furthermore, *per se* treatment with sodium cromoglycate and diethyldithiocarbamic acid did not modulate the frequency of head dips and rearing in the normal rats (Figs. 1 and 2).

Effect of sodium cromoglycate and diethyldithiocarbamic acid on line crossing (total motor activity) and rearing in open field test

Line crossings are taken as an indicator of motor activity and the frequency of rearing reflects the exploration of novel surroundings. In restraint subjected rats, the number of line crossings decreased significantly as compared to the normal control group. Treatment with sodium cromoglycate (50 mg/kg, *ip*) and diethyldithiocarbamic acid (150 mg/kg, *ip*) significantly attenuated the restraint stress-induced decrease in the line crossings and the frequency of rearing. However, treatment with sodium cromoglycate (25 mg/kg, *ip*) and diethyldithiocarbamic acid (75 mg/kg, *ip*) did not modulate stress-induced changes in the number of line crossings and frequency of rearing in a significant manner. Furthermore, *per se* treatment with sodium cromoglycate and diethyldithiocarbamic acid did not modulate the line crossings and the frequency of rearing in the normal rats (Figs. 3 and 4).

Effect of sodium cromoglycate and diethyldithiocarbamic acid on following and avoidance in the social interaction test

In restraint subjected rats, the non-social behavior (avoiding the partner) was observed at higher frequency as compared to the normal control group, which exhibited social behavior (following the partner). Treatment with sodium cromoglycate (50 mg/kg, *ip*) and diethyldithiocarbamic acid (150 mg/kg, *ip*) significantly attenuated restraint stress-induced non-social behavior. However, treatment with sodium cromoglycate (25 mg/kg, *ip*) and diethyldithiocarbamic acid (75 mg/kg, *ip*) did not modulate stress-induced changes in the non-social behavior in a significant manner. Furthermore, *per se* treatment with sodium cromoglycate and diethyldithiocarbamic acid did not modulate the social behavior in normal rats (Tab. 1).

Discussion

Acute stress has been reported to produce behavioral alterations, including a decrease in spontaneous activity, a decrease in exploratory behavior and an alteration in social behavior. Furthermore, chronic exposure to stress has been documented to induce a state of depression both in animal models [57, 63] and in humans [12]. In the present study, restraint of female rats for 3.5 h resulted in significant behavioral alterations, including decreased spontaneous activity, decreased orientational-investigating activity and an al-

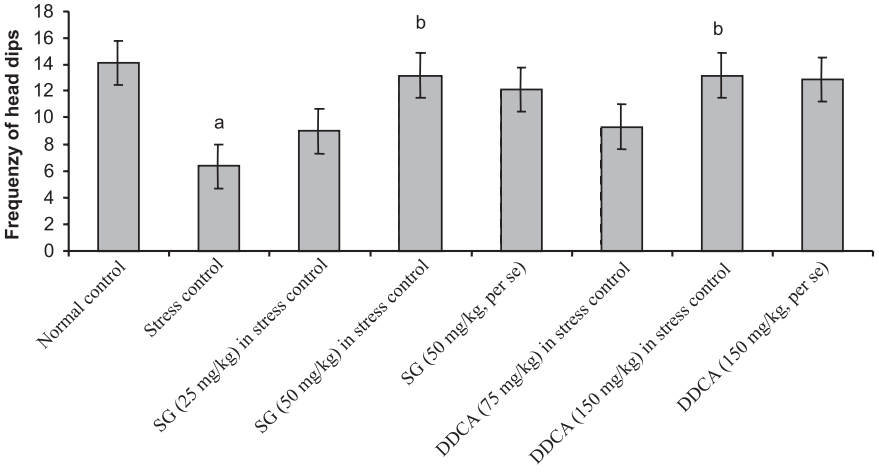

Fig. 1. Effect of sodium cromoglycate and diethyldithiocarbamic acid on frequency of head dips in time interval of 10 min in the hole-board test in restraint stress subjected rats. SG – sodium cromoglycate, DDCA – diethyldithiocarbamic acid. Results are represented as the mean ± SEM with n = 6 in each group. [a] $p < 0.05$ as compared to the normal control group, [b] $p < 0.05$ as compared to the stress control group

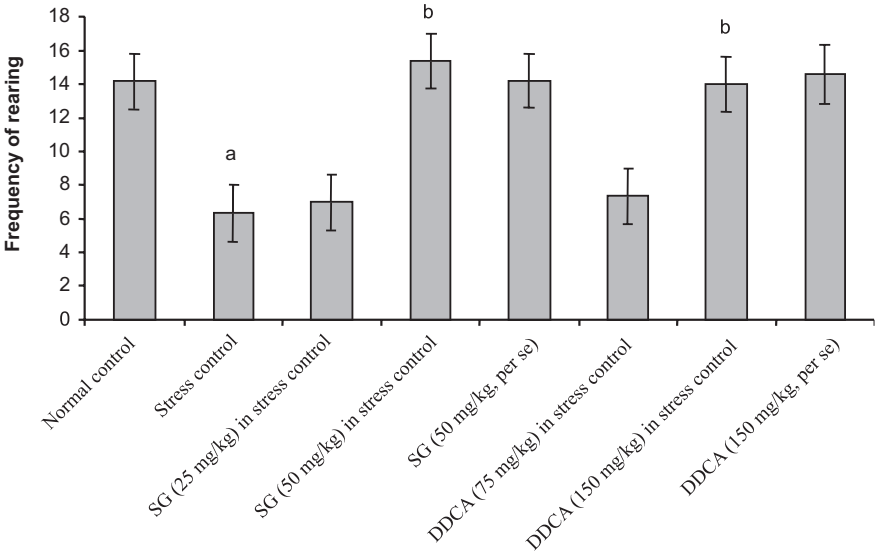

Fig. 2. Effect of sodium cromoglycate and diethyldithiocarbamic acid on frequency of rearing in time interval of 10 min in the hole-board test in restraint stress subjected rats. SG – sodium cromoglycate, DDCA – diethyldithiocarbamic acid. Results are represented as the mean ± SEM with n = 6 in each group. [a] $p < 0.05$ as compared to the normal control group, [b] $p < 0.05$ as compared to the stress control group

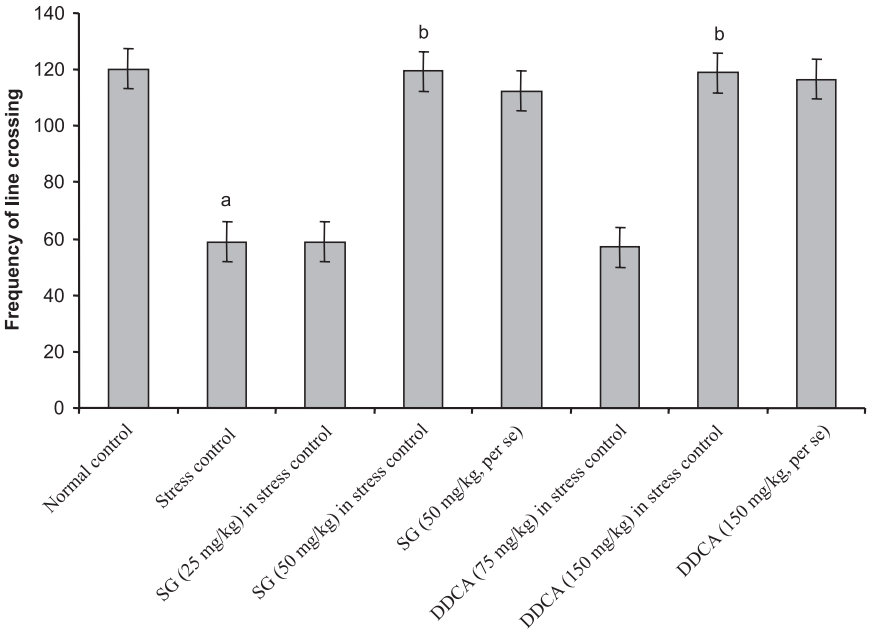

Fig. 3. Effect of sodium cromoglycate and diethyldithiocarbamic acid on line crossings in time interval of 10 min in the open field test in restraint stress subjected rats. SG – sodium cromoglycate, DDCA – diethyldithiocarbamic acid. Results are represented as the mean ± SEM with n = 6 in each group. [a] $p < 0.05$ as compared to the normal control group; [b] $p < 0.05$ as compared to the stress control group

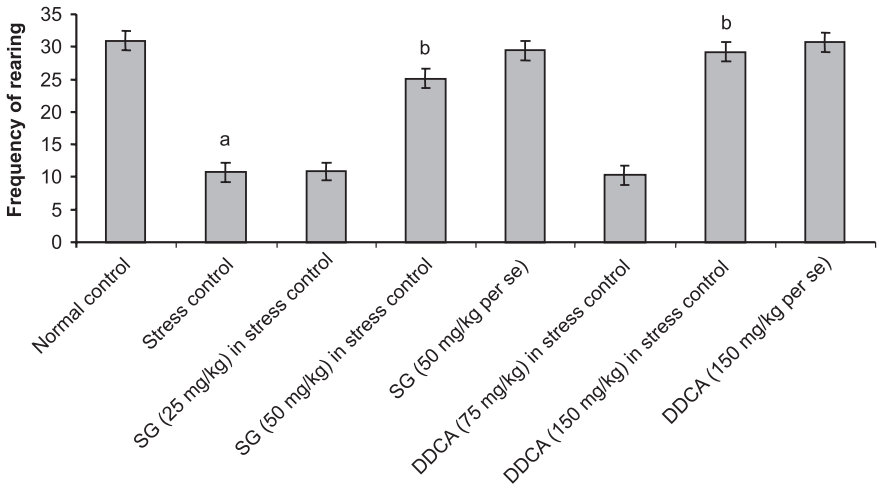

Fig. 4. Effect of sodium cromoglycate and diethyldithiocarbamic acid on frequency of rearing in time interval of 10 min in the open field test in restraint stress subjected rats. SG – sodium cromoglycate, DDCA – diethyldithiocarbamic acid. Results are represented as the mean ± SEM with n = 6 in each group. [a] $p < 0.05$ as compared to the normal control group, [b] $p < 0.05$ as compared to the stress control group

Tab. 1. Effect of different interventions on following and avoidance (s) in the social interaction test in restraint stress subjected rats over a time interval of 10 minutes. DDCA – diethyldithiocarbamic acid. Results are represented as the mean ± SEM with n = 6 in each group. [a] $p < 0.05$, as compared to normal control; [b] $p < 0.05$ as compared to the stress control group

Experimental group	Social interaction test	
	Following (s)	Avoidance (s)
Normal control	593.0 ± 2.1	7.0 ± 2.1
Stress control	78.3 ± 10.6	521.7 ± 10.6
Sodium cromoglycate (25 mg/kg) in stress control	282.8 ± 11.3	317.2 ± 11.4
Sodium cromoglycate (50 mg/kg) in stress control	595.3 ± 2.1	4.7 ± 2.1
Sodium cromoglycate (50 mg/kg *per se*)	599.9 ± 0.6	0.1 ± 0.6
DDCA (75 mg/kg) in stress control.	182.8 ± 13.2	417.2 ± 13.2
DDCA (150 mg/kg) in stress control	594.3 ± 3.0	5.6 ± 3.0
DDCA (150 mg/kg, *per se*)	599.2 ± 0.4	0.79 ± 0.4

tered social behavior in the hole-board, open field and social interaction tests in terms of a decrease in the frequency of head dips and rearing, a decrease in the total line crossings and rearing, a decrease in the time of following and an increase in the time of avoidance. Restraint-induced stress has been one of more commonly employed models for the induction of acute stress in rats [31, 56] and this type of physical stress has been useful for studying stress-induced neurodegeneration and post-traumatic disorders [55].

Mast cells are multi-effector cells that have been localized in different brain regions [54], including the hypothalamus and pituitary, and their degranulation has been associated with the activation of the HPA axis [10, 35]. There have been reports documenting the activation and degranulation of mast cell during stress conditions [11, 20, 25, 26]. The brain localized mast cells have been demonstrated to mediate the expression of anxiety-like behavior in mice [42]. It has been reported that individuals with chronic self-injury have significantly more degranulated mast cells [58]. Induction of an asthmatic or food allergy response in mice has been associated with a mast cell-dependent increase in anxiety-like behavior and activation of the HPA axis [1, 13, 33]. Furthermore, the patients afflicted with systemic mastocytosis, a disease characterized by an increase in the number of mast cells, report low arousal states and lethargy [37]. In the present investigation, pre-treatment with sodium cromoglycate significantly attenuated restraint stress-induced behavioral alterations. Sodium cromoglycate is a mast cell stabilizer, and the role of mast cells has been de-

scribed in stress-related behavioral alterations. Therefore, it may be proposed that the mast stabilizing activity of sodium cromoglycate is responsible for its observed anti-stress effects in the present investigation. Sodium cromoglycate has been reported to produce gastro-protection against water immersion restraint stress (WIR)-induced gastric ulceration [60]. However, this is the first report describing its ameliorative role in restraint stress-induced behavioral alterations in rats.

Previous reports have demonstrated that mast cell mediators such as histamine and serotonin mediate behavioral changes, including anxiety, arousal and emotions [16, 27]. Furthermore, mast cells are rich sources of neuroinflammatory mediators, including chemo-tactic factors, prostaglandins, and cytokines such as IL-1, 2 ,5, 6 and TNF-α [4, 36, 48]. A number of studies have shown increased levels of cytokines during stressful states [9, 40, 68], which consequently produce behavioral alterations [23]. Therefore, it may be tentatively proposed that mast cell-derived neuroinflammatory mediators trigger the cascade of events that lead to behavioral alterations during stressful conditions. Furthermore, sodium cromoglycate has been reported to produce anti-inflammatory actions by reducing the release of different pro-inflammatory mediators from mast cells [24]. Therefore, sodium cromoglycate-mediated anti-stress effects may possibly be attributed to the decrease in the release of neuro-inflammatory mediators from the mast cells by virtue of its mast cell stabilization activity.

Furthermore in the present study, pretreatment with diethyldithiocarbamic acid, a selective NF-κB inhibitor, also attenuated restraint stress-induced behavioral alterations. NF-κB is a widely expressed transcription factor in the CNS, including the hippocampus, hypothalamus and pituitary [28, 40, 67]. The studies have suggested that activation of NF-κB produces different behavioral changes [41, 49]. Acute stress has been documented to trigger signal transduction pathways involving activation and potentiation of NF-κB in different brain parts of rats [9, 40]. The stress-induced activation of NF-κB has also been documented in humans and transgenic mice subjected to psychological or immobilization stress, respectively [3]. Chronic unpredictable stress has been shown to enhance the activation of NF-κB in response to inflammatory stimuli [32, 40], and social stress has been reported to increase NF-κB signaling in healthy subjects and produce an exaggerated response in depressed patients [3, 43]. Recently, the critical role of NF-κB signaling in anti-neurogenic and behavioral actions of stress has been described [30]. Therefore, it may be possible to suggest that stress triggers a signal transduction pathway involving NF-κB activation and eventually produces behavioral alterations.

Mast cell-derived neuroinflammatory mediators have been reported to potentiate NF-κB activity [14, 22, 39, 40]. Furthermore, NF-κB is also expressed in mast cells and plays an important role in mediating the release of inflammatory mediators [38]. Based on these reports, it may be tentatively proposed that stressful conditions trigger degranulation of mast cells and release neuro-inflammatory mediators, which subsequently activate a cascade of signaling pathways involving the potentiation of NF-κB activity. Alternatively, it may also be possible that stressful stimuli directly potentiate NF-κB activity, which in turn trigger the release of neuro-inflammatory mediators from mast cells and hence induce the behavioral changes. Nevertheless, further studies are required to demonstrate direct evidence of mast cell degranulation and increased NF-κB activity in the brains of rats subjected to restraint stress.

Conclusion

Sodium cromoglycate and diethyldithiocarbamic acid ameliorates restraint stress-induced behavioral alterations in rats, and this process may possibly be attributed to mast cell stabilization and inhibition of NF-κB activity.

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