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Neurochemical and behavioral profile of corticosterone: a dose related study

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Abstract: During stressful events, glucocorticoids are secreted peripherally and act on central monoaminergic systems particularly modulating serotonin (5-hydroxytryptamine; 5-HT) functions, social behavior, aggression and responses to stress. Studies on rat models show that exogenous corticosterone could alter brain serotonin metabolism as well as functional responses. It has been reported by others that increased serotonergic neurotransmission increases memory consolidation and vice versa. It is therefore suggested that corticosterone could regulate 5-HT induced hippocampal excitability and cognitive functions. Responses to 5-HT could be affected by hyper- or hypocorticism. The present study was designed to monitor effects of various doses of glucocorticoides on memory and levels of neurotransmitters in rat brain. In this study we administered different doses of corticosterone to monitor dose related effects on brain 5-HT. Water maze test revealed that the low dose of corticosterone improved short term memory with no effect at other two doses. Whereas, at all the three doses corticosterone impaired long term memory. Effects of corticosterone on memory consolidation in relation with 5-HT metabolism are discussed. Findings would be helpful for extending therapeutics in neurodegenerative disorders.

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INTRODUCTION

Secretion of corticosteroid hormones is the most important endocrine attribute of the stress response. Mechanisms influencing function and regulation of Hypothalamic Pituitary Adrenal (HPA) axis are responsible for the respective behavioral and neurobiological changes[1]. A circadian pattern of corticosteroid secretion is followed by the adrenal glands[2]. In nocturnal animals such as rats, corticosterone level is low in early hours of the day and high in the evening. Increased plasma corticosterone levels fall neatly between two secretary episodes of the testosterone[3]. Corticosteroids act via mineralocorticoid (MR) and glucocorticoid (GR) receptors which differ in their affinity for corticosteroids. As a result, decreased corticosterone levels activate brain MRs, while high levels activate GRs along with MRs[4].

The effects of corticosterone releasing factor (CRF) are principally excitatory and limited to a subpopulation of serotonergic neurons. A dose-response study suggests that 5-HT could regulate the sensitivity of proliferating dentate gyrus cells to corticosterone[5].Acute, as well as chronic administration of corticosterone could reverse depressive behavior in animals[6]. Repeated administration of corticosterone at a dose of 20 mg/kg for a period of 5 weeks has been reported to increase immobility in tail suspension and forced-swim tests[6]. The present study was designed to monitor effects of various doses of glucocorticoides on memory and levels of neurotransmitters in rat brain.

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats weighing 180(±20) gm were housed individually in a quite room with free access to standard rodent diet cubes and water for at least seven days before starting the experiment.

Drugs

Corticosterone suspension was prepared in 0.9% NaCl (along with Brij-35) and injected subcutaneously at the doses of 10, 25 & 50mg/kg. Control animals were injected with 0.9% NaCl solution.

Experimental protocol

Twenty four rats were randomly assigned to four groups each containing six animals each: (i) saline (ii) low corticosterone (10mg/Kg), (iii) moderate corticosterone (25mg/Kg) and (iv) high corticosterone (50 mg/Kg) injected groups. Animals were injected with saline or corticosterone respectively. Morris water maze test (MWM) was performed 5 h after first injection to monitor short term memory. Second injection was given at the same day after MWM test. On day 2, MWM test was performed to monitor long term memory (10 hrs after second injection). Third injection was given on day 2 after MWM test. Animals were decapitated after 1 hr of third injection. Plasma and brain samples were collected and stored at –70 oC for neurochemical analysis by HPLC-EC.

Behavioral assessment

Home cage activity

Transparent perspex cages (26x26x26cm) were used to monitor the activity in familiar environment. Floor of the apparatus was covered with sawdust. Activities were monitored in a parallel design. Rats were placed individually in cages for 30 minutes pre-injection, to get them familiarized with the environment, and number of cage crossing were counted for 10 minutes.

Open field activity

Activities were monitored in open field 40 min post 1st injection. The apparatus consists of square area (76x76 cm) with walls 42 cm high. The floor was equally divided into 25 squares by lines. Rats were placed in the central square and squares crossed with all four paws were counted for five minutes.

Testing animals in Morris water maze

Apparatus consisted of transparent rectangular glass tank (60x30cms) filled with room temperature-water opacified with powder milk, to the depth of 12cm. A wooden platform (15x13cms) was hidden 2cm below the surface of water in a fixed location. The experiment was performed after 30 minutes of injections. Initially the rats were trained and during the training session each rat was placed into the water facing the wall of the tank and allowed 120 seconds to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 10 seconds. If it failed to find the platform within the allowed time it was guided gently onto the platform. Memory functions of rats were tested by recording the retention latency (RL; the time taken by each rat to locate the hidden platform 24hrs after training). The cut off time for each session was 2 minutes.

Brain dissection

Conscious rats were decapitated and brain was removed within 30 sec from skull. The skin covering the skull was cut along the midline and removed to expose the dorsal skull plates. The plates were spilt by introducing one blade of a pair of scissors along the midline. The plates were then twisted and turned out across the lateral border to expose the brain. The membrane covering the brain was removed with the help of fine forceps. The brain then taken out using spatula and was dipped in ice-cold saline. Cerebellum was discarded with forceps and hippocampus samples were dissected out as described earlier[7].

HPLC-EC analysis

A 5µODS (ECHPHERE) separation column of 4mm i.d. and 250mm length was used. The solvent system was methanol (14%), octyl sodium sulphate (ODS; 0.023%) and EDTA (0.05%) in 0.1M phosphate buffer of pH 2.9. Electrochemical (EC) detection of brain 5-HT and 5-HIAA was achieved on Shimadzu L-EC 6A detector at an operating potential of +0.8V.

Statistical analysis

The results are given as means $\pm$ SD. Behavioral data on the effect of corticosterone on home cage activity in control and test animals was analyzed by one-way ANOVA. Post-hoc comparisons were done by Newman-Keuls test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Figure 1 shows basal values of animals in the MWM training before assigning them to various groups. Values of different groups were not statistically different from each other.

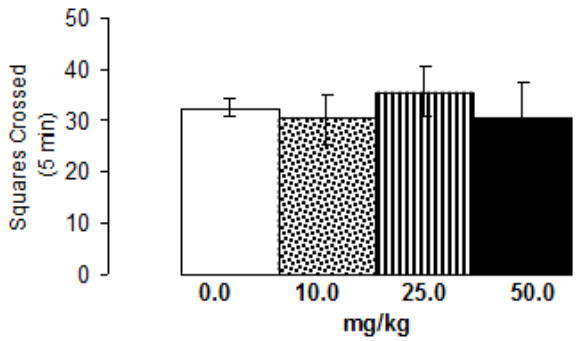

Figure 1: Basal activities of rats in the water maze. Values are means$\pm$SD (n=6).

Figure 2a shows effects of various doses of corticosterone (10, 25 & 50 mg/kg) on water maze test (short term memory). One-way ANOVA showed significant ($F=3.73$, $df=3,20$, $p<0.05$) effects of corticosterone on water maze test short term memory. Post hoc analysis by Newman-Keuls test showed that low dose of corticosterone increased ($p<0.01$) short term memory as compared to saline and moderate dose injected animals.

Figure 2b shows effects of various doses of corticosterone (10, 25 and 50mg/kg) on water maze test (long term memory). One-way ANOVA showed significant ($F=18.22$, $df=3,20$, $p<0.01$) effects of corticosterone on water maze test long term memory. Post hoc analysis by Newman-Keuls test showed that corticosterone decreased ($p<0.01$) long term memory at all doses. Results in low dose injected animals were comparable to moderate dose injected rats.

Figure 3 shows effects of various doses of corticosterone (10, 25 & 50 mg/kg) on 5-HT metabolism in the hippocampus. One-way ANOVA ($df=3,20$) showed that the effects of corticosterone administration on 5-HT ($F=17.78$) & 5-HIAA

(F=5.59) levels were all significant (p<0.01). Post hoc analysis by Newman-Keuls test showed that 5-HT levels were decreased (p<0.01) in animals injected with low (10mg/kg) as well as high (50mg/kg) dose of corticosterone as compared to both saline injected controls & moderate (25mg/kg) dose injected animals. Animals injected with moderate (25mg/kg) dose corticosterone showed decreased (p<0.01) levels of 5-HIAA as compared to saline injected controls. Whereas, both low (10mg/kg) as well as high (50mg/kg) doses of corticosterone did not significantly altered the levels of 5-HIAA.

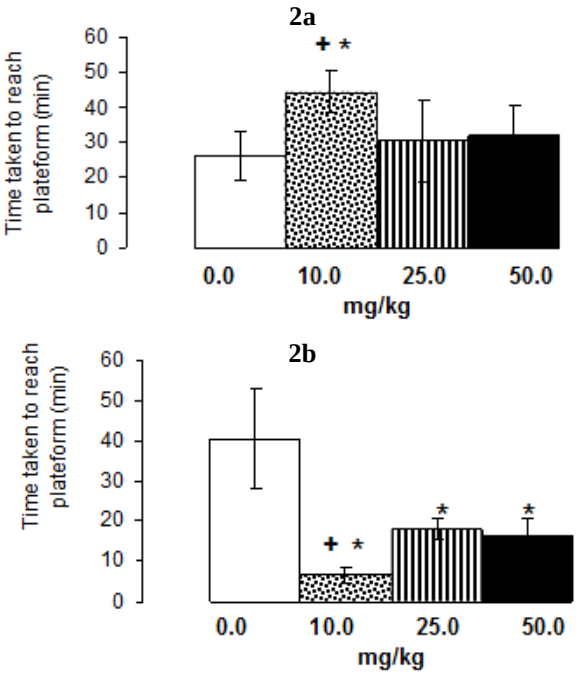

Figure 2: Effects of low, moderate and high doses of corticosterone (10, 25 & 50 mg/kg) on water maze activity (long term memory) in rats 10 hr after second injection. Values are means±SD. Significant differences by Newman-Keuls test: *p<0.01 and +p<0.01 from respective saline and moderate dose injected animals following one-way ANOVA.

DISCUSSION

Increased 5-HT metabolism in the hippocampus is involved in the consolidation of learning and memory[8,9]. In present study we found that Low and high doses of corticosterone decreased (p<0.01) 5-HT, while moderate dose decreased (p<0.01) 5-HIAA levels in hippocampus (Figure 4). A single dose of corticosterone (1 mg/kg) increased the 5-HT content in the hypothalamus, mesencephalon and amygdala. Significant increase was observed at 15 min post injection and elevated 5-HT levels return to normal between 60 and 180 min. Plasma corticosterone level plays a crucial role in regulating the activity of serotonergic system in limbic brain regions[10].

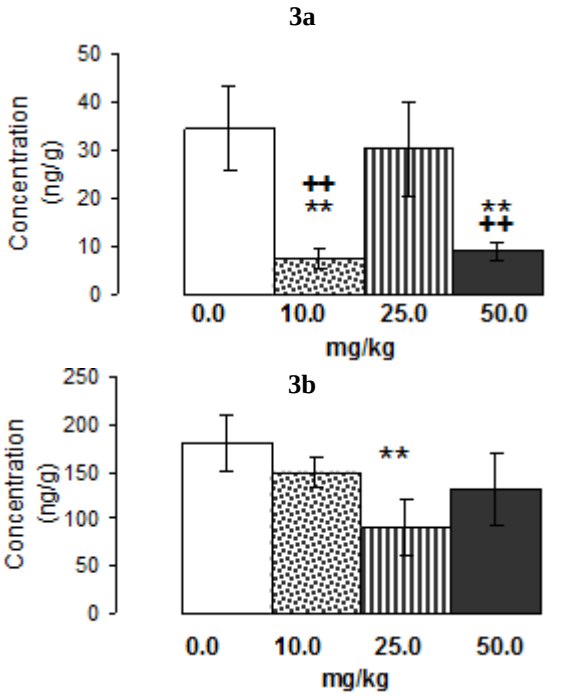

Figure 3: Effects of low, moderate and high doses of corticosterone (10, 25 & 50 mg/kg) on 5-HT metabolism in the hippocampus of rats. Values are means±SD. Significant differences by Newman-Keuls test: **p<0.01; ++p<0.01 from saline and moderate dose injected animals respectively, following one-way ANOVA.

Changes in glucocorticoid level influence serotonergic neurotransmission, especially in the hippocampus. Both adrenalectomy (ADX) and corticosterone treatment modulate responses to 5-HT1A receptor stimulation in the hippocampal neurons[11,12,13]. The ADX animals show increased expression of 5-HT1A receptor mRNA as well as increased sensitivity of 5-HT1A receptors in the hippocampus. Both of these effects could be prevented by low concentrations of corticosterone[14,15,1617]. All these studies show that hippocampal 5-HT1A receptors are under influence of glucocorticoids.

Corticosterone has been shown to down regulate 5-HT1A receptors and decrease the expression of 5-HT1A receptor mRNA after repeated administration[14,18,19]. These changes were not observed in other studies; particularly in the CA1 region[20,21]. This could be due to different effect of 5-HT1A receptor binding in dorsal and ventral part of CA1 region.

Glucocorticoid hormones have been reported to elevate memory consolidation of hippocampus-dependent learning[22,23]. In present study Water maze

test revealed that the low dose of corticosterone improved short term memory with no effect at other two doses (Figure 2). Whereas, at all the three doses corticosterone impaired long term memory (Figure 3). Since low dose of corticosterone can improve the short term memory. It is suggested that repeated administration of corticosterone could be beneficial for treating memory loss in patients with neurodegenerative disorder.

Short term memory improvement following acute administration of corticosterone at low dose (10 mg/kg) might be due to the increased functions of 5-HT. However, long term administration of corticosterone at all three doses impaired long term memory. This impaired memory was well related with decreased 5-HT metabolism. Results therefore support the notion that effect of corticosterone on memory is well related to 5-HT metabolism. Future studies on effects of single administration of corticosterone on brain 5-HT metabolism should be monitored.

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