

ORIGINAL ARTICLE

Plasma Levels of Monoamines and Behavioral Stress: Animal Model of Depression

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Abstract: *Background:* The functions of the monoamine neurotransmitters dopamine (DA) and serotonin (5-HT) in the etiology and treatment of depressive disorders are currently an area of intense research. Much of this research has focused on a relevant animal model of depression as a means of understanding the biological mechanisms underlying human anxiety and depression. Our studies examine whether changes in plasma monoamines after single and repeated stress are associated with altered behavioral responses to inescapable shocks (IS) in a well established animal model known as learned helplessness (LH). *Methods:* We examined the effects of single and repeated stress on the plasma levels of cortisol, dopamine and serotonin using High Performance Liquid Chromatography with electrochemical detection (HPLC-EC). *Results:* We found that while levels of cortisol were increased but the levels of monoamines were significantly decreased in plasma after single and repeated stress. *Conclusion:* This study indicates that these two monoamine neurotransmitters systems play important roles in both the pathophysiology of depression.

Key Words: Behavioral stress, Cortisol, Dopamine, Learned Helplessness, Serotonin,

Abbreviations: 5-HT, 5-Hydroxytryptamine or Serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; DA, Dopamine; ET, escape test; HVA, homovanilic acid IS, inescapable shock; LH, Learned Helplessness; NLH, Non Learned Helplessness; TC, Tested Control.

Introduction

Stress is a common precipitating factor for depression, and it is well documented that stress activates the hypothalamic-pituitary-adrenal (HPA) axis [1-3]. The HPA has a negative feedback system that prevents excessive hormonal deviation from the baseline. The elevated plasma cortisol and monoamines levels are thought to be related to LH behavior and severity of depression. Learned helplessness (LH) is a behavioral depression following inescapable stress. This paradigm is used in the preclinical identification and development of potential antidepressant drugs. It is also a useful tool in the search for the better understanding of the role of specific monoamines and receptor subtypes implicated in depressive states [4].

It has long been known that exposing organisms to a series of uncontrollable stressors produces behavioral, neurochemical and endocrine sequelae that resemble the characteristics of a number of psychiatric disorders [5-6]. This has typically been studied by exposing animal subjects to a series of inescapable tail shocks or foot shocks, a procedure which results in learned helplessness (LH) behavior. This paradigm has been proposed as a valid animal model of human depression [7-9].

Indeed, following chronic, inescapable stress various physiological parameters such as weight loss, sleep activity, libido and cognitive function are remarkably similar in “learned helplessness” or “behavioral depression” and in human depression [10]. The catecholamine system is also related to the pathophysiology of depression and the mechanism of antidepressants [11]. The dopamine and serotonin system is of interest because it plays a role in hedonia, motivation and suicidal ideations in psychiatric disorders [12]. Depressed patients who later commit suicide exhibit reduced dopaminergic and serotonergic activity compared to depressed patients who never attempt suicide [13]. There are few reports about plasma levels of dopamine metabolite homovallinic acid (HVA) and serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in depressed patients [14]. The neurotransmitter serotonin has long been known to be a major factor in the neurobiology of depression [15] although recently dopamine has also been implicated in the etiology and treatment of the disorder [16]. Animal behavioral models find an association of depressive behaviors with altered dopamine functioning of the mesolimbic pathway [17]. Plasma levels of monoamine neurotransmitters suggest their involvement in the behavioral changes as well as the molecular signaling pathways in depression.

Since human depression has been hypothesized to be related to some dysfunction in monoaminergic neurotransmission [18], monoaminergic neurochemistry has been extensively investigated with regard to LH. Increased noradrenergic turnover, decreased norepinephrine brain levels, up-regulation of β -adrenergic receptors have been noted as a result of exposure to uncontrollable shocks [19]. This study was undertaken to test the hypothesis that LH behavior is associated with alterations in plasma monoamines, to examine how long these changes persist after inescapable shocks and to determine whether repetition of shocks would prolong the duration of the behavioral deficits and any changes in plasma monoamines levels. Our study is critical not only in establishing whether plasma monoamines are involved in depressive behavior but also in providing evidence from the repeated stress group results of the potential role of monoamines in other stress related disorders, such as PTSD. In the present investigation, we examined plasma levels of cortisol, dopamine and serotonin to determine the relationship among them and correlate them with the severity of depression in animal model of LH and behavior. We will discuss the relationship among them in correlation to LH behavior and severity of depression.

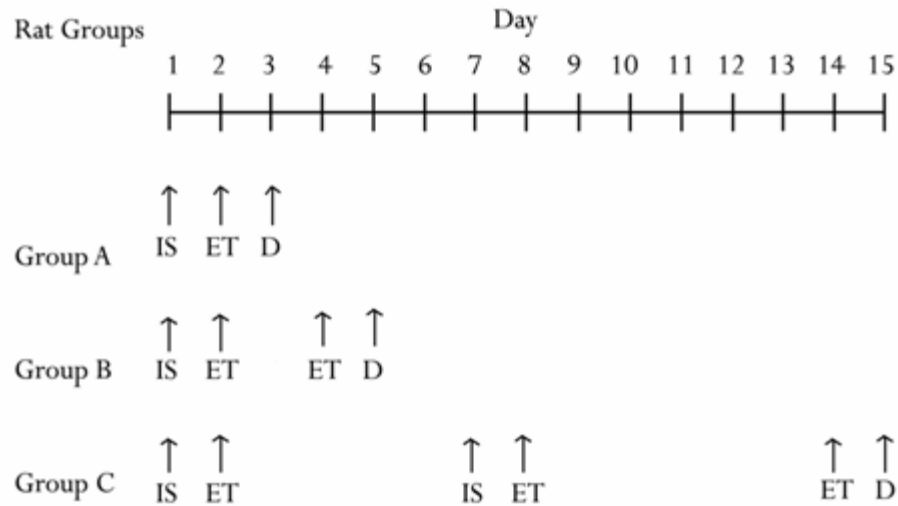
Material and Methods

Animals: Male Sprague-Dawley rats were used in present experiment. Rats weighing 275-300 gm were housed in pairs in standard plexiglas cages, with food and water ad libitum. All experiments were performed under strict guidelines of Indian Committee for the Purpose of Control and Supervision of Experiments (CPCSEA) and conducted with approval of the Institutional Animal Ethics Committee (IAEC) of RPM College.

Behavioral Procedures: Learned helplessness induction by inescapable shock (IS) and escape test (ET) paradigms for the experimental groups are provided in figure 1.

Fig-1: Inescapable shock and escape test paradigms. The detailed experimental protocol is given in the Behavioral Procedures section of Materials and Methods

IS= Inescapable shock; ET=Escape test; D= Decapitation



Learned helplessness induction by IS and ET were performed by the procedure described by [7]. Rats were divided into three groups designed as group A, B and C. Group A rats were given IS on day 1 and tested for escape latency on day 2. Group B rats were given IS on day 1 and tested for escape latency on day 2; and again on day 4. Groups C rats were given on day 1 and tested for escape behavior on day 2; these animals were given another IS on day 7 and tested for escape behavior on days 8 and 14. All the animals were decapitated 24h after last escape testing.

Inescapable stress: The rats were placed in Plexiglas tubes with the rat's tail extending from the rear of the tube and shocks were delivered by means of computer controlled shock generator (Model ENV-410B, Med Associate, Lafayette, Indiana, USA). Electrodes were pasted to the rat's tail. The inescapable shocks consisted of 100 random shocks and were delivered for 5s at the intensity of 1.0mA with a mean interval of 60s. A tested control (TC) group was placed in plexiglas tubes but was not subjected to shocks.

Shuttle box apparatus: Behavioral testing occurred in a shuttle box measuring 46 cm long X 20.7 cm wide X 20 cm high (Model ENV-413, Med Associate, Lafayette, Indiana, USA). Scrambled 0.6 mA foot shocks were administered through a grid floor made of stainless steel. The shuttle box was divided into two equal halves with an aluminum partition that contained an archway that allowed passage from one side to other.

Shuttle-box-procedure: Twenty four hours after exposure to inescapable shocks, rats were placed in a shuttle boxes and administered five 0.6 mA foot shocks that were delivered on a 1 min variable-interval schedule and could be terminated by crossing to the other side one time [fixed ratio-1 (FR-1) trials]. This procedure was followed by 25 trials FR-2 trials during which rats were required to cross the shuttle box twice

to terminate the shock. Shocks were terminated automatically after 30s if there is no response within that time. There was a 5 min interval between FR-1 and FR-2. Shuttle escape latencies and escape failure were recorded automatically by a computer attached to a generator and shuttle box. In the FR-2 trial with escape latency > 20 sec was defined as an escape failure. Rats with 10-25 failure were considered to be deficient in the escape response. Rats were divided into two groups based on the mean latency observed after FR-2: (1) Rats with mean latency \geq 20 sec (termed learned helplessness, LH) and (2) rats with mean latency < 20s (termed as nonlearned helplessness, NLH). In our study, we found that above 50% of all the rats tested became LH rats. The rats that were confined to Plexiglas tubes but were not shocked were also tested.

Determination of plasma cortisol levels: Trunk blood was collected on ice, centrifuged and plasma was stored at -80 °C. Plasma cortisol levels were measured by a commercially available radioimmunoassay kit (ICN Biomedical Inc, Cleveland, OH, USA).

Blood: 1.0-1.5 ml of fresh blood was immediately transferred into a 5 ml EDTA (7.2 mg) tube containing Na metabisulphite (20 μ l, 2M) as an antioxidant and kept at 4 °C. The plasma was separated immediately by centrifugation (4 °C, 2000 rpm) for 30 minutes. The plasma was deproteinated with 50 μ l of 4 M HClO₄, centrifuged (4 °C, 5000 rpm) and the supernatant collected for analysis [20].

Extraction of plasma monoamines: The deproteinated plasma was added to 1.5 ml Tris-HCL buffer pH 8.6-8.7 and then adsorbed onto acid-boiled dry alumina [25 mg] kept at 65 °C. The alumina was washed three times with Millipore water containing 0.001 EDTA and 0.1M Na metabisulphite. The monoamines were eluted from the alumina (dried) with 100 μ l of 0.6M H₃PO₄, mixed for 5 minutes, then centrifuged for 5 minutes (4 °C, 2000 rpm). The eluent was transferred to a micro centrifuge tube for HPLC analysis [20].

Determination of plasma monoamines by HPLC with ECD: The protein in the sample was denatured by storage in the frozen state and separated by centrifugation at 800g at 4 °C. 2ml of the soup was taken in 15ml capacity centrifuge tube and treated with 200 μ l of internal standard solution, 400 μ l of 0.5M Tris-HCL, pH-8.6. This was followed by addition of 20mg of activated alumina. The content of tube was shaken gently for 15 mins in a spiral mixture. Tube was centrifuged at 600g for 2 min. The supernatant was removed and the alumina was washed three times by buffer solution, centrifuging each time at 600g for 2 min. DA and 5-HT was eluted from the alumina into 50 μ l of alumina eluting solution containing 400 μ M NAHSO₃ in 0.6 M perchloric acid. The elute was centrifuged at 800g for 3 min. 20 μ l of supernatant was collected and injected into the chromatograph and compared with the injected reference standard solution of DA and 5-HT (Waters model HPLC System).

Statistics: Statistical analyses were performed using SPSS software for window version 15 (SPSS). Results expressed \pm mean S.D. The comparison of the data between normal control and LH subjects was performed using ANOVA. Equal variance was assumed and $p < 0.001$ was considered significant.

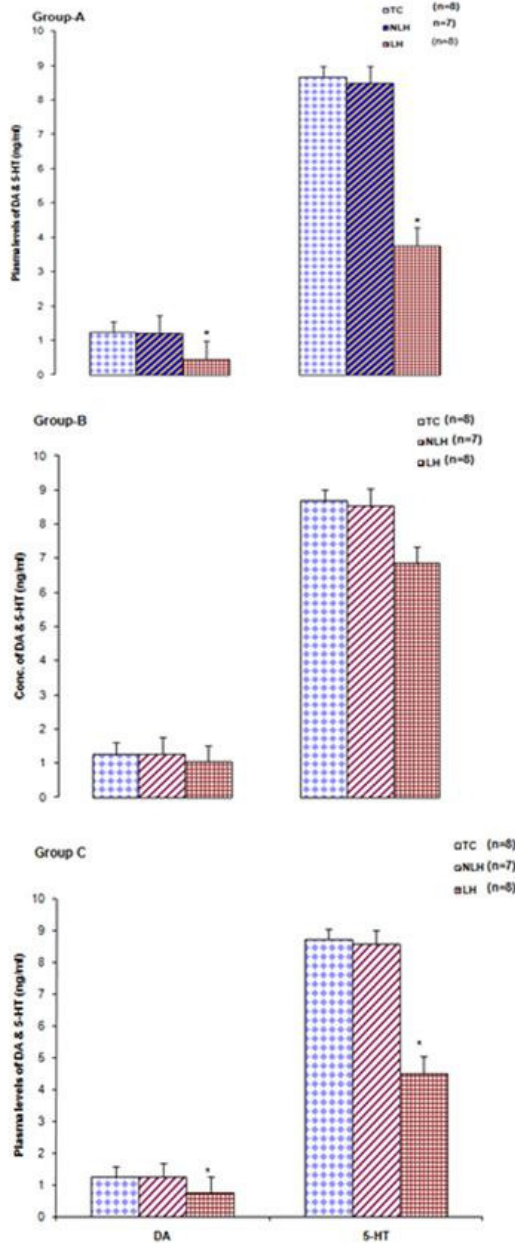
Results

The effect of learned helpless paradigms in rats: We did not observe any significant differences in mean escape latency after FR-1 trials in any experimental paradigm (data not shown). Escape latencies after 25 FR-2 trials in the different groups were given in table-1. In group A, LH rats showed significantly higher mean escape latency ($P < 0.001$) than NLH and TC rats, whereas the escape latency of NLH rats was not significantly different from that of TC rats. The escape latencies (in seconds) group-A rats were as follows: (TC = 6.28 ± 2.03 , NLH = 7.25 ± 3.75 , LH = 24.35 ± 3.48). Similarly in group B (IS on day 1 and escape test on day 2 again on day 4), the mean escape latency was significantly higher on day 2 in LH rats ($p < 0.001$) compared with TC or NLH rats; (TC = 5.32 ± 3.21 , NLH = 4.10 ± 2.25 , LH = 25.95 ± 3.62), however on day 4, the escape latency of LH rats was similar to that of NLH or TC rats ($P < 0.094$) (TC = 5.75 ± 2.39 , NLH = 4.06 ± 1.75 , LH = 7.88 ± 3.86). In group C (IS on day 1 and day 7), the mean escape latency was significantly higher in LH rats ($P < 0.001$) compared with the NLH group at all time intervals (TC = 4.30 ± 2.37 , NLH = 5.05 ± 2.58 , LH = 26.50 ± 3.18). None of the group showed significant differences in mean escape latency between TC and NLH rats (Table-1). The LH rats of group A and C failed to escape 16-22 times, whereas NLH and TC rats escaped rapidly with only 1-3 failures.

Group	Escape Latency (Seconds)		ANOVA			
	TC	NLH	LH	df	F	P
Group A Rats ^a						
Day 2	6.28 ± 2.03	7.25 ± 3.75	24.35 ± 3.48^d	2,20	57.73	<0.001
Group B Rats ^b						
Day 2	5.32 ± 3.21	4.10 ± 2.25	25.95 ± 3.62^d	2,20	95.18	<0.001
Day 4	5.75 ± 2.39	4.06 ± 1.75	7.88 ± 3.86	2,20	2.77	0.094
Group C Rats ^c						
Day 2	5.70 ± 3.22	5.11 ± 1.87	25.00 ± 3.07^d	2,20	98.66	<0.001
Day 8	6.20 ± 3.11	4.78 ± 2.33	25.99 ± 3.72^d	2,20	87.31	<0.001
Day 14	4.30 ± 2.37	5.05 ± 2.58	26.50 ± 3.18^d	2,20	127.76	<0.001
Data are mean \pm SD, TC Tested Control; NLH= Nonlearned helpless; LH= Learned Helpless; ANOVA, Analysis of Variance						
^a Rats were given inescapable shock (IS) on day 1 and tested for escape latency on day 2.						
^b Rats were given inescapable shock (IS) on day 1 and tested on day 2 and day 4.						
^c Rats were given inescapable shock (IS) on day 1 and tested for escape latency on day 2; these rats were given another IS on day 7 and tested on day 8 and day 14.						
^d $p < 0.001$ compared with TC or NLH						

The effect of learned helpless on plasma cortisol levels: Plasma cortisol levels were measured in rats of group A and group C and were as follows ($\mu\text{g/dl}$): group A rats: TC = 8.2 ± 1.2 , NLH = 8.3 ± 1.2 ; LH = 16.4 ± 2.3 . Plasma cortisol levels did not differ in TC and NLH. Plasma cortisol levels were significantly increased in LH rats compared to TC and NLH rats. In case of group C rats: TC = 8.0 ± 1.3 , NLH = 8.2 ± 1.3 ; LH = 12.5 ± 1.5 . Plasma cortisol levels were significantly increased in LH rats

Fig-2: Plasma concentration of dopamine and serotonin after inescapable shocks in LH stress paradigm in group A, B and C. Plasma monoamines were significantly decreased in LH rats in comparison to tested control rats. P is less than 0.02. Values are \pm mean.



of both group A and C compared to TC and NLH rats. Group-A ($df=2, 20, F= 2.49, P= 0.001$) and group-C ($df=2, 20, F= 2.38, P= 0.001$) The magnitude of increase of plasma cortisol in LH rat of group A is more than the LH rats of group C. We did not measure plasma cortisol levels in group B rats since we did not find differences in escape latencies in this group of rats.

The effect of learned helplessness on plasma levels of dopamine and serotonin: Statistical analysis revealed a significant effect of LH on plasma monoamine levels. In group A, it was observed that exposure to a single stress causes significant decrease in DA and 5-HT levels in LH rats as compared with TC and NLH rats. Plasma monoamines levels were measured in pg/ml. In Group-A, the mean DA and 5-HT levels were 1.24 ± 0.31 and 8.68 ± 0.52 for the control vs. 0.45 ± 0.22 and 3.75 ± 0.45 for the LH rats. In group- B, the mean DA and 5-HT levels were 1.25 ± 0.30 and 8.67 ± 0.53 for the control vs. 1.05 ± 0.32 and 6.85 ± 0.52 for the LH rats which are almost similar to group-A rats. In Group-C, the mean DA and 5-HT levels were 1.23 ± 0.3 and 8.71 ± 0.5 for the control vs. 0.72 ± 0.25 and 4.48 ± 0.54 for the LH rats. LH rats showed decreased levels of plasma dopamine and serotonin in all the groups compared to tested control TC and NLH rats. Positive correlations with monoamine levels were found with LH rats compared with TC. (Fig. 2)

Discussion

Investigations into the neurobiology of major depressive disorder have traditionally focused on cortisol and the monoamine neurotransmitters dopamine and serotonin. Our behavioral and analytical findings demonstrate selective and significant changes in plasma dopamine and serotonin levels after acute shock stress that persists when the stress is repeated. We observed a clear behavioral difference between LH and NLH rats, because the escape latency in LH rat was significantly different from that of NLH or TC rats. Whereas NLH rats showed escape latency similar to that of TC rats, the escape latency of LH rats was significantly higher than that of NLH or TC rats. The higher escape latency was present in LH rats exposed to IS on day 1 and tested for escape latency on day 2, but not rats tested on day 4, suggesting that the behavioral deficit does not persist for 4 days after a single IS paradigm. These observations were similar to those of [7-9], who found that behavioral deficit persists only until day 2 but not until day 4 after IS. When we subjected rats to another IS on day 7 in addition to that of first IS and tested them on day 14, the escape latency was significantly higher in LH rats compared with NLH or TC rats. Our observations thus suggest that exposure to a second IS produces a longer lasting behavioral deficit. There was a significant increase in the levels of cortisol in LH rats compared to TC, NLH rats after exposure to single and repeated stress in this paradigm. Interesting results emerged when we determined the levels of dopamine and serotonin in plasma of LH, NLH and TC rats. The LH rats given IS on day 1 and tested for escape latency on day 2 showed a significant decrease in dopamine and serotonin compared with NLH or TC rats. On the other hand, in LH rats given IS twice, once on day 1 and again on day 7 and tested on day 8 and day 14, there were found decreased levels of dopamine and serotonin compared to TC and NLH of same group. Magnitude of decrease of monoamines is more in case of single stress than the repeated stress of same group and inter group.

The behavioral model used in these studies takes advantage of the inherent variability between individual rats in their responsiveness to a regimen of inescapable shocks. This experiment was carried out in plasma and compared LH rats with unaffected rats receiving the same treatment condition (NLH). The inclusion of the tested control group (TC) in our comparison allows for the assessment of the effect of shocks and these control rats are not subjected to shock and escape test. However, a study of this type, one can correlate between the behavioral deficits exhibited by the LH rats and the changes in plasma conc. of cortisol and DA, 5-HT. Although no causal inferences can be made, we believe that the changes in plasma cortisol, DA, 5HT conc. demonstrated in these experiments influence the induction of LH behavior.

Our findings of increased levels of cortisol and decreased DA, 5HT are relevant to stress related disorders, particularly to post traumatic stress disorder (PTSD) [21]. For example, chronic treatment with monoamine oxidase inhibitor (MAO) for a long period causes the increases of their levels in plasma and alleviates depressive symptoms. These data suggests that presynaptic DA and 5-HT regulation may be involved in the modulation of LH behavior.

Conclusion

The role of DA and 5-HT circuit dysfunction plays in the pathophysiology of depression remains an open one. The most fruitful investigation may be with the subjects non responding with the behavior treatment. Clinical trials with pure DA and 5-HT acting compounds as therapy for augmentation in SSRI/SNRI would also be valuable. Further elucidation of the role of DA and 5-HT dysfunctions are clearly warranted as psychiatry strives to find ways to improve outcomes of patients with depressive disorders.

Finally, these data may be of direct relevance to human psychopathology. It has been argued that the LH paradigm provides a useful model for affective disorders, such as major depressive illness. The present data add further support to the importance of cortisol, DA and 5-HT levels in plasma which is synthesized from its respective neurons and therefore reinforce the notion that DA and 5-HT may play particularly important roles in the pathophysiology of clinical depression.

Acknowledgements

This research was financially supported by the University Grants Commission (UGC), Kolkata, INDIA, UGC, MRP (Ref: F. PSW-040/08-09).

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