Hippocampal Serotonin in a Rat Model of Learned Helplessness

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Abstract: Serotonin (5-hydroxytryptamine, 5-HT), acting via hippocampus, is thought to be critical for the neuroadaptation that alleviates adverse effects of stress on emotion and behavior. On the other hand, animal studies show that acute exposure to a stress-inducing situation increases brain serotonin metabolism and elicits learned helplessness often described as animal model of depression. In the present study it was hypothesized that acute stress-induced increases of brain serotonin metabolism are followed by a decrease in particularly hippocampal serotonin that makes significant contribution to stress-induced behavioral deficits. Effects of 2h restraint stress were therefore determined on the metabolism of serotonin in the hippocampus, raphe and cortex immediately and 24 h after the termination of stress period. We report that acute exposure to 2h restraint stress increased 5-HT metabolism in the cortex and raphe region but no effect was produced in the hippocampus. Exposure to 2h restraint stress elicited anxiety like behavior in light dark transition test monitored next day. Animals killed 24 h after the termination of stress period exhibited a decrease in the concentration of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus but not in the cortex and raphe. A role of serotonin in the elicitation of stress-induced behavioral deficits in the rat model of learned helplessness is discussed.

Key Words: Light dark transition test, Learned helplessness, Serotonin, Hippocampus, Stress.

INTRODUCTION

A dysfunctional 5-hydroxytryptamine (5-HT; serotonin)-ergic system is a vulnerability factor for major depressive disorder and other forms of affective illnesses [1]. A role of hippocampus in responses to stress emerged because acute exposure to an episode of 2h restraint stress increased 5-HT turnover in the hypothalamus, midbrain and cortex but the increases did not occur in the hippocampus [2]. Conversely, repeated daily exposure to 2h/day restraint, which produced behavioral adaptation, increased 5-HT turnover in the hippocampus only and not in other brain regions. It was suggested that an increase in serotonin neurotransmission via hippocampus is involved in adaptation to stress [2]. Later studies also consistently showed that hippocampus may mediate adaptation to severe inescapable stressor by the facilitation of serotonergic neurotransmission. Acute exposure to an elevated platform enhanced 5-HT overflow in the prefrontal cortex but not dorsal hippocampus whereas repeated daily exposure to the same stressor increased extracellular 5-HT in the dorsal hippocampus but not the prefrontal cortex [3]. In another study rats received inescapable foot shock and were tested in a shuttle box 24 h later. Pre stressed animals exhibited impairment of escape responses. This effect was prevented by bilateral intra hippocampal injection of zimelidine, a serotonin reuptake blocker but not by desipramine, a noradrenaline reuptake blocker [4]. Rats receiving a variety of chronic unpredictable mild stressors for 3 weeks showed a variety of depression-like behavioral changes which were suppressed or blocked by intra-hippocampal injection of 5-HT [5].

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These lines of evidence led us to hypothesize that reduced 5-HT neurotransmission in the hippocampus makes significant contributions to restraint-induced behavioral deficits. To critically test this hypothesis, we have monitored the metabolism of 5-HT in the hippocampus of restrained rats immediately and 24 h after the termination of restraint period.

MATERIALS AND METHODS

Animals

Locally bred male albino Wistar rats, weighing 180-220g purchased from HEJ Research Institute, Karachi, Pakistan were housed individually under a 12-hours light and dark cycle (lights on at 6:00 h) with free access to cubes of standard rodent diet and tap water 5 days before starting the experiment. All animal experiments were conducted in accordance with guidelines laid down by the NIH and approved by the institutional Ethics and Animal Care Committee.

Experimental Protocol

Experiment 1: Effects of 2h Acute Restraint Stress on 5-HT Metabolism:

Twelve rats were randomly assigned to unrestrained and restrained groups of 6 each. The animals assigned to restrained group were immobilized on wire grids for two h (between 09:00 and 11:00 h). The animals assigned to unrestrained group were left in their home cages during this period. Animals of restrained group were killed immediately after the termination of restraint period. Animals of

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unrestrained group were also killed at the same time. Brains were removed immediately after decapitation and desired regions dissected out were stored at -70^{0}C for the estimation of 5-HT and 5-HIAA by HPLC-EC.

Experiment 2: Effects of 2h Restraint Stress on the Activity of Rats in Light-Dark Transition Test and 5-HT Metabolism 24 h after the Termination of Stress Period

Twelve rats were randomly assigned to unrestrained and restrained groups of 6 each. The animals assigned to restrained group were immobilized on wire grids for two h (between 09:00 and 11:00 h). The animals assigned to unrestrained group were left in their home cages during this period. Behavior of rats (both unrestrained and restrained) in light dark transition test was monitored next day between 09:00 to 10:00 h. Animals were killed between 10:00 to 11:00 h to collect and store desired brain regions for the estimation of 5-HT and 5-HIAA.

Restraining Procedure

The animals were restrained as described earlier [2] by taping them to a wire grid of 10" x 9" fitted with a Perspex plate of 9" x 6.5". Restraint stress was produced by pressing the forelegs of the rat through the gaps in the metal grid and taping them together with zinc oxide plaster. Hind limbs were also taped and head of the animal rested on the Perspex plate.

Light-Dark Transition Test

The test procedure was essentially same as described earlier [7,8]. The apparatus used in the present investigation was a two compartment light-dark box. Both the light (made up of transparent plastic) and dark (made up of black plastic) compartments measured 26 x 26 x 26 cm. Access between the compartments was provided by a 12 x 12 cm passageway. The experiment was performed in a quiet, air-conditioned room and the apparatus placed under white light. An animal was introduced to the apparatus by placing in the light compartment. Time spent in the light compartment and entries in the light compartment were monitored for a cut off time of 5 min.

Brain Dissection Technique

The technique used was essentially same as described earlier [2,9,10]. A fresh brain was dipped in ice cold saline and placed with its dorsal side up in the moulded cavity of a brain slicer. A fine fishing line wire was inserted in to the slots of slicer to get slices of 1mm thickness. The slices were transferred to Petri dish kept on ice and desired brain regions identified with the aid of stereotaxic atlas. From the slice containing cortex olfactory material was discarded. Hippocampal material (CA1-4 fields + subiculum + dentate gyrus) was dissected out with a sharp scalpel. Raphe material was obtained by taking punches of 0.5mm diameter from slices containing dorsal and dorsal plus median raphe.

Neurochemical Analysis

Concentration of 5-HT and 5-HIAA in brain regions was determined by HPLC-EC as described before [8]. A 5 µ shim-pack ODS separation column of 4.5mm internal diameter and 15 cm length was used. The mobile phase was 0.1M phosphate buffer of pH=2.9, containing 14% methanol, 0.023% OSS and 0.005% EDTA. Electrochemical detection was done at an operating potential of 0.8V (glassy carbon electrode vs. Ag/AgCl reference electrode).

Statistical Analysis

Data on the effects of restraint stress on the levels of 5-HT and 5-HIAA were analyzed by t-test. Effects of restraint on the behavior of rats in light dark transition test were also analyzed by t-test.

RESULTS

Fig. (**1**) shows acute effects of 2h restraint stress on 5-HT and 5-HIAA concentrations in the cortex, hippocampus and raphe. T-test showed that 5-HT as well as 5-HIAA

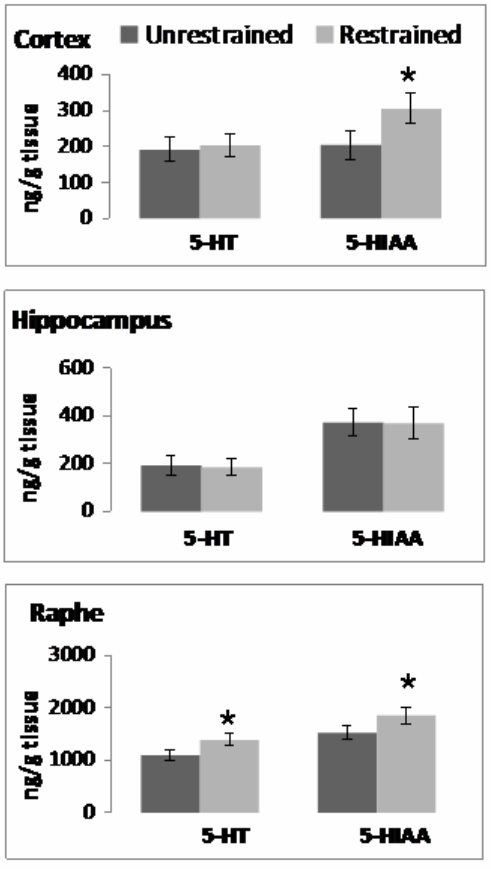

Fig. (1). Effects of 2h restraint stress on the levels of 5-HT and 5-HIAA in the cortex, hippocampus and raphe. Values are means ± SD (n=6) immediately after the termination of stress period. Significant differences by t-test: *P<0.01 from unrestrained animals.

concentrations increased in the raphe. Only 5-HIAA levels significantly increased in the cortex while no effect on 5-HT or 5-HIAA concentrations occurred in the hippocampus.

The effect of 2h restraint stress on the behavior of rats in light-dark transition test, monitored 22 h after the termination of stress period, is shown in Fig. (**2**). Restrained animals exhibited a large and significant decrease in the number of entries in light compartment (t-test). Time passed in light compartment was also significantly smaller in restrained than unrestrained animals.

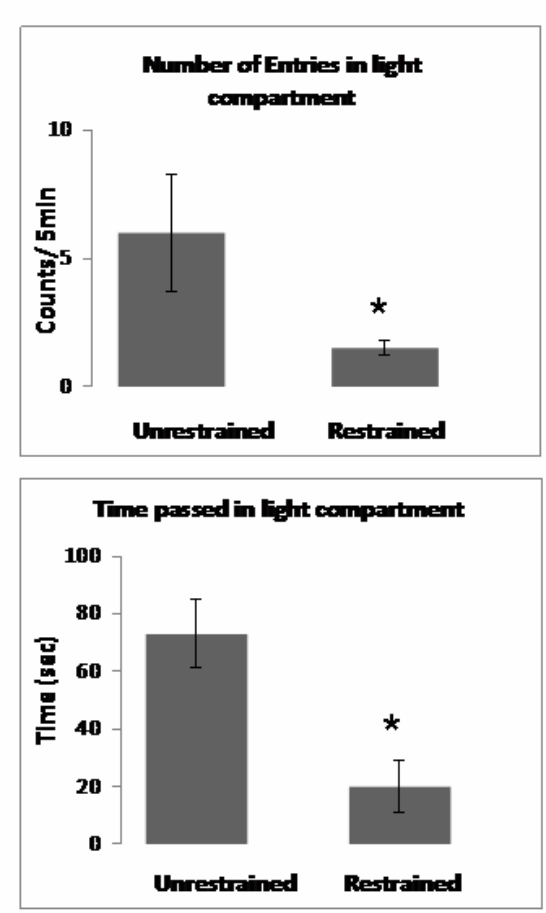

Fig. (2). Effects of 2h restraint stress on the behavior of rats in light-dark transition test. Values are means ± SD (n=6) 22 h after the termination of stress period. Significant differences by t-test: *P<0.01 from unrestrained animals.

Fig. (**3**) shows the effects of 2h restraint on the metabolism of 5-HT in the cortex, hippocampus and raphe, 24 h after the termination of stress period. Restrained and unrestrained animals exhibited comparable values of 5-HT and 5-HIAA in the cortex and raphe region. The levels of 5-HT as well as 5-HIAA were smaller in the hippocampus of restrained than unrestrained animals.

DISCUSSION

The present study shows that rats restrained for 2h on day 1 when tested for behavior in light-dark transition test exhibited a decrease in time passed in the light compartment (Fig. **2**). Number of entries in the light compartment also decreased. The results suggest that exposure to restraint stress produces behavioral deficits comparable to other controllable stressors.

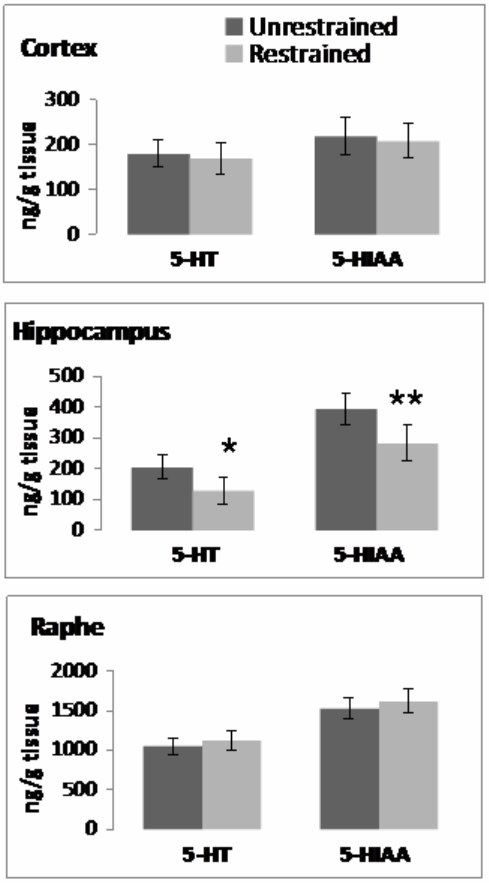

Fig. (3). Effects of 2h restraint stress on the levels of 5-HT and 5-HIAA in the cortex, hippocampus and raphe. Values are means ± SD (n=6) 24 h after the termination of stress period. Significant differences by t-test: *P<0.05, **P<0.01 from unrestrained animals.

Based upon the clinical evidence that links stressful life events with depressive episodes [11-13]; several animal models exhibiting stressor controllability and learned helplessness have been developed [14-17]. The most common animal model of 'stress and coping' is that of 'learned helplessness' [18-19]. With it, animals are exposed to either controllable or uncontrollable stressful events and later, they are tested on a new task in which all animals are given the opportunity to control the stressor, usually by escape. In most reports, animals that are exposed to uncontrollable stressful events do not learn to escape during testing on the new task [20-21]. This behavior has been equated with a sense of 'giving up', experienced by humans with major depression [22].

The learned helplessness paradigm was not developed to provide an animal model of depression or anxiety but it was shown in later studies that the model is sensitive to both antidepressants [23-24] and anxiolytics [25-27]. The

paradigm is widely used to understand neural mechanism and degree of behavioral adaptation to an uncontrollable stressor [28-30].

Animals exposed to other unpredictable and uncontrollable stressor e.g restraint stress, elevated platform and forced swimming also show coping deficits for aversive but escapable situations [31-32]. Chronic mild stress also causes behavioral changes in animals that parallel symptoms of depression [33-34]. The present results are consistent that acute exposure to restraint stress elicits behavioral deficits that can be seen 24 h after the termination of stress period and are comparable to the deficits produced by an uncontrollable stressor.

Other authors have shown that 5-HT turnover is enhanced following exposure to various stressors such as exercise and foot shock although brain levels of 5-HT are not always altered [35-37]. It has been also shown that stress-induced increases of brain serotonin are caused by an increase in the availability of tryptophan, the precursor of 5-HT [35-37], or an increase in the activity of tryptophan hydroxylase, the rate limiting enzyme of 5-HT biosynthesis [38]. Microdialysis also showed an increase in extracellular levels of serotonin in different areas of the brain following exposure to different types of stressors [39]. In the present study rats killed immediately after the termination of 2h restraint period exhibited an increase in 5-HT metabolism in the cortex and raphe but increases did not occur in the hippocampus (Fig. **1**). In addition, the present study shows that rats killed 24 h after the termination of stress period did not show an increase in 5-HT metabolism in the cortex and raphe while 5-HT levels decreased in the hippocampus (Fig. **3**) suggesting that a decrease in 5-HT particularly in the hippocampus is involved in restraint-induced behavioral deficits.

In conclusion the present study shows that raphe hippocampal serotonin neurotransmission may have important consequences on the ability of an organism to cope stress demand. Stress-induced exaggerated feedback control via pre or postsynaptic 5-HT-1A receptors decreasing the availability of 5-HT in the hippocampus may impair adaptation to lead to behavioral depression.

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