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INJECTED TRYPTOPHAN INCREASES BRAIN BUT NOT PLASMA TRYPTOPHAN LEVELS MORE IN RESTRAINED RATS

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ABSTRACT:

Immobilization stress given for one hour decreased cumulative food intake and growth rate in rats. Activity in an open field scored next day was also smaller in the restrained animals.

Stress is known to enhance brain serotonin (5-hydroxytryptamine; 5-HT) metabolism. It is however not clear, whether this enhancement is caused by an increase in the activity of tryptophan hydroxylase, the rate limiting enzyme of 5-HT biosynthesis or availability of the precursor tryptophan to the brain. In order to determine the utilization of tryptophan via serotonin path way in brain during stress; rats were injected with saline or tryptophan (50 mg/kg, i.p.). A group of both saline and tryptophan injected animals was restrained for one hour, while another group left unrestrained. The animals were killed immediately after the termination of stress period to collect plasma and brain samples.

Injected tryptophan resulted in 3-4 fold rise of plasma tryptophan in both unstressed and stressed animals in one hour. Stress alone however had no effect on plasma total tryptophan concentration. Free tryptophan in plasma, brain tryptophan, 5-HT and 5-hydroxyindole acetic acid (5-HIAA) all increased by both stress and tryptophan injection. The increases of brain tryptophan, 5-HT and 5-HIAA but not of free tryptophan in plasma were considerably greater in restrained animals given tryptophan load. The results suggest that enhancement of brain serotonin metabolism during stress is caused by an increase in the availability of tryptophan to the brain as well as an increase in the activity of the enzyme tryptophan hydroxylase. Factors affecting the availability of tryptophan to the brain are discussed.

INTRODUCTION

Stress is defined as a bodily response caused by unidentified stimuli. It has been shown experimentally that alterations of physiological functions under emotional stress depend particularly on the central nervous system. Such consequences are to a great extent related to specific features of brain metabolism. Various stress procedures, including immobilization have been shown to enhance rat brain serotonin (5-hydroxytryptamine; 5-HT) metabolism (Bliss *et al.,* 1968, Curzon *et al.,* 1972, Lobacheva, 1980 and Culvan *et al.,* 1980). However it is not clear whether this is caused by an increase in the availability of tryptophan to the brain (Fernstrom, 1983) or an increase in the activity of tryptophan hydroxylase, the rate-limiting enzyme in serotonin biosynthesis (Ichiyama *et al.,* 1968). The present paper is concerned with investigating the role of the precursor, tryptophan, in the enhancement of brain serotonin metabolism during immobilization. This was determined by comparing the effects of a tryptophan load on brain serotonin metabolism in stressed and unstressed rats. Plasma levels of total and free tryptophan were determined to investigate a role of plasma tryptophan in the availability of tryptophan to the brain.

MATERIALS AND METHODS

Locally bred male and female albino Wistar rats weighing 150-200g were housed individually with free access to cubes of standard rat food and water for at least five days before the start of the experiment.

The rats were randomly assigned to unstressed and stressed groups. However equal number of male or female animals were kept in each group. Food intake and body weight of the rats were monitored daily. The rats assigned to the stressed group were restrained for 2 hours on a wire grid, those assigned to the unstressed group were left in their home cages. On the next day activity of both the groups was scored in an open field. Effects of tryptophan load on brain 5-HT metabolism during stress were investigated by injecting (i.p.) 50mg/kg/ml tryptophan or saline to previously restrained or unrestrained groups. Previously restrained rats were then again restrained for 1 hour while unrestrained rats were injected and kept back in their home cages. Restraint was terminated at the end of 1 hour period and rats were killed immediately. The experiment was carried out in a balanced design such that the animals injected with saline or tryptophan were alternately restrained or left unstressed and then killed accordingly. Blood was collected in heparinized centrifuge tubes and centrifuged to collect the plasma for the estimation of total and free tryptophan. Brain was taken out immediately after decapitation and stored at $-70^{o}C$ until analysis of 5-HT, 5-HIAA and tryptophan concentrations.

Chemicals:

Chemicals purchased from Sigma Chemical Company or Merck were used in all experiments.

Biochemical Estimations:

Determination of Total and Free Tryptophan in Plasma:

Plasma total and free (ultrafilterable) tryptophan concentrations were determined by the fluorimetric method of Denckla and Dewey (1967) as modified by Bloxam and Warren (1974).

Determination of 5-HT, 5-HIAA and Tryptophan in Brain:

The procedure used for the analysis was essentially as Curzon *et al.* (1981). 5-HT and 5-HIAA were condensed with O-phthalaldehyde and tryptophan with formaldehyde to form respective fluorescent compound.

STATISTICAL ANALYSIS

Data on the effect of stress on food intake and growth rate were analyzed by student's t-test (two-tailed). Scores of activity in an open-field were compared by Mann-Whitney 'U'-test. The effect of tryptophan load on brain 5-HT metabolism and plasma total and free tryptophan in restrained and unrestrained rats was analyzed by two ways ANOVA (factor 1=tryptophan load, factor 2=stress). Individual comparisons were made using Newman-Keuls Statistics. Differences between groups were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Studies on experimental animals have shown that uncontrollable stress produces neurochemical and behavioural deficits (Weiss *et al.,* 1981). Comparable deficits in behaviour were found in the present study where 2h immobilization resulted in a slight (6.26%) but significant ($P<0.01$) decrease of growth rate. Food intake was also decreased ($P<0.01$) in

restrained rats (Fig. 1). Rats subjected to immobilization stress were found to have lower ambulatory activity (P<0.01) in an open-field (Fig. 2). No significant difference in the defecations scores was however observed between the rats of the two groups.

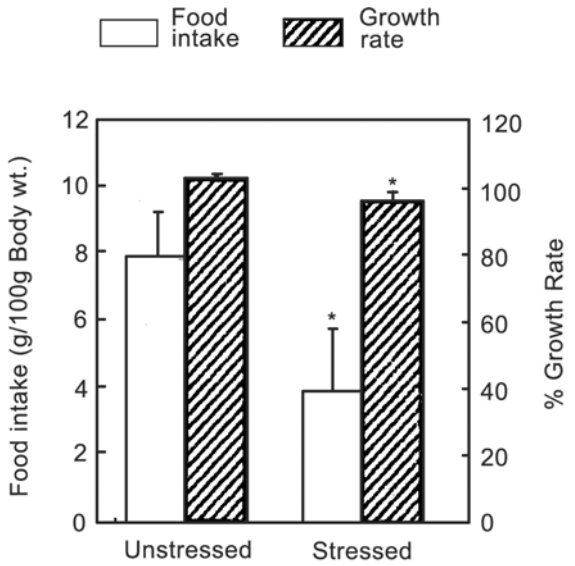

Fig. 1: The effect of immobilization stress on food intake (g/100 gm body weight) and % growth rate of rats. Values represent means ± S.D. (n=16).
*Statistically significant difference between control and stressed group (P<0.01) by Student's (two-tailed) t-test analysis.

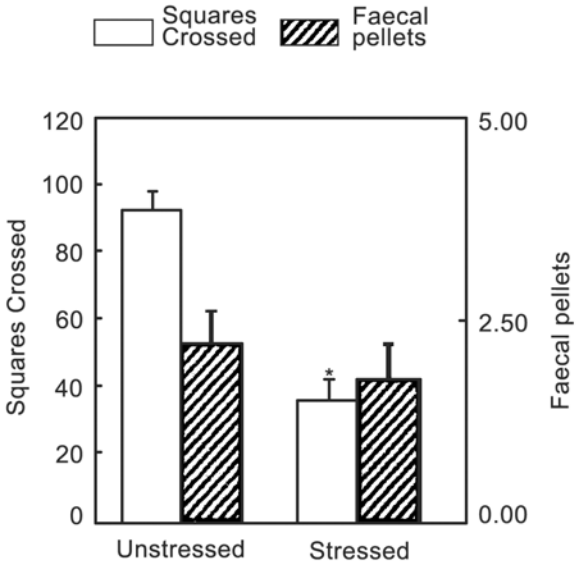

Fig. 2: Effect of immobilization stress on the activity of rats in an open field. Values represent means ± S.E.M.(n=16).
*Statistically significant difference between control and stressed group (P<0.01) by Mann-Whitney 'U'-test.

Serotonin is synthesized by an essential amino acid tryptophan. Plasma tryptophan is transported to the brain via a carrier-mediated mechanism shared by all large neutral amino acids (LNAAs). Factors like total tryptophan, free tryptophan and LNAAs other than tryptophan in plasma have been shown to have some role in determining brain levels of tryptophan (Fernstrom and Wurtman, 1971; Bloxam *et al.,* 1980). Estimations of LNAAs were not performed in the present study.

Plasma total tryptophan concentration increased slightly but non-significantly in saline injected (28.33%) and tryptophan injected (14.15%) rats when they were restrained. However, plasma free tryptophan increased 89.73% in saline injected and 19.65% in tryptophan injected animals. Tryptophan injection caused 3-4 fold rise of total tryptophan concentration in plasma. Stress alone did not affect plasma total tryptophan concentration in saline or tryptophan injected animals. Two way ANOVA also showed a significant effect of tryptophan injection. Effects of stress procedure and of interaction between tryptophan and stress were not significant. An increase of plasma free tryptophan occurred following both tryptophan injection and stress. Two way ANOVA showed significant effect of tryptophan injection and stress procedure. Interaction of these two factors was not significant.

Tryptophan hydroxylase, the rate limiting enzyme of 5-HT biosynthesis, exists unsaturated with its substrate (Eccleston *et al.,* 1965; Fernstrom and Wurtman, 1971). Therefore brain levels of tryptophan have been shown to modulate 5-HT synthesis in many circumstances (Lobacheva, 1980; Mohammed and Taghride, 1982 a & b).

Table 1

The effect of immobilization stress on plasma total and free tryptophan concentrations (ug/ml) in controls and tryptophan injected (50 mg/kg i.p.) rats.

	Saline Injected		**Tryptophan Injected**		**Two Way ANOVA**		
	Un-stressed	Stressed	Un-stressed	Stressed	Trypto-phan	Stress	Tryptophan Stress Inter-action
Total Tryptophan % increase from respective unstressed controls	12.00 ± 1.09	15.40 ± 1.67 28.33	41.83 ± 6.91**	47.75 ± 10.02** 14.15	F=152.1 P<0.01	F=3.4 n.s	F=0.24 n.s
% increase from respective saline injected controls	–	–	248.58	210.06	–	–	–
Free Tryptophan % increase from respective unstressed controls	4.48 ± 0.98	8.50 ± 1.0^{+} 89.73	17.05 ± 2.98**	20.4 ± 3.63**,$^{+}$ 19.65	F=149.42 P<0.01	F=13.59 P<0.01	F=0.105 n.s
% increase from respective saline injected controls	–	–	280.58	140.0	–	–	–

**P<0.01 from respective saline injected rats; $^{+}$P<0.05 from respective unstressed controls by Newman-Keuls Q statistics following two-way ANOVA (df: 1,20). Values are means ± S.D. (n=6).

Table 2
The effect of immobilization stress on brain indole concentrations (ng/g) in controls and tryptophan injected (50 mg/kg i.p.) rats.

	Saline Injected		**Tryptophan Injected**		**Two Way ANOVA**		
	Un-stressed	Stressed	Un-stressed	Stressed	Trypto-phan	Stress	Trypto-phan Stress Inter-action
Tryptophan % increase from respective unstressed controls	2650.00 ± 920.3	5773.50 ± 1663.6^{++} 85.20	12613.33 ± 1230.5** 	20041.60 ± 2423.10**,$^{++}$ 55.20	F=320.25 P<0.01	F=60.72 P<0.01	F=10.10 P<0.01
% increase from respective saline injected controls	–	–	452.77	363.25	–	–	–
5-HT % increase from respective unstressed controls	780.83 ± 258.2	1175.08 ± 329.26^{+} 50.49	1301.85 ± 192.40* 	2250.41 ± 317.45**,$^{++}$ 72.86	F=48.85 P<0.01	F=34.57 P<0.01	F=5.89 P<0.01
% increase from respective saline injected controls	–	–	66.72	91.51	–	–	–
5-HIAA % increase from respective unstressed controls	615.06 ± 49.52	1044.89 ± 164.94^{++} 69.88	1010.76 ± 307.52** 	1724.63 ± 325.57**,$^{++}$ 70.62	F=153.38 P<0.01	F=166.6 P<0.01	F=45.309 P<0.01
% increase from respective saline injected controls	–	–	64.33	65.05	–	–	–

**P<0.05, **P<0.01 from respective saline injected rats; $^{+}$P<0.05, $^{++}$P<0.01 from respective unstressed controls by Newman-Keuls Q statistics following two-way ANOVA (df: 1,20). Values are means ± S.D. (n=8).

Various stress situations including immobilization have been shown to enhance rat brain serotonin metabolism (Kennett and Joseph, 1981 and Dunn, 1988). Similarly in the present study 2h immobilization increased rat brain serotonin metabolism. In the present study stress showed a profound effect (P<0.01) on brain tryptophan, 5-HT and 5-HIAA concentrations in both control (85.2%, 50.49% and 69.88% respectively) and tryptophan injected (55.2%, 72.86% and 70.62% respectively) animals. Tryptophan load caused a six-fold rise of brain tryptophan concentration in unstressed rats. Two-fold rise of brain tryptophan occurred due to stress in saline injected animals. The effects of tryptophan load and stress were additive. Two way ANOVA showed significant effects of stress, tryptophan injection and significant interaction between the two factors. Since increases were observed in levels of both 5-HT and 5-HIAA and there was an associated increase of tryptophan, the results tend to suggest that an increase in the availability of tryptophan may

have a primary role in the stress-induced increases of 5-HT metabolism. The effect of tryptophan load on the enhancement of brain 5-HT metabolism is also consistent with previous reports (Fernstrom and Wurtman, 1971). Increases of free tryptophan in plasma (Table 1) but not of total tryptophan and associated increases of brain tryptophan concentration (Table 2) suggest that stress-induced increases of free tryptophan may be at least in part involved in the enhancement of brain serotonin metabolism.

Parallel to brain tryptophan changes were the changes in brain levels in 5-HIAA. The levels of 5-HT were also increased by stress in both saline injected and tryptophan injected groups. Percentage increases in the latter group were greater and more significant. Somewhat greater effects of stress on the increases of 5-HT concentration in the tryptophan injected rats suggest that together with the availability of tryptophan, factors like kinetic properties of the rate limiting enzyme, tryptophan hydroxylase, may also be involved in the stress-induced increases of 5-HT metabolism. Indeed an increase in the activity of enzyme occurred following electric shock, etherization or cold stress (Azmitia and McEwen, 1974).

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