Indian J Physiol Pharmacol 1993; 37(1) : 8-12

REGIONAL METABOLISM OF 5-HYDROXYTRYPTAMINE IN BRAIN UNDER ACUTE AND CHRONIC HEAT STRESS

SANGITA DEY, P. K. DEY* AND H. S. SHARMA

Neurophysiology Unit,
Department of Physiology,
Banaras Hindu University,
Varanasi - 221 005

(Received on March 31, 1992)

Abstract : The metabolic alteration of 5-HT in four different regions of rat brain and plasma was studied under acute and chronic heat stress. A generalised elevation of 5-HT in all the brain regions along with high plasma level was observed in animals subjected to 4 hour heat stress at 38°C. Such elevation of brain 5-HT may be due to entry of plasma 5-HT into the brain owing to breakdown of blood-brain barrier (BBB). In heat adapted rats, where BBB remained unaffected, no increase in brain 5-HT was observed, rather a significantly low level was maintained both in plasma and brain tissue.

Key words: heat stress 5-HT metabolism brain 5-HT stress adaptation

INTRODUCTION

A number of studies have reported that prolonged exposure of animals to heat can precipitate brain oedema, gastric haemorrhagic lesions, hyperthermia, increased blood-brain barrier permeability (BBB), diminished cerebral blood flow and prostration behaviour (1,2,3,4). Most of these pathophysiological sequlae have been shown to be associated with increase of 5-HT in plasma and in the whole brain (5, 6, 7, 8).

Several reports indicate that different regions of brain do not respond to stress in the same manner in terms of 5-HT metabolism, and also that the responses of different regions of the brain are distinctly different under conditions of acute stress and in stress adapted condition (9,10).

Our earlier studies were concerned with 5-HT measurement in whole brain under different durations of acute heat stress and in chronic heat stress (1,3,5). The present study deals with the changes in 5-HT level in several parts of the rat brain both under acute heat stress and in heat adapted animals.

METHODS

Inbred CF rats of either sex (8-9 weeks old, weighing 60-80 gm) were housed at control ambient temperature (28°±1°C) with a 12 hour light/dark cycle. Rat ± feed and tap water were freely supplied.

Heat stress : All heat stress experiments began between 9.00 and 10.00 hour. Three separate groups of animals were exposed to heat stress for 1, 2, and 4 hour in B.O.D. incubator (Adair Dutt & Co., New Delhi) at 38°C (relative humidity 45-47%; wind velocity 28.6 cm/sec). Changes in body temperature (before and after heat exposure) and haemorrhagic lesions in the stomach were recorded in each animal as indices of stress (11). The occurrence of salivation and prostration as qualitative assessment of heat stress was noted during the entire period of heat exposure at 1 hour interval.

Chronic heat stress : Two separate groups of animals were exposed to 38°C daily for 1 hour and 4 hour respectively for 1 week, followed by 4 hour acute heat exposure on day 8. The rectal temperature was daily noted and stomach was examined for haemorrhagic lesion on day 8. The signs for behavioral prostration, locomotor activity and salivation were noted every day.

Drug administration : 75 µg of 5,6 dihydroxytryptamine (5, 6, DHT) was administered into right lateral ventricle of brain through stereotaxically implanted cannula described earlier (12). Then the animals were divided into three groups. One group served as experimental control (n = 5). The second and 3rd group were exposed to 4 hour acute heat exposure 14 days

after 5,6,DHT administration. The 5-HT estimation in plasma and brain were done in 2nd group (n = 4); 3rd group was examined for BBB permeability (n = 5). The stomach lesions were examined in all groups. Parachlorophenylalanine (PCPA 100 mg/kg/day) was administered intraperitoneally for 3 successive days into 15 rats which were then divided into three groups. One group served as experimental control (n = 5). On day 4, 2nd and 3rd group were exposed to 4 hour heat vexposure. 5-HT measurement in plasma and brain was carried out in 2nd group (n = 4) and 3rd group was examined for BBB permeability (n = 5). All groups were examined for haemorrhagic lessions in stomach wall.

BBB permeability : Evans blue which forms Evans blue-Albumin complex in circulation was used as tracer for BBB permeability. The dye (0.3 ml/100 gm of a 2% solution in 0.9% saline) was injected through a polythene cannula (PE 25 USA), within 5 minute after termination of stress, into external branch of right jugular vein under urethane anaesthesia (0.8 gm/kg, i.p.). Five minute after dye infusion, the brain was perfused with 0.9% saline through the heart to remove blood from the vessels. The brain was removed, and after naked eye inspection of extravasation of dye, the quantity of dye which entered the brain was measured colorimetrically according to the method of Harada et al (13) and expressed as mg%.

Gastric lesions : The procedure was described earlier (4). In brief, the stomach was taken out, opened along the greater curvature, rinsed with normal saline and pinned on a flat surface. The number of haemorrhagic spots and streaks were counted with the help of a magnifying lens (x 20).

5-HT measurement : 5-HT in plasma (including platelets) and brain was flurometrically measured (1,14). At the end of each set of experiment, the animals were quickly decapitated and the trunk blood was collected in ice-cold polythene tube and the plasma was separated immediately and stored at –70°C. The whole brain was quickly removed and transferred to glass slide placed on ice slab. The parieto-occipital Cortex (P-O cortex), hippocampus, cerebellum and midbrain were quickly dissected out and stored at –70°C. Dunnett's test for multiple group comparisons was used to evaluate statistical significance of the data.

Drugs : 5, 6, DHT was purchased from Sigma Chemical Co., USA.

RESULTS

Acute heat stress : The results are shown in Table I. A brief exposure to heat stress (1 hr) produced a significant diminution of plasma 5-HT level which underwent reverse modification with longer heat exposure. Thus, following 2 and 4 hour heat stress a rise of plasma 5-HT from the initial diminished level, and a profound increase of the same above the control level occurred respectively. Following 1 hr heat

TABLE I : Effects of acute heat stress on plasma and brain 5-HT levels (Mean ± SEM).

Expt.	*Rise in Rect. Temp.*	*Plasma 5-HT (µg/ml)*	5-HT (µg/gm)			
			P-O Cortex	*Cerebellar cortex*	*Hippocampus*	*Midbrain*
Control (5)		0.31 ± 0.076	0.796 ± 0.128	0.113 ± 0.048	0.48 ± 0.06	0.736 ± 0.116
Heat Stress 1 hr (5)	1.4 ± 0.20	0.156*** ± 0.023	0.594* ± 0.135	0.359*** ± 0.019	0.129*** ± 0.007	0.416** ± 0.062
Heat Stress 2 hr (5)	2.12 ± 0.30	0.247 ± 0.083	0.692 ± 0.144	0.214 ± 0.068	0.476 ± 0.176	0.562 ± 0.049
Heat Stress 4 hr (5)	3.44 ± 0.24	1.128*** ± 0.170	2.310*** ± 0.96	1.536*** ± 0.32	1.226*** ± 0.30	0.98 ± 0.06

*P < 0.05; ** P < 0.01; *** P < 0.001

Figures in parentheses indicate number of animals used.

exposure, 5-HT level was significantly diminished in P-O cortex, midbrain and hippocampus, whereas cerebellar region showed significant increase. Like the plasma concentration profile, following 2 hour heat exposure, 5-HT level began to increase in all brain regions from the initial diminished level; with 4 hour head exposure, except in midbrain region, 5-HT level showed highly significant increase in other three brain regions.

The increase in rectal temperatures following 1, 2, and 4 hr head exposure was 1.4+0.2°, 2.12+0.30° and 3.44+0.24°C respectively. Gastric lesions (2-5 in number) appeared during 2 hour heat exposure, and such lesions occurred extensively (10-24 in number) with 4 hour heat exposure. Profuse salivation, prostration and marked inhibition of spontaneous locomotion were the predominant features in 4 hour heat stress group.

5, 6, DHT and PCPA pretreatment and heat stress: The results are shown in Table II. On exposure of 5,6,DHT pretreated rats to 4 hr heat stress, an increase of 5-HT in whole brain was observed inspite of 5-HT neurones being damaged as reflected by depletion of brain 5-HT. The results further showed that i.c.v. administration of the drug did not interfere with the marked increase of plasma 5-HT with 4 hr heat exposure. BBB permeability showed a significant increase as evident from dye concentration in brain (control 0.23 + 0.15; experimental group 12.+0.14 mg%) and 9-10 gastric haemorrhagic spots were observed in heat exposed drug-pretreated group.

PCPA treatment of control rats led to a marked lowereing of 5-HT in both plasma and brain. Following exposure of PCPA-pretreated rats to 4 hr heat stress, there were no significant increase of 5-HT in plasma and whole brain. Also, gastric lesions and breakdown of BBB permeability were absent in this group. The rectal temperature rose to 1.63 ± 0.26°C.

Chronic heat stress: The results are shown in Table III. In animals subjected to 1 hour daily heat exposure for 7 days followed by 4 hour acute heat stress on day 8, the plasma 5-HT level remained almost within normal range. In case of brain, unlike that of 4 hour acute heat exposure, 5-HT concentration did not increase in P-O cortex except in cerebellum and hippocampus.

Exposure of animals to more severe heat stress i.e. 4 hr daily heat exposure for 7 days followed by 4 hr acute heat exposure on day 8, half of the animals in this group (n = 10) died between 2nd and 4th day of exposure..The rest 50% animals survived and showed adaptation to heat stress. 5-HT concentration in different brain regions and plasma of such heat adapted rats remained significantly low in contrast to those of acute 4 hour heat exposure. The adapted animals did not show gastric lesions and increased BBB permeability and less rise of rectal temperature was noted. The prostration behaviour gradually disappeared completely by the 7th day of exposure.

DISCUSSION

Earlier reports (1, 2, 15) have shown that acute heat stress leads to increased BBB permeability, brain

TABLE II: Effect of 5, 6 DHT (I.C.V.) and PCPA (I.P.) administration on plasma and brain 5-HT level under acute heat stress (Mean ± SEM).

Expt.	*Rise in Rect. Temp.*	*Plasma 5-HT (µg/ml)*	*Whole brain 5-HT (µg/gm)*
Control		0.29 ± 0.08	0.68 ± 0.06
5, 6 DHT Treatment (5)		0.30 ± 0.07	0.165 ± 0.04***
5, 6 DHT Treatment plus 4 hr heat stress (4)	2.5 ± 0.7	1.50 ± 0.38***++	0.33 ± 0.38***++
PCPA Treatment (5)		0.10 ± 0.05***	0.39 ± 0.08***
PCPA Treatment plus 4 hr heat stress (4)	1.63 ± 0.26	0.14 ± 0.05	0.49 ± 0.13

***P < 0.001; The figure in parenthesis indicate number of animals. ++Compared to Exptal. Control

TABLE III : Plasma and brain 5-HT levels following chronic heat stress (Mean ± SEM).

Expt.	*Rise in Rect. Temp.*	*Plasma 5-HT (μg/ml)*	5-HT (μg/gm)			
			P-O Cortex	*Cerebellar cortex*	*Hippocampus*	*Midbrain*
Control (5)		0.31 ± 0.076	0.796 ± 0.128	0.113 ± 0.048	0.48 ± 0.15	0.736 ± 0.116
7 days chronic (+) + Heat exposure (4)	2.10 ± 0.25	0.24 ± 0.07	0.816 ± 0.306	0.816*** ± 0.306	0.75** ± 0.079	0.892 ± 0.146
7 days chronic (++) Heat exposure (5)	2.0 ± 0.40	0.09*** ± 0.03	0.11*** ± 0.003	0.106*** ± 0.002	0.245*** ± 0.20	0.193*** ± 0.002

(+) The rats were exposed to 1 hr at 38°C for 7 days followed by 4 hr heat exposure on day 8.
(++) The rats were exposed to 4 hr at 38°C for 7 days followed by 4 hr heat exposure on day 8.
The figure in parenthesis indicate the number of animals.

oedema, diminished cerebral blood flow, gastric lesions, diarrhoea and prostration; and these sequelae were mainly manifested when animals were exposed to 38° for 4 hour. Many of these heat induced symptoms are shown to be mediated through excessive increase of 5-HT in circulating blood induced by exposure to heat; parripasu a great increase of 5-HT in whole brain also occurred under such heat stress (2, 5). The increased BBB permeability was mainly evident in P-O cortex and cerebellar cortex.

The present study was aimed to find out the regional specificity of 5-HT metabolism in rat brain to both acute and chronic heat stress. The present findings that a rapid decline of 5-HT in P-O cortex, hippocampus and midbrain as well as in circulating blood observed following a short period of heat exposure (1 hr) could be attributed to enhanced monoamine oxidase activity (MAO) induced by short term exposure (6). It was earlier shown that brief exposure to heat stress had no significant effect on BBB permeability (1).

However, a significant increase of 5-HT in circulating blood as well as in different brain regions (except midbrain) with concomittant increase in BBB permeability was found when the rats were exposed to heat stress for a prolonged period (Table I). This raises the question as to whether plasma 5-HT, owing to the breakdown of BBB, has contributed towards a high level of brain 5-HT. Such an extraordinary increase of 5-HT in cerebral cortical region under prolonged heat stress is somewhat difficult to account for on the basis of increased synthesis and transport of 5-HT from brainstem raphe nuclei to the cerebral cortex because of absence of a rise in brainstem 5-HT level. Secondly, although MAO activity undergoes significant inhibition under prolonged heat stress (3,6), this is unlikely to contribute to such a great elevation of brain 5-HT. A plausible explanation for the high cerebral 5-HT level appears to be the leakage of plasma 5-HT due to the opening of the blood-brain-barrier by prolonged heat stress. Likewise the occurrence of significantly high level of brain 5-HT in 5,6, DHT pretreated rats is most probably due to entry of plasma 5-HT into the brain as significantly elevated plasma 5-HT level was observed alongwith increased BBB permeability. As expected, PCPA pretreated rats did not show elevated 5-HT level in brain as PCPA treatment caused significant reduction of plasma and brain 5-HT and thus preventing the opening of BBB.

A significant adaptive change in 5-HT metabolism accompanied the adaptation of rats to chronic heat stress. Unlike the findings with 4 hr acute heat stress, there was no increase of 5-HT in plasma and brain regions except in cerebellum and hippocampus following adaptation to moderate heat stress (1 hr exposure daily for 7 days). On being adapted to severe heat stress (4 hr exposure daily for 7 days), the metabolic adaptation of 5-HT was tuned mainly towards attenuated 5-HT level in brain and in circulation which may be related to adaptive enhancement of MAO activity observed in earlier experiments (3,6).

We conclude that brain biogenic amines measured in various stress conditions should be interpreted with

caution, when the stress condition is accompanied by increased BBB permeability. Secondly, the adaptation to heat stress is accompanied by metabolic adaptation of 5-HT.

ACKNOWLEDGEMENTS

The authors are thankful to Indian Council of Medical Research, New Delhi for financial assistance and Council of Scientific & Industrial Research, New Delhi for JRF to Sangita Dey.

REFERENCES

1. Sharma, HS. Blood-Brain barrier (BBB) in stress. Ph.D Thesis. Banaras Hindu University, Varanasi, 1982.
2. Sharma HS, Dey PK. Influence of long term acute heat exposure on regional blood-brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. *Brain Res* 1987; 424: 153-162.
3. Dey PK, Sharma HS, Ashok Kumar. Modification of plasma and brain 5-HT level, BBB permeability, brain oedema, RCBF and lung MAO activity under acute and chronic heat stress in rats. In: Brain and psychophysiology of Stress. Eds: K.N. Sharma, W. Selvamurthy and Neena Bhattacharya. *Indian Council of Medical Research, New Delhi* 1988; pp. 38-47.
4. Sharma HS, Dey PK. Probable involvement of 5-hydroxytryptamine in increased permeability of blood-brain barrier under heat stress in young rats. *Neuropharmacology* 1986; 25: 161-167.
5. Sharma HS, Dey PK. Role of 5-HT in increased permeability of blood-brain barrier under heat stress. *Indian J Physiol Pharmacol* 1984; 28: 259-267.
6. Sharma HS, Dey PK. Ashok Kumar. Role of circulating 5-HT and lung monoamine oxidase activity in physiological processes of heat adaptation in conscious young rats. *Biomedicine* 1986; 6: 31-40.
7. Sharma HS, Dey PK. Regional cerebral blood flow and blood-brain barrier changes following intracarotid infusion of serotonin: Influence of drugs. Presented in Second International Symposium on 'Pharmacology of Cerebral ischaemia. *Hamburg (Germany)* October 3-5, 1988.
8. Sharma HS, Olsson Y, Dey PK. Changes in blood-brain barrier and cerebral blood flow following elevation of circulating serotonin level in anaesthetised rats. *Brain Res* 1990; 517: 215-225.
9. Adell A, Garcia-Marquez C, Armario A, Gelpi E. Chronic stress increases serotonin and noradrenaline in rat brain and sensitized their responses to a further acute stress. *J Neurochem* 1988; 50: 1678-1681.
10. Kenett G, Chauloff F, Marcou M, Curzon G. Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin. *Brain Res* 1986; 382: 416-421.
11. Paulson OB, Sharbrough FW. Physiologic relationship between the EEG and the regional cerebral blood flow. *Acta Neurol Scand* 1974; 50: 194-220.
12. Ray AK, Dey PK. Morphine analgesia following its infusion into different liquor spaces in the rat brain. *Arch Internat. Pharmacodyn* 1980; 246: 108-117.
13. Harada AM, Takeuchi M, Fukato T, Katagiri K. A simple method for the quantitative extraction of dye extravasated into the skin. *J Pharm Pharmacol* 1971; 23: 218-219.
14. Snyder SH, Axelrod J, Zweig M. A sensitive and specific flurorescence assay for tissue serotonin. *Biochem Pharmacol* 1965; 14: 831-835.
15. Sharma HS, Olsson Y, Dey PK. Influence of serotonin on regional blood-brain barrier permeability and cerebral blood flow in the rat. In Neurotransmission and cardiovascular function I. Eds: J. Seylaz and ET Mackenzie. *Elsvier Science Publishers B.V. (Biomedical Division)* 1989; pp. 253-255.