

ROLE OF 5-HT ON INCREASED PERMEABILITY OF BLOOD-BRAIN BARRIER UNDER HEAT STRESS

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Summary : Exposure of young rats (9-10 wks) to chronic summer heat (36°C) or acute heat (38°C, 4hr) increased the BBB permeability to Evans blue albumin complex (MW 68,000) and ¹³¹I-sodium (MW 154) in different brain regions which correlated well with the increased level of 5-HT in plasma and brain. This increased permeability of BBB and the increased 5-HT level were prevented by pretreatment with p-CPA, indomethacin and diazepam. Cyproheptadine and vinblastine pretreatment however, prevented only the increased permeability of BBB, the plasma and brain 5-HT level continued to remain high. These results indicate a probable role of 5-HT as one of the factors leading to the increased permeability of BBB in young rats following heat stress.

Key words : blood-brain barrier (BBB) diazepam heat stress 5-HT
Evans blue albumin (EBA) vinblastine ¹³¹I-sodium p-CPA
indomethacin cyproheptadine mean arterial blood pressure (MABP)

INTRODUCTION

Heat-stress is frequent among children during summer months in many parts of India which often leads to serious clinical problems. Several patho-physiological symptoms like brain oedema, subarachnoid haemorrhage and alteration in EEG pattern have been reported following summer heat in children (19, 26). The aetiology of these neurological changes still remains to be elucidated. It is imperative that the homeostatic regulation of the fluid environment of the brain is likely to undergo severe alteration during heat exposure. Such alteration of the regulation of fluid compartments of brain may result from the functional/structural breakdown of the BBB, which may be instrumental in precipitating the above neurological consequences.

Since the BBB is nature's protective device which maintains the composition of extracellular fluid strictly within narrow limit, in the present study, the state of BBB was

examined following heat exposure in model situation with particular reference to 5-HT which is well known to increase the BBB permeability under various pathophysiological conditions (9, 30).

MATERIAL AND METHODS

Experiments were carried out on inbred CF rats of either sex body weight ranging from 60-80 g (9-10 wks old) and 200-250 g (24-26 wks old). The animals were housed at controlled room temperature ($24 \pm 1^\circ\text{C}$) with 12 hr light and 12 hr dark schedule. Rat feed and tap water were supplied *ad libitum*.

Exposure to heat :

(a) *Chronic exposure to summer heat* : During the late period of the month of May and throughout the period of June, the room air temperature gradually reached to $34-36^\circ\text{C}$. Animals were exposed to this ambient air temperature of Varanasi (relative humidity 50-55%, wind velocity 24.5 cm/sec) from 8.00 AM to 6.00 PM for one week.

(b) *Acute heat exposure* : Animals were exposed to controlled high ambient air temperature maintained at either 36°C or 38°C for 2 and 4 hr in B.O.D. incubator (relative humidity 45-47%, wind velocity 28.6 cm/sec) (13).

The stress response was assessed by recording body temperature before and after heat exposure as well as the number of haemorrhagic spots in stomach at post-mortem examination (23).

The BBB permeability was studied using Evans blue-albumin (EBA, MW 68,000) (22) and ^{131}I -sodium (MW 154) (3). The 5-HT level in plasma and brain tissue was measured in another separate groups of rats (24, 25).

Control group : The animals maintained at $24 \pm 1^\circ\text{C}$ room temperature served as control

Drug treatments : The details of drug treatment has been mentioned in the Results.

Drugs used : Parachlorophenylalanine (p-CPA), EBA, indomethacin (Sigma Chemical Co., U.S.A.), diazepam (Ranbaxy Lab., India), vinblastine (Eli Lilly and Co., U.S.A.), cyproheptadine (Metck Sharp & Dhome, India). The drugs in powder form were dissolved in physiological saline and injected at pH 7.4.

Statistical analysis of data : The unpaired Student's 't' test was applied to evaluate the statistical significance of the data obtained.

RESULTS

Chronic heat exposure : The chronic exposure of 5 young rats to summer heat at 32-33°C had experienced a mild thermal load as evidenced from the small rise in mean rectal temperature by $0.84 \pm 0.23^\circ\text{C}$. No increased BBB permeability was observed in all these animals. On the other hand, 18 young rats chronically exposed to higher summer heat of 34-36°C, the BBB permeability was increased in 11 animals, and also experienced greater degree of thermal stress because the mean rectal temperature was increased by $2.5 \pm 0.46^\circ\text{C}$. This increased BBB permeability at 34-36°C was limited to young rats only because the old rats (n=8) subjected to similar exposure did not show increased permeability although, the rise of mean rectal temperature of $1.80 \pm 0.23^\circ\text{C}$ was observed.

Acute heat exposure : The effect of thermal stress was further examined in a controlled manner through exposure of animals in BOD chamber at different temperature ranges. The results show that continuous exposure of young rats for 4 hr at 38°C led to the increased BBB permeability and the rise in mean rectal temperature in these animals was $3.51 \pm 0.41^\circ\text{C}$. Few animals died also following the rise of rectal temperature beyond 41°C. The stress symptoms were also very prominent in these animals as indicated by profuse salivation, prostration and development of large numbers of gastric haemorrhagic spots of about 30.12 ± 4.54 including microhaemorrhages in 4 animals

The pattern of extravasation of dye, though showed minor differences in individual animals (Fig. 1), but in general, the dorsal cerebral cortex and in few cases, the whole cerebellar cortex was stained. No significant difference in pattern was observed in the extravasation of dye in rats either exposed to chronic summer heat or acute heat in BOD incubator. A quantitative estimation of extravasation of dye in brain tissue has been shown in Table I.

The permeability of ^{131}I -sodium was studied in 13 areas of brain. A significant increase in radioactivity was observed in all 13 areas following 4 hr heat exposure at 38°C again only in young rats

The measurement of 5-HT in both plasma and brain show that following 4 hr heat exposure at 38°C in young rats the plasma and brain 5-HT increased by 207% and 467% from the control value respectively (Table I). This points out a good correlation between increased BBB permeability and increased 5-HT level.

However, the old animals following their exposure to a similar condition neither showed increase in BBB permeability nor increase in 5-HT and signs of prostrations. The

rise in mean rectal temperature was also less ($2.83 \pm 32^\circ\text{C}$) and only microhaemorrhages were present in the stomach. On the other hand, the increased permeability of BBB was not observed in young animals following their acute heat exposure either for 2 hr or 4 hr at 36°C or at 38°C for 2 hr (instead of 4 hr). The measurement of 5-HT both in plasma and brain showed that none of these groups exhibited any significant increase from the control value. The stress symptoms developed were of lesser degree in these groups.

HIGH AMBIENT TEMPERATURE 4hr
(in B.O.D. incubator at 38°C)
Relative humidity 45-47%.

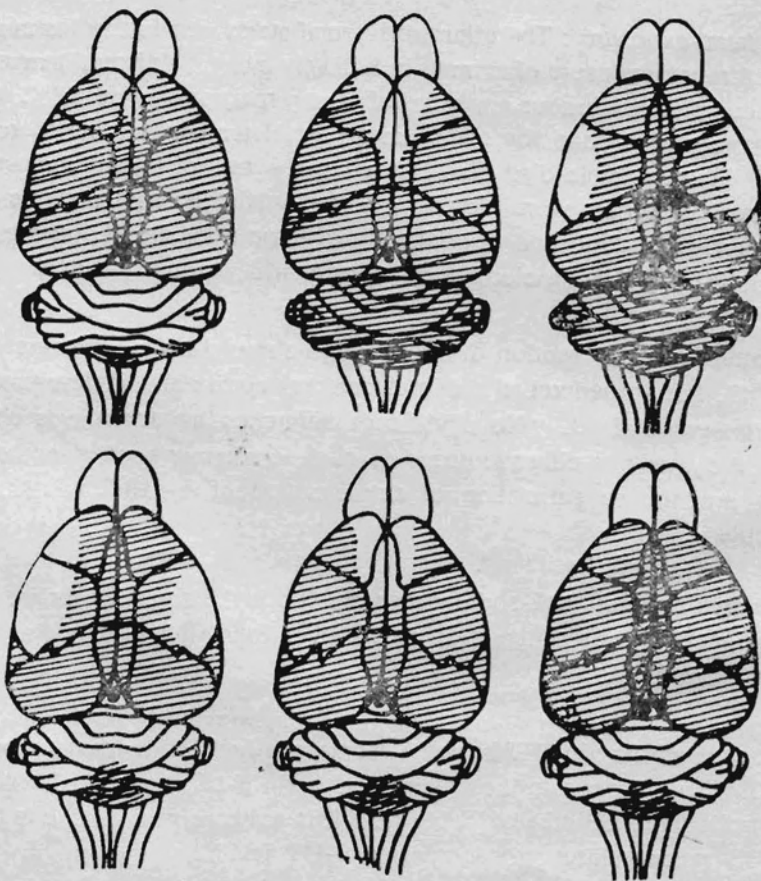


Fig. 1 : Shows extensive extravasation of Evans blue dyen in cerebral cortex of 6 individul young rats (9-10 wks) following actue heat exposure at 38°C for 4 hr in BOD incubator.

TABLE 1: Shows effect of acute heat exposure on BBB permeability as well as 5-HT level in plasma and brain in young rats and its modification with drugs.

Treatment	Brain EBA	5-HT level	
		Plasma ($\mu\text{g/ml}$)	Brain ($\mu\text{g/g}$)
Unstressed control (n=8)	0.17 \pm 0.12	0.20 \pm 0.08	0.68 \pm 0.06
Heat Exposure 38°C, 2 hr (n=6)	0.25 \pm 0.08	0.21 \pm 0.22	0.73 \pm 0.13
Heat Exposure 38°C, 4 hr (n=10)	2.10 \pm 0.96***	0.89 \pm 0.01***	3.86 \pm 0.39***
p-CPA+Heat Exposure 38°C, 4 hr (n=6)	0.19 \pm 0.08	0.13 \pm 0.17	0.48 \pm 0.12
Indomethacin+Heat Exposure 38°C, 4 hr (n=6)	0.35 \pm 0.25	0.40 \pm 0.23	0.72 \pm 0.02
Diazepam +Heat Exposure 38°C, 4 hr (n=5)	0.27 \pm 0.08	0.40 \pm 0.26	0.62 \pm 0.04
Cyproheptadine+Heat exposure 38°C, 4 hr (n=5)	0.30 \pm 0.08	1.42 \pm 0.72***	4.99 \pm 1.20***
Vinblastine+Heat Exposure 38°C, 4 hr (n=5)	0.29 \pm 0.11	1.69 \pm 0.07***	1.72 \pm 0.12***

Values are expressed as mean \pm SD.

***P<0.001 (unpaired Student's 't' test).

Thus, the animal group exposed to 38°C for 2 hr, the mean rectal temperature was increased by 2.80 \pm 0.71°C and accompanied by 8.34 \pm 2.45 gastric haemorrhagic spots in 4 animals and microhaemorrhages in 2 animals were observed. This animal group also became lethargic and lay prostrate in the cage and showed moderate salivation. The other animal group exposed for 4 hr at 36°C showed a mean increase of rectal temperature by 1.84 \pm 0.34°C and the prostration and salivation developed to a mild degree.

Drug treatment: The influence of pretreatment with selective drugs was examined only in young animal groups subjected to 4hr heat stress at 38°C, because this experimental condition lead to both increase in BBB permeability and 5-HT in plasma and brain.

(a) *p-CPA (5-HT synthesis inhibitor)*: Six animals were administered intraperitoneally p-CPA (100 mg/kg) for 3 days. On the fourth day the animals were exposed to heat stress. The increase in BBB permeability as well as increase in 5-HT level in plasma and brain were not observed following heat stress (Table 1).

(b) *Indomethacin (PG synthesis inhibitor)*: Six animals were administered intraperitoneally indomethacin (10 mg/kg) 30 min before exposure to heat stress. There was no increase in BBB permeability and 5-HT level in plasma and brain following exposure to heat stress (Table 1).

(c) *Diazepam (anti-stress drug)* : Five animals were administered diazepam (4 mg/kg) subcutaneously 30 minute before exposure to heat stress. The animals did not exhibit either increase in BBB permeability or 5-HT level in plasma and brain following heat stress (Table I).

(d) *Cyproheptadine (5-HT receptor blocker)* : Five animals were administered cyproheptadine intraperitoneally (15 mg/kg) 30 minute before heat exposure. The results showed that the pretreatment with cyproheptadine prevented the occurrence of increased BBB permeability but the 5-HT level in plasma and brain was not attenuated, rather the values were even little more higher from those of untreated heat-stressed group (Table I).

(e) *Vinblastine (anti-mitotic drug)* : Five animals were administered vinblastine (0.7 mg/kg) intravenously 48 hr before exposure to heat stress. This treatment completely prevented the occurrence of increased BBB permeability but have no effect on the increase in plasma and brain 5-HT following heat exposure (Table I).

DISCUSSION

The chronic exposure of young rats to higher summer heat of 34-36°C or acute exposure at 38°C has been observed to develop stress symptoms in animals. An increased permeability of BBB was observed in 61% of animals chronically exposed to summer heat and in case of acute exposure at 38°C for 4 hr, all the animals exhibited increased permeability to tracers. Such increased permeability was found to be more extensive with ¹³¹I-sodium as compared to EBA dye, which may be due to smaller molecular size of the radioactive tracer.

The absence of increased permeability in young animals following 2 hr exposure at 38°C as well as exposure of old animals at 38°C for 4 hr indicates that the duration of heat stress and the age of animals are important factors for affecting permeability of BBB under heat stress.

Several earlier reports (3,7,30) have suggested that the BBB dysfunction observed in few stress conditions is related with acute hypertension. Therefore, the mean arterial blood pressure (MABP) was continuously monitored in separate groups of rats through indwelling polythene catheter (PE 25) in right carotid artery chronically implanted 2 days before exposure to heat stress. The results (not reported here) indicates that hypertension may not be a contributory factor for BBB dysfunction under heat stress, because MABP remained to a small degree below the control value during the period of increased permeability following 4 hr heat stress. Moreover, this mild hypotension itself is also

not a contributory factor for BBB dysfunction because such hypotension also occurred in heat-stressed *old animals* as well as in indomethacin treated heat-stressed young animals, in which increased permeability was not shown. Thus, the dysfunction of BBB following heat-stress appears to be unrelated with hypertension or hypotension.

On the other hand, a close parallelism has been observed between 5-HT level in plasma as well as in brain and permeability of BBB under heat stress. Thus the occurrence of profound increase in both plasma and brain 5-HT has always been evident in young animals (heat exposure for 4 hr at 38°C) which also exhibited marked increase in permeability. Whereas, the young animals (2 hr heat exposure at 38°C) not showing increased permeability also had not shown increase in 5-HT level. This probable relationship of 5-HT with alteration of BBB permeability has been further strengthened from the results obtained with various drug treatments. Thus those drugs (p-CPA, indomethacin and diazepam) which have prevented the occurrence of increase in 5-HT in plasma and brain in heat-stressed groups, no increased permeability occurred in these animals.

That the increased plasma 5-HT level, in order to bring increased permeability of cerebral vessels, needs binding with specifically 5-HT receptors located in cerebral vessels (7) has been indicated from the results obtained with cyproheptadine treatment. Cyproheptadine is a classical 5-HT receptor antagonist (27). Thus, pretreatment of animals with cyproheptadine prevented the extravasation of protein tracers in cerebral tissues in spite of association with marked rise of plasma 5-HT following heat stress. This finding points out that the high level of plasma 5-HT under heat stress may not result in increased permeability unless the 5-HT receptors are available for free binding with plasma 5-HT.

The prevention of BBB dysfunction with indomethacin pretreatment suggested that the PGs are also involved. The cerebral capillaries have been shown to contain PGs as well as cAMP synthesis and catabolic enzymes (1,2,5,10,11,12,20,21,31). It has fairly been established that local accumulation of PG substances and cAMP in cerebral microvessels of rat lead to marked vasodilatation of cerebral microvessels accompanied by increased vesicular transport of tracer substances (8,14-16, 17, 21, 28, 29).

It has also been established that 5-HT stimulates the PG synthesis and release in tissues, which in turn is known to stimulate cAMP formation (4,6,12). All these informations considered together, it may be suggested that the increase in plasma 5-HT level stimulates the PG synthesis in cerebral vessels. The PGs then induce marked vasodilatation of cerebral vessels alongwith stimulation of cAMP synthesis. The increased level of cAMP in cerebral vessels, in turn, then finally stimulates vesicular transport of tracers manifested as increased BBB permeability.

That the vesicular transport is the major mechanism of increased permeability under heat stress appears likely from the results obtained with vinblastine which is known to damage the vesicular transport (18). Thus the exposure of vinblastine treated animals to 4 hr heat stress at 38°C did not show increased BBB permeability although this heat stress resulted in marked increase in plasma and brain 5-HT level.

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