ELSEVIER

Behavioural Brain Research 72 (1996) 189–196

BEHAVIOURAL BRAIN RESEARCH

Short communication

Probable involvement of serotonin in the increased permeability of the blood–brain barrier by forced swimming. An experimental study using Evans blue and ^{131}I-sodium tracers in the rat¶

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Received 16 May 1994; revised 22 April 1995; accepted 5 May 1995

Abstract

The possibility that endogenous serotonin (5-hydroxytryptamine, 5-HT) participates in alteration of the blood–brain barrier (BBB) following short-term forced swimming (FS) exercise was examined in a rat model. Subjection of conscious young (age 8–9 weeks, 80–90 g) animals to continuous FS (at a water temperature of 30±1°C) for 30 min, increased the permeability of the BBB to Evans blue albumin (EBA) and ^{131}I-sodium in six and nine brain regions, respectively. The EBA staining was noted in posterior cingulate cortex, parietal, occipital cortices, cerebellar vermis, medial lateral cerebellar cortices and dorsal surface of hippocampus. In addition to these brain regions, the BBB permeability to ^{131}I-sodium was further extended to caudate nucleus, thalamus and hypothalamus. This effect of FS on the BBB permeability was absent in adult (age 24–30 weeks, 300–400 g) animals. Measurement of 5-HT showed a profound increase of plasma and brain in young rats by 180% and 250%, respectively, from the control group. Adult animals showed only a minor increase in brain and plasma 5-HT levels. In young animals, pretreatment with *p*-CPA (a 5-HT synthesis inhibitor) and indomethacin (a prostaglandin synthesis inhibitor) prevented the FS induced increase in BBB permeability and 5-HT levels. Destruction of serotonergic neurons with 5,7-dihydroxytryptamine (5,7-DHT) reduced the breakdown of the BBB and attenuated the brain 5-HT level without affecting the plasma 5-HT. Cyproheptadine, ketanserin (5-HT$_2$ receptor antagonists) and vinblastine (a vesicular transport inhibitor) prevented the increased permeability of the BBB alone. The plasma and brain 5-HT continued to remain high. These observations suggest that (i) 5-HT plays an important role in the breakdown of BBB permeability in FS, (ii) this effect of 5-HT on BBB permeability is mediated by 5-HT$_2$ receptors, and (iii) FS induced increase in BBB permeability is age dependent.

Keywords: Forced swimming; Blood–brain barrier; Serotonin (5-HT); *p*-Chlorophenylalanine; Cyproheptadine; Indomethacin; Ketanserin; 5,7-Dihydroxytryptamine (5,7-DHT); Vinblastine; 5-HT$_2$ receptor; Prostaglandin; Ageing

1. Introduction

Blood–brain barrier (BBB) is a physiological dynamic regulatory barrier which maintains a constant composition of the fluid microenvironment of the brain [5]. Alterations of BBB permeability occurs in a wide variety of neurological diseases and pathological conditions such as Alzheimer's disease, vascular dementia and encephalomyelitis [15,21]. There are indications that altered serotonin metabolism occurs in a wide variety of neurological diseases which may selectively increase the permeability of the BBB [4,14]. This is apparent from the fact that intravenous, intracarotid infusion; or topical application of serotonin in small amounts increases the permeability of the BBB to various tracers [36,43,48,49].

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¶This paper is dedicated to Professor Yngue Olsson on the occasion of his 60th birthday.

SSDI 0166-4328(96)00170-0

Forced swimming (FS) is a severe stressful condition in which alteration of serotonin metabolism occurs [11,14,37,40,45]. Thus a possibility exists that altered metabolism of 5-HT may increase the permeability of the BBB in FS. There are reports that 5-HT is involved in the physiological mechanisms of stress [6,16,27,30,33,34]. Thus the metabolism of 5-HT is altered following various stressful conditions [6,43,45]. We earlier reported a marked increase in the permeability of the BBB following immobilization and heat stress which appears to be mediated by increased levels of serotonin in brain and plasma which is in line with these observations [41,42]. Altered metabolism of 5-HT occur in FS [33]. Therefore, the present study was undertaken to examine the status of BBB permeability following FS in a rat model. Since, the effect of stress on BBB permeability and serotonin metabolism are age dependent [32,45], the age related changes of the BBB permeability and serotonin level in FS were also examined.

Furthermore, in view of the fact that prostaglandins (PGs) are implicated as a first mediator of stress response [17], and may stimulate 5-HT synthesis in the brain [20], the influence of PGs on the BBB permeability and 5-HT level in FS was examined by inhibiting its synthesis with indomethacin [51].

2. Experimental procedures

2.1. Animals

Experiments were carried out on 97 inbred Charles–Foster (71 male and 26 female) rats weighing between 80–90 g (age 8–9 weeks) and 250–300 g (age 30–32 weeks) housed at control ambient temperature ($22 \pm 1°C$) with a 12/12 h light/dark schedule. The rat feed and tap water were supplied ad libitum.

2.2. Continuous forced swimming

The experiments were commenced between 08.00 and 09.00 h. Rats were individually placed in a Corning glass cylinder (140 cm height, 20 cm internal diameter) containing water (18 cm depth) maintained at $30 \pm 1°C$ [8,19,44]. When rats or mice are forced to swim in a restricted pool they quickly acquire an immobility response [18,24,37]. To avoid this response, water was manually stirred with a glass rod from time to time (about 6–7 times within a 30-min session) which allowed the animals to swim continuously in the pool during the entire swimming session.

2.3. Stress response

Rectal temperature, excretion of faecal pellets and occurrence of haemorrhagic spots in the stomach were used as indices of stress in these animals [41,42,44,45]. The body temperature was recorded before and after swimming in each animal using a thermistor probe (Yellow Springfield, USA) (inserted about 5 cm deep into the rectum) connected to a telethermometer (Electromed, England). The number of faecal pellets excreted by the individual animal during the swimming session was counted. The haemorrhagic spots in the wall of the stomach was examined at post-mortem [40–42].

2.4. Physiological variables

The mean arterial blood pressure (MABP) was recorded from the common carotid artery through an indwelling polythene catheter (PE 25, USA) implanted two days prior to the experiment [31]. At the time of recording, the arterial catheter was connected to a pressure transducer (Strain gauge, Statham P 23, USA) which led to a chart recorder (Electromed, England). The arterial pH, PaO_2 and $PaCO_2$ were measured using a radiometer apparatus (Copenhagen).

2.5. Blood–brain barrier permeability

The BBB permeability was measured using Evans blue and ^{131}I-sodium tracers [28,31,41–45]. These tracers bind to serum albumin in vivo. Thus, extravasation of these tracers into the brain represents mainly the leakage of tracer-protein complex [28,38]. Evans blue (0.3 ml/100 g of a 2% solution in 0.9% saline) and ^{131}I-sodium (10 μCi/100 g) were injected intravenously immediately after termination of FS in animals under urethane anaesthesia (1 g/kg, i.p.) into the right femoral vein manually through a needle puncture. To study the reversibility of the BBB permeability, the tracers were injected in separate groups of animals 2 h period after in vivo.

Fifteen min later, the animals were perfused with 0.9% saline through the heart to remove the blood from the vessels [43]. After inspection of EBA extravasation, the brain was bisected and divided into 14 anatomical regions (Fig. 2). The samples (ranging between 54 and 135 mg) obtained from one half of the brain were placed in tared plastic vials that were immediately reweighed. The radioactivity was determined in a 7.62-cm well type gamma counter at the energy window of 500–800 keV. The extravasation of ^{131}I-sodium was expressed in percentage of the activity in whole blood as follows: CPM/mg brain tissue over CPM/mg blood × 100 [43].

The blood sample was collected from the heart at the end of the experiment. The whole blood rather than the serum was used. There was no significant difference in whole blood tracer concentration between controls and stressed rats either following 5 min, 15 min or 30 min FS.

The EB dye entered into the brain was measured colorimetrically [18,43]. For this purpose, the remaining half of the brain was homogenised in a mixture of acetone (3.5 ml) and 0.5% sodium sulphate (1.5 ml), pH 7.4 to extract the dye. The suspension was left

overnight at 4°C. On the next day, the samples were centrifuged at a low speed (900 *g*) for 10 min. The extracted dye in the mixture was filtered and measured in a spectrophotometer (Beckman, Germany) at 620 nm [18].

2.6. Measurement of 5-HT

The 5-HT in plasma and brain was measured using a sensitive and specific fluorometric assay [41,42]. In brief, about one ml of whole blood was collected before sacrifice from the right auricle after cardiac puncture and the plasma was separated by centrifugation at low speed (900 *g*) for 30 min at room temperature. The plasma (0.5 ml) was diluted to 4 ml in 0.4 N ice cooled perchloric acid (PCA). The whole brain sample was homogenised in 8 ml PCA. Both plasma and brain samples were centrifuged at 4°C to separate out proteins. One ml of aliquots from plasma or brain homogenates was used for 5-HT extraction. The extraction of 5-HT was done using butanol in a salt saturated and alkaline medium (pH 10). The purification of 5-HT extract was carried out using *n*-heptane (Extra pure, Merck). The fluoropores were developed by incubating the sample with ninhydrin at 75°C for 30 min. The fluorescence was measured in duplicate cooled samples at room temperature using a Aminco-Bowman spectrophotofluorometer (USA) at excitation 385 nm and emission 490 nm wave lengths.

2.7. Control group

The normal animals of same age group served as controls.

2.8. Effect of drugs

Since 30 min FS increased the BBB permeability to EBA and ^{131}I-sodium in young animals, the effect of pretreatment of the following drugs was examined in young animals at this time point only.

2.8.1. p-Chlorophenylalanine (p-CPA, 5-HT synthesis inhibitor, Sigma Chemical, USA)

p-CPA (100 mg/kg) was administered intraperitoneally in animals daily for 3 consecutive days. On the 4th day, these animals were subjected to FS. The drug at this dose will induce a long-lasting depletion of 5-HT in the CNS [23].

2.8.2. Cyproheptadine (a 5-HT$_2$ receptor antagonist, Periactin, Merck, Sharp and Dohme, UK)

Cyproheptadine (15 mg/kg) was injected intraperitoneally 30 min prior to the onset of FS. This dose of the drug is sufficient to block 5-HT$_2$ receptors in vivo [1,48].

2.8.3. Ketanserin (a specific 5-HT$_2$ receptor antagonist, Janssen Pharmaceuticals, Belgium)

Ketanserin (1 mg/kg) was administered intraperitoneally 30 min before FS. This dose is high enough to block endogenous 5-HT$_2$ receptors located on cerebral vessels without affecting the release or metabolism of 5-HT [2,11,14].

2.8.4. 5,7-dihydroxytryptamine (5,7-DHT, a serotonin neurotoxin, Sigma Chemical, USA)

The compound was dissolved in physiological saline containing 0.1% ascorbic acid. The drug (175 μg in 10 μl) was injected into the right lateral ventricle under stereotaxic guidance (coordinate 1.5 mm right to mid line, 2.5 mm caudal to bregma, and about 3 mm deep from the skull surface) ($n=15$). All these procedures were carried out in the dark in order to avoid the exposure of light to the solution. The tip of the cannula was lying over the surface of hippocampus. The position of cannula placement was confirmed at autopsy. Two weeks after the i.c.v. injection, the animals were subjected to 30 min FS. This dose and time period is sufficient to destroy more than 80% of the serotonergic nerve terminals in the CNS before FS [12,14]. The drug-treated animals were drowsy and calm after injection of this drug before FS. Few animals in which the cannula tip was located within the tissue ($n=4$) were discarded.

2.8.5. Indomethacin (a prostaglandin synthetase inhibitor, Sigma Chemical, USA)

The drug in powder form was dissolved in physiological saline with a pinch of sodium bicarbonate and the pH was adjusted to 7.4. The drug was administered in a dose of 10 mg/kg intraperitoneally 30 min before FS. This dose of the drug is sufficient to inhibit the endogenous prostaglandin synthesis within 30 min for a long time period [39,51].

2.8.6. Vinblastine (a vesicular transport inhibitor, Eli Lily and Co, Indianapolis, USA)

This drug was injected in a dose of 0.8 mg/kg, intravenously 48 h before the FS through a polythene cannula implanted into the right jugular vein for this purpose. The drug in this dose will inhibit the function of microtubules and thus interfere with intracellular vesicular transport mechanisms [7,25].

2.9. Statistical analysis

The quantitative data obtained were fed to a Apple Macintosh personal computer. Stat View II (Abacus concepts, USA) software was used. ANOVA and Dunnett test were applied to evaluate the statistical significance of the data [9]. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Stress symptoms

Subjection of young animals to 30 min FS resulted in development of profound hypothermia and stress symptoms (Table 1). These symptoms were much less severe in animals subjected to either 5 min or 15 min FS. The body temperature was restored near normal values in animals after a rest period of 120 min following 30 min FS (Table 1). Adult animals subjected to similar FS showed minor but significant changes in the body temperature and excretion of faecal pellets compared to the same age group of normal rats (Table 1).

3.2. Physiological variables

The MABP following 30 min FS in young animals showed a mild decrease. The PaO_2 was significantly increased, whereas the $PaCO_2$ showed a mild decline. The arterial pH was not altered from the control value (Table 1). On the other hand, a mild hypertension was seen at the end of 5 min and 15 min after FS without affecting the PaO_2, $PaCO_2$ and the arterial pH. The altered physiological variables following 30 min FS approached near normal values in animals after a rest of 120 min (Table 1). Adult animals showed minor but significant changes in these variables compared to the same age group of normal rats (Table 1).

3.3. Blood–brain barrier permeability

The BBB permeability to EBA and ^{131}I-sodium was markedly increased in young animals after 30 min FS (Fig. 1). This increase in the BBB permeability following 30 min FS was not dependent on sex of the animals. Thus there was no significant difference in the extravasa-

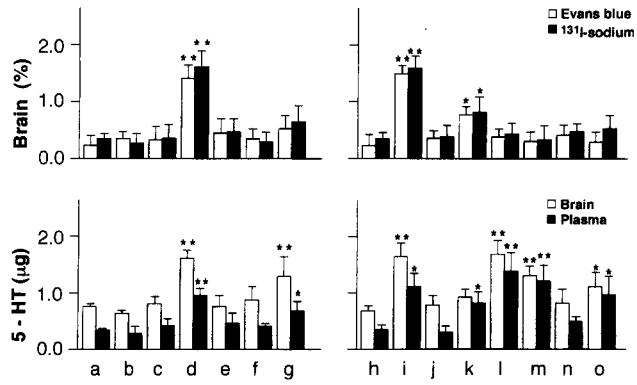

Fig. 1. Blood–brain barrier permeability (upper panel) and 5-HT levels in plasma and brain (lower panel) following forced swimming in young and adult rats (left). The influence of drugs on blood–brain barrier permeability and 5-HT levels in young rats following 30 min forced swimming are shown right. $^{*}P<0.01$, $^{**}P<0.01$, compared with control. ANOVA and Dunnet's test. a, control young; b, 5 min FS; c, 15 min FS; d, 30 min FS; e, 2 h rest after 30 min FS; f, control adult; g, 30 min FS adult; h, control young; i, 30 min FS untreated; j, p−CPA+FS; k, 5,7-DHT+FS; l, ketanserin+FS; m, cyproheptadine+FS; n, indomethacin+FS; o, vinblastine+FS.

Table 1. Stress symptoms and physiological variables in animals subjected to forced swimming (FS) and their modification with drugs. The animals were subjected to FS in a glass cylinder filled with water (18 cm depth) at 30±1°C. The drugs were injected before FS and the parameters were measured 30 min after FS.

Type of the Experiment	n	Stress symptoms			Physiological variables			
		Rect Temp °C	Faecal Pellets no	Haemorrhagic spots in stomach no	MABP torr	Arterial pH	PaO_2 torr	$PaCO_2$ torr
Control group								
Young animals	5	36.86±0.23	4±2	nil	104±6	7.38±0.04	79.34±0.54	34.68±0.67
Adult animals	5	37.54±0.12	3±2	nil	110±8	7.37±0.02	80.56±0.43	35.42±0.78
Forced Swimming								
Young animals								
5 min	5	34.56±0.42*	6±4	nil	120±6*	7.38±0.06	79.38±0.38	35.03±0.12
15 min	5	32.48±0.53**	20±8*	10±4$^{\Delta}$	114±8	7.36±0.08	80.32±0.23	33.89±0.22
30 min	8	30.42±0.48**	40±8*	44±12	83±8*	7.36±0.06	81.36±0.44*	33.22±0.24*
30 min + rest 120 min	5	36.58±0.37	4±3	38±8$^{\Delta}$	114±10	7.37±0.07	80.56±0.37	34.37±0.34
Adult animals								
30 min	6	32.38±0.27**	20±8**	8±5$^{\Delta\Delta}$	88±6**	7.36±0.05	81.58±0.35	34.03±0.28
Drug treatments + 30 min Forced swimming								
Young animals								
p-CPA	5	31.56±0.43**	30±12**	20±8$^{\Delta}$	85±5**	7.37±0.04	80.76±0.34	33.86±0.35
Indomethacin	5	32.65±0.36**$^{\Delta}$	35±8**	46±12	82±6**	7.37±0.08	80.48±0.32	33.78±0.47
Cyproheptadine	6	32.29±0.48**$^{\Delta}$	28±12**	36±8	84±4**	7.37±0.04	80.82±0.35	34.26±0.66
Ketanserin	5	31.87±0.36**	20±8**$^{\Delta}$	28±6$^{\Delta}$	86±4**	7.37±0.08	80.28±0.37	34.42±0.28
5,7-DHT	6	30.68±0.38**	36±14**	34±14	82±8**	7.36±0.11	81.54±0.54	33.87±0.36
Vinblastine	5	30.76±0.25**	33±15**	40±12	84±6**	7.36±0.08	81.06±0.34	34.08±0.18

Values are expressed as mean ± SD

* = P<0.05, ** = P<0.01, compared from control group; $^{\Delta}$ = P < 0.05, $^{\Delta\Delta}$ = P < 0.01, compared from 30 min FS in young rats, ANOVA and Dunnet's test.

tion of Evans blue or radioactive iodine between male or female rats subjected to FS. The BBB permeability to EBA was noted in six brain regions viz; posterior cingulate cortex, parietal and occipital cortices, cerebellum (vermis, cerebellar cortex) and the dorsal surface of the hippocampus. In addition to the above brain regions, the BBB permeability to ^{131}I-sodium was extended to caudate nucleus, thalamus and hypothalamus (Fig. 2).

This increase in BBB permeability was absent in animals after a rest period of 120 min (Fig. 1) indicating that FS-induced increased BBB permeability was reversible in nature. No extravasation of either tracer was noted in animals following 5 min or 15 min FS (Fig. 1). Adult animals subjected to 30 min FS did not result in extravasation of tracers in the brain (Fig. 1).

3.4. 5-HT level

Plasma and brain 5-HT levels following 30 min FS in young rats showed a significant increase of the amine (Fig. 1). This increase in 5-HT level was absent following 5 min and 15 min period of FS (Fig. 1). No significant increase in the plasma or brain 5-HT levels in young animals could be seen after a rest period of 120 min following 30 min FS (Fig. 1). The plasma and brain 5-HT levels in adult animals following 30 min FS showed a minor but significant increase from the same age group of normal rats (Fig. 1).

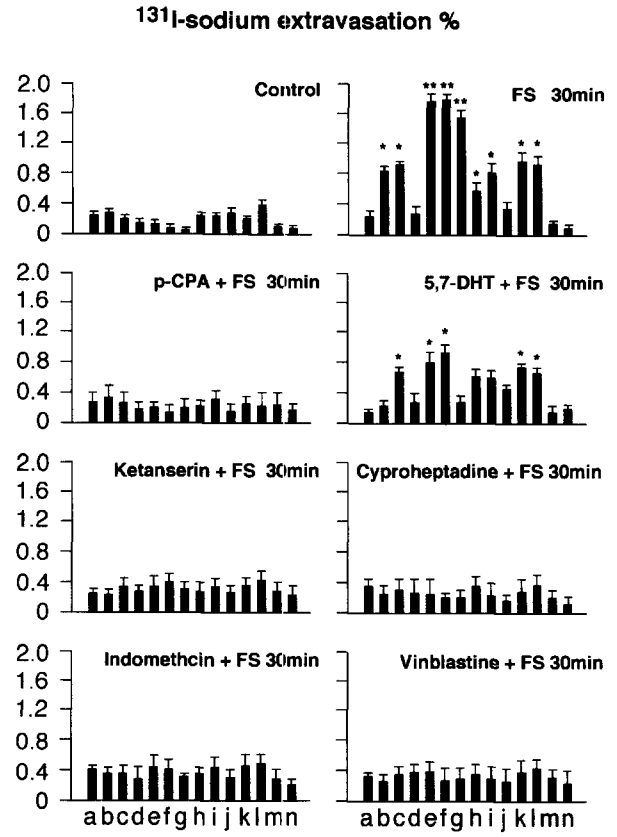

Fig. 2. Regional blood–brain barrier permeability changes to radioactive iodine following 30 min forced swimming in young rats and their modification with drugs. $^{*}P<0.05$, $^{**}P<0.01$ compared with control group. ANOVA and Dunnet's test. a, frontal cortex; b, parietal cortex; c, occipital cortex; d, cingulate cortex anterior; e, cingulate cortex posterior; f, cerebellum vermis; g, cerebellum lateral; h, caudate nucleus; i, hippocampus; j, colliculi; k, thalamus; l, hypothalamus; m, medulla; n, brain stem.

3.5. Effect of drugs

Since 30 min FS increased the permeability of the BBB and 5-HT levels in young rats, the effects of selective drugs on these parameters were examined in young animals only.

3.5.1. p-CPA (a 5-HT synthesis inhibitor)

Pretreatment with p-CPA prevented the increased BBB permeability in all the brain regions examined (Fig. 2). There was no increase in 5-HT levels in plasma and brain (Fig. 1). The stress symptoms and the physiological variables were similar to the untreated groups (Table 1).

3.5.2. Cyproheptadine (a 5-HT$_2$ receptor antagonist)

Cyproheptadine pretreatment prevented the increased permeability of the BBB (Fig. 2) without affecting the plasma and brain 5-HT levels (Fig. 1). The physiological variables and stress symptoms were not affected (Table 1).

3.5.3. Ketanserin (a specific 5-HT$_2$ receptor antagonist)

Pretreatment with ketanserin prevented the breakdown of the BBB permeability to both tracers (Fig. 1 and Fig. 2). However, the increased plasma or brain 5-HT level was unaffected (Fig. 1). The stress symptoms and physiological variables were very similar to the untreated group (Table 1).

3.5.4. 5,7-Dihydroxytryptamine (a 5-HT neurotoxic drug)

Destruction of 5-HT neurons in the CNS did not prevent the occurrence of the BBB permeability following FS (Fig. 1). However, the magnitude of permeability increase was considerably reduced (Fig. 1 and Fig. 2). The plasma and brain 5-HT levels were significantly lowered in the drug treated animals (Fig. 1). The physiological variables and stress symptoms remained unaffected (Table 1).

3.5.5. Vinblastine (a vesicular transport inhibitor drug)

Pretreatment with this drug prevented the extravasation of tracers in brain (Fig. 1 and Fig. 2). The plasma and brain 5-HT levels remained high (Fig. 1). The physiological variables and stress symptoms were unaffected (Table 1).

4. Discussion

The salient new findings of the present investigation show that 30 min FS increased the permeability of the BBB to protein tracers in specific brain regions of young rats. This increased permeability following FS was reversible in nature. This is evident with the fact that the breakdown of the BBB permeability was no longer evident in animals subjected to 120 min rest period after 30 min FS. Furthermore, there was no increase in the permeability of the BBB following 5 and 15 min FS in young rats and following 30 min FS in adult rats. This indicates that the duration of FS and age of animals are important factors in inducing the breakdown of BBB permeability.

The functional clinical significance of this finding may be that children exposed to stressful environments are more vulnerable to mental abnormalities than adults [14]. Apparently increased serotonin level may induce a breakdown of the BBB in children or infants exposed to stressful environments.

We used two different protein tracers to examine the permeability of the BBB. These protein tracers will bind to serum albumin after introduction into the systemic circulation [41,42]. Evans blue in the dose used here will bind mostly to serum albumin by 68% whereas, the binding of ^{131}I-sodium to albumin will be less than 50% [28,38]. Neither bound nor the unbound forms of either tracer cross the normal BBB in young or adult rats [41–45]. A significantly higher permeation of ^{131}I-sodium across the cerebral vessels following FS compared to EBA may be due to a difference in the molecular size of the tracers and/or due to a difference in the proteins to which they bind in vivo [31,43].

The probable mechanisms of increased permeability following FS is not clear. However, it appears that serotonin may play an important role. This is evident with a parallel rise of 5-HT in plasma and brain at the time of increased BBB permeability. Serotonin is a potent vasoactive agent which is known to modify the permeability of the BBB by a specific receptor mediated mechanism [1,11,14,35,36]. Intracerebroventricular, intravenous and intracarotid application of 5-HT will increase the BBB permeability to proteins [36,43,48–50]. In the present study both plasma and brain 5-HT was elevated from the control value which alone or in combination may modify the BBB permeability following FS (cf. [43]).

A minor but significant increase of 5-HT levels in plasma and brain in adult animals following FS did not influence the BBB permeability. This suggests that the cerebral vessels of adult animals are less responsive to serotonin due to receptor down regulation with advancing age [14,32]. However, a reduced accumulation of 5-HT in adult animals may be due to a difference in stress response on serotonin metabolism [32], a conclusion which requires additional investigation.

That 5-HT is involved in the physiological mechanisms of increased BBB permeability following FS is further supported by the results obtained with drug treatments. Thus blockade of increased 5-HT level in plasma and brain with *p*-CPA; and antagonism of 5-HT$_2$ receptors with cyproheptadine or ketanserin in spite of high plasma and brain 5-HT levels, prevented the increased permeability of the BBB following FS. These observations show that binding of serotonin to 5-HT$_2$ receptors is important in the breakdown of the BBB permeability following FS.

The probable mechanism by which serotonin after binding to 5-HT$_2$ receptors will induce a breakdown of the BBB permeability is not clear. It appears that 5-HT induced intracellular signalling such as stimulation of phosphoinositide metabolism is not playing an important role in the increased permeability of the BBB in FS. This is evident with the fact that 5-HT induces stimulation of phosphoinositide metabolism in rat cerebral cortex slices without affecting the density or affinity of 5-HT$_2$ receptors [34]. This effect of 5-HT is inhibited by FS [34]. Thus it may be that 5-HT after binding with 5-HT$_2$ receptors may initiate some other signalling mechanisms leading to the increased BBB permeability. One possibility would be that 5-HT after binding to its 5-HT$_2$ receptor may act on the cerebral vessels through prostaglandins (PGs) [10,20] and/or cyclic adenosine monophosphate (cAMP) [3,22,36,48,50].

This is evident with the observation that, indomethacin (a potent PG synthesis inhibitor) [39,51], in a concentration high enough to block the PG synthesis in the cerebral vessels [3,10,13,51] completely prevented the increased vascular permeability. This blockade of the BBB permeability in FS by indomethacin suggests that prostaglandins are also involved. Prostaglandins have been implicated as a first mediator of stress and the inhibition of their release with indomethacin will prevent the stress response in animals [17]. In addition, PGs are known stimulator of 5-HT synthesis [20]. Therefore, it seems plausible that indomethacin prevents the release of PGs in response to FS, and thus prevents the increase of 5-HT in plasma and brain [47].

The cerebral capillaries contain all necessary enzymes for synthesis and catabolism of PGs as well as cAMP [3,13,16]. Local accumulation of PGs in cerebral capillaries leads to marked vasodilatation of cerebral vessels accompanied with increased vesicular transport of tracer substances [10,16]. Furthermore, 5-HT has the capacity to stimulate the synthesis and release of PGs in brain [6,14,39]. It may be that the increase in plasma 5-HT following FS stimulates PG synthesis in the cerebral vessels. The PGs then stimulate cAMP synthesis leading to an increase in vesicular transport across the cerebral microvessels [3,11,22,35,49,50].

The results obtained with vinblastine in the present study are in agreement with the above hypothesis. Vinblastine is effective in reducing tracer permeability by inhibiting the microtubule function [7,25]. Microtubules are associated with transcellular transport of tracer substances. A complete blockade of extravasation of tracers in FS by vinblastine suggest that vesicular transport play major role in tracer transfer across the microvessels [25,43]. However, ultrastructural studies using electron dense tracer lanthanum are needed to clarify this point.

A simple mechanical effect of vasospasm caused by 5-HT in the vascular endothelium of young rats that has not yet matured its cell to cell binding matrix in increasing the BBB permeability in FS appears to be less likely [1,2,11,14,25]. This is because of the fact that the serotonin levels in plasma were very high following 30 min FS in young rats pretreated with either cyproheptadine, ketanserin or vinblastine but the increased BBB permeability was not present in these drug treated rats.

The mechanisms underlying accumulation of serotonin in plasma and brain following FS is not clear. Activation of central serotonergic neurons resulting in increased release of the amine from nerve terminals and a decrease in synaptic re-uptake or degradation are important factors [14,25]. However, it appears that serotonergic neurons do not contribute to the accumulation of serotonin in brain or plasma. This is evident with the results obtained with 5,7-DHT. This compound specifically degenerates 5-HT nerve terminals [12]. Thus release of 5-HT from serotonergic nerve terminals are impaired by this drug treatment. In FS, this drug treatment did not abolish the increased level of 5-HT in brain. Thus it seems quite likely that mast cells and pineal gland could be other potential source for brain serotonin [11,14]. Additionally, 5-HT in brain may come from plasma due to a breakdown of the BBB permeability. The increased plasma serotonin may result from disintegration of platelets or alteration in binding or its re-uptake mechanisms [1,4,14].

Apart from 5-HT levels, marked alterations in catecholamines in FS are well documented in the literature [8,19,33,40]. However, it appears that catecholamines do not participate in the mechanisms of FS induced breakdown of the BBB [46]. This is evident with the findings that destruction of either peripheral or central nor-adrenergic neurons by 6-hydroxydopamine (6-OHDA), a noradrenergic neurotoxin did not reduce the increased permeability of BBB in FS (unpublished observations). It may be that catecholamines contribute to other stress symptoms associated with FS such as alterations in cardiovascular response or thermoregulation [40].

The changes in MABP, blood gases or body temperature by itself do not contribute to the increased permeability of the BBB [26,29]. This is apparent because similar changes were seen in drug treated animals in which no increase in the permeability was observed. The early hypertension was not severe enough to impair the BBB permeability because EBA injected before the onset of FS did not result in blue staining of the brain (results not shown). Thus, it is quite likely that these physiological variables seen in our animals following FS could be coupled to the water temperature, activation of hypothalamic-pituitary-adrenal axis and/or related physiological compensatory mechanisms [19,40].

Thus if all the above information will be taken together, a hypothesis may be put forwarded as follows. Subjection of young animals to 30 min FS induces an early release and accumulation of serotonin in plasma. The increased level of serotonin then acts on 5-HT_2 receptors located on cerebral vessels. After binding to 5-HT_2 receptors, the amine will stimulate the synthesis of prostaglandins. Prostaglandins in turn may further stimulate the accumulation of cAMP. An accumulation of cAMP in cerebral vessels will enhance vesicular transport resulting in extravasation of tracers across cerebral vessels. Obviously, this mode of tracer transport may be influenced by various pharmacological agents.

Acknowledgment

This research was supported by grants from the University Grants Commission, New Delhi, India; Swedish Medical Research Council projects no. 2710, 9459, 10357; Göran Gustafsson Foundation, (formerly the Torsten and Ragnar Söderberg Foundation), Sweden and The Alexander von Humboldt Foundation, Bonn, Germany. The secretarial assistance of Aruna Misra, Angela Ludwig and Katherin Kern are highly appreciated with thanks.

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