Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 2, pp. 375-378, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00121-2

Influence of Acute Exposure to Heat on the Blood-Brain Barrier Permeability During Acute Hypertension

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Received 8 March 1994

ÖZTAŞ, B. *Influence of acute exposure to heat on the blood-brain barrier permeability during acute hypertension.* PHARMACOL BIOCHEM BEHAV **52**(2) 375-378, 1995.—In mild hyperthermic rats with acute hypertension induced by intravenous injection of adrenaline, changes in blood-brain barrier permeability to macromolecules were investigated using Evans blue as indicator. Evans blue albumin extravasation was determined macroscopically, and a quantitative estimation with spectrophotometer using homogenized brain to release the dye was also performed to evaluate the macroscopic findings. Four groups of rats were studied: group I: control normothermia; group II: acute exposure to heat; group III: normothermia + acute hypertension; group IV: acute exposure to heat + acute hypertension. The rats were anesthetized with diethyl-ether. Body temperature was increased by elevating ambient temperature in the vented box covered with a 3 mm thick black copper plate. The colonic temperature was increased to 39 ± 0.5°C. During adrenaline-induced acute hypertension the mean arterial blood pressure increased in both normothermic and mild hyperthermic animals. Mean values for Evans blue dye were found to be 0.20 ± 0.04 mg% whole brain in normothermic control rats and 0.30 ± 0.1 mg% in hyperthermic rats ($p < 0.05$). Mean values for Evans blue dye in the whole brain were found to be 0.63 ± 0.2 mg% in the normothermic rats and 0.40 ± 0.2 mg% in the mild hyperthermic rats during adrenaline-induced hypertension ($p < 0.05$). Our results show that the extravasation of Evans blue albumin was less pronounced in the brains of mild hyperthermic rats compared to normothermic rats after adrenaline-induced acute hypertension.

Blood-brain barrier Hypertension Hyperthermia

THE BLOOD-BRAIN barrier (BBB) maintains the homeostatic regulation of the fluid environment of the brain (15,22). The neurons, glial cells, and brain extracellular fluid are separated from the blood by the BBB (22). BBB is represented by continuous capillaries of the cerebral microcirculation, in which the endothelial cells are sealed by tight junctions to form a complete cellular layer (15,22). The BBB can be opened by an increase in blood pressure induced by intravenous injection of vasoactive subtances such as norepinephrine, epinephrine, metaraminol, phenylephrine, and angiotensin (3,11,16, 17). These drugs may be given with no effect on the BBB if the increase in pressure is prevented or reduced (11,22). On the other hand, several earlier reports have suggested that the dysfunction of the BBB observed in some stress conditions is related to acute hypertension (14,26). But Sharma and Dey indicate that hypertension may not be a contributory factor in the dysfunction of the BBB under heat stress (24,25). Morphologic brain lesions after heat stroke have generally been vascular in origin and include edema, hemorrhage, congestion, and thrombosis (5,6,9). On the other hand, heat stress is important during summer months, and this also often leads to brain edema, subarachnoid hemorrhage, and alteration in the EEG pattern in human beings (5,6). The etiology of these neurological changes still remains to be elucidated. It is likely that the homeostatic regulation of the fluid environment of the brain undergoes severe alteration during heat exposure (25). Therefore, in the present study, the state of BBB was examined to answer the following questions; 1) does acute exposure to heat (moderate hyperthermia) influence the BBB permeability? and 2) Does acute exposure to heat have a protective or aggravating effect on BBB permeability during acute adrealine-induced hypertension?

METHOD

Adult male Wistar rats weighing between 230-280 g, were used in this study. They were anesthetized with diethyl-ether. A femoral artery was cannulated for recording the mean arte-

rial blood pressure. Mean arterial blood pressure was recorded by connecting the arterial catheter to a stain gauge transducer (Ugo-Basil). The experiments were performed between 0900-1100 h. The femoral vein was cannulated for intravenous injections of tracer or drugs. Evans blue tracer (4 ml/kg of a 2% solution in saline) (16) was given intravenously as an indicator of the BBB function. Evans blue dye does not permeate cerebral vessels in normal animals. This tracer binds to serum albumin after their introduction in the circulation, and when it is exudated in pathologic conditions, it is chiefly the tracer-protein complex that exists in the edema fluid (4). One molecule of Evans blue will for instance bind 12 molecules of albumin (22).

Acute Exposure to Heat

After cannulation, the animal was placed in a 20 × 20 × 10 cm vented box covered with a 3 mm-thick black copper plate. Rectal temperature was measured with a thermometer, inserted 3-4 cm into the rectum, and taped to the animals tail. The ambient temperature was elevated by directing a 250 W infrared lamp onto the copper plate. The animals rectal temperature was increased to 39 ± 0.5°C under these conditions. After the desired temperature was reached (15-20 min, depending on the weight of the animals), the rats were taken out of the box. After the rectal temperature 39°C was attained, the temperature of the heat exchanges was controlled and maintained for the experimental duration. Control animals were similarly treated, but without exposure to ambient heat. Their rectal temperature was 37°C.

Four groups of rats were studied. Group I: normothermic control (n = 10); group II: acute exposure to heat (n = 11); group III: normothermia + acute hypertension (n = 17); group IV: acute exposure to heat + acute hypertension (n = 14).

The first and second groups of rats served as control for the permeability of BBB and recevied just Evans blue. The animals were injected with Evans blue about 10 min after their body temperature attained 39 ± 0.5°C. In the third and fourth groups of animals, after control blood pressure had been recorded, Evans blue was injected, and after 5 min 40 μg/kg adrenaline was administered IV (18,20). At the end of experiments, i.e., 30 min after Evans blue and drug injection, all the rats were killed by perfusion through the heart with saline to avoid artificial staining of the brain during removal. Then brains were removed. After macroscopic inspection of the brain surface for the extravasation of Evans blue albumin, the brains were cut by hand in 2-3 mm-thick coronal blocks, and the presence and distrubution of Evans blue leakage were also inspected. Barrier opening was graded as follows from observations and extent of Evans blue staining. Grade 0: no staining; grade 1+: faint and localized staining; grade 2+: moderate blue staining; grade 3+: extensive, dark blue staining (19,22). In another separate group of rats, a quantitative estimation of dye in the brain was also carried out according to the method of Harada et al. (8) in the same experimental conditions. Each quantitative group consisted of 12 animals. Briefly, the brain was removed and bisected at the midline. Each half cerebrum and cerebellum were placed in tared tubes that were immediately reweighed. Hemispheres and cerebellum were homogenized with 5 ml of phosphate-buffered saline containng a 5% solution of 1 N NaOH. The homogenized brains were centrifuged (10,000 rpm for 5 min) and a spectrophotometrical analysis at 620 nm of wavelength was performed to measure the amount of resolved dye (8,12). Macroscopical findings described above supported this quantitative estimation with a spectrophotometer.

Statistical Analysis of Data

Data are expressed as mean ± SD. Values for individual groups were compared by Student's t-test. A p-value less than 0.05 was considered to be significant.

RESULTS

The degree of BBB breakdown and mean arterial blood pressure before and after drug administration are presented in Table 1. In all rats, a single rapid intravenous administration of 40 μg/kg adrenaline resulted in an immediate increase in mean arterial blood pressure. The initial mean arterial blood pressure was 95 ± 9 mmHg in normothermic animals and 90 ± 6 mmHg in acute exposure to heat. These pressures rapidly increased to 168 ± 10 mmHg in normothermic and 166 ± 11 mmHg in acute exposure to heat after adrenaline injection (Table 1). The duration of increased blood pressure was 3.7 ± 1.3 min in normothermic rats and 1.3 ± 0.4 min in rats treated with acute exposure to heat during adrenaline-induced hypertension. The duration difference between normothermic and mild hyperthermic animals was found statistically significant ($p < 0.01$). The term duration was used for the period of time this elevated blood pressure was retained (17).

BBB Permeability Changes

No Evans blue albumin extravasation was seen in the brains from normothermic control rats except in the pineal body, pituitary gland, and choroid plexus regions, in which capillaries are known to be leaky. Mean Evans blue dye content in moderate hyperthermic rats are elevated above those of sham-exposed animals (Fig. 1). The mean values for Evans blue dye were found to be 0.20 ± 0.04 mg%, and 0.30 ± 0.1 mg% whole brain in normothermic and moderate hyperthermic animals, respectively ($p < 0.05$). In normothermic animals, the adrenaline-induced hypertension resulted in BBB breakdown restricted regionally to the posterior parietal, occipital cortex, and frontal cortex in a symetric fashion. No deep brain areas like thalamus, n. caudatus, hypothalamus, and brain stem were ever affected.

In moderate hyperthermic group (IV) only five animals showed BBB disruption after acute arterial hypertension. The others did not shown any Evans blue albumin extravasation. The animal had a seizure after adrenaline injection and showed extreme BBB disruption in two hemispheres and thalamus, hypothalamus, and n. caudatus. The animal with seizure was omitted from data. Mean value for Evans blue dye was found to be 0.63 ± 0.2 mg% in normothermic plus adrenaline injected group and 0.40 ± 0.2 mg% in moderate hyperthermic animals ($p < 0.05$).

DISCUSSION

Evans blue albumin extravasation was determined as a macroscopic finding. However, quantitative estimation with a spectrophotometer using homogenized brain to release the dye was also performed to evaluate the macroscopic findings. Macroscopic findings correlated well with this quantitative estimation with spectrophotometer. Kajiwara et al. (12) and our previous studies have also confirmed that macroscopic findings correlated well with the spectrophotometer (20). The questions of whether hyperthermia lowers or increases the permeability of the BBB has not yet been unequivocally decided (1,7). Williams et al. (21,22), studied the BBB in animals using HRP as a tracer after hyperthermia. Ultrastructural studies revealed no significant extravasation of HRP indicative of microwave or ambient heat-induced disruption of the BBB.

TABLE 1

MEAN ARTERIAL BLOOD PRESSURE (MABP) AND DEGREE OF BLOOD-BRAIN BARRIER (BBB) BREAKDOWN DURING EXPERIMENTAL GROUPS

Experimental Groups	(*n*)	MABP (mm Hg)		Degree of BBB				
		Initial	Maximum	Duration	0	1	2	3
Normothermic Control (I)	10	108 ± 11	–	–	10	–	–	–
Hyperthermic Control (II)	11	102 ± 9	–	–	6	5	–	–
Normothermia + Adrenaline (III)	17	95 ± 9	168 ± 10**	3.7 ± 1.3	7	5	5	–
Hyperthermia + Adrenaline (IV)	14	90 ± 6	166 ± 11*	1.3 ± 0.4†	9	3	1	1

Values are expressed as the mean ± SD.
*In comparison to initial value $p < 0.01$.
†In comparison to normothermia + adrenaline group $p < 0.01$.
n = Number of animals.

Preston (21) has also studied the BBB using ^{14}C sucrose and ^{125}I-bovine albumin as tracer after hyperthermia. From this study, it appeared that hyperthermia caused an apparent reduction in permeation of sucrose across the BBB. Sharma and Dey (24,25) have shown that exposure of young rats to chronic summer heat or acute heat increased the permeability of the BBB to Evans blue albumin complex in different regions of the brain.

The partly contradictory results may be explained by different methods of heating, different tracers and temperature ranges, different times of exposure to heat, and evaluation methods (13,28). Some reports have shown that the duration of heat stress and the age of animals are important factors for affecting the permeability of the BBB under heat stress (24,25,29). In the present study, slight alteration of BBB permeability was observed in mild hyperthermic rats.

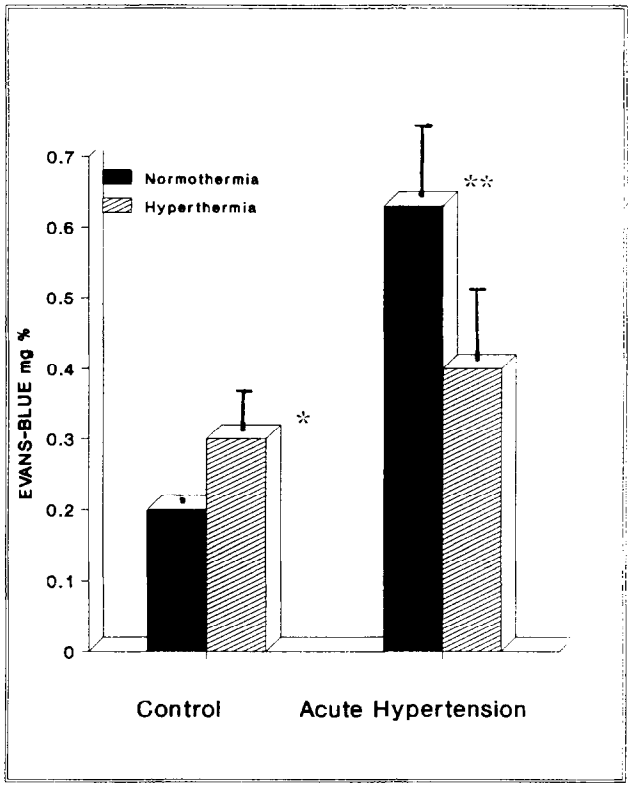

FIG. 1. Evans blue content (mg% whole brain) in the control and adrenaline-induced acute hypertension during normothermic and hyperthermic animals. *$p < 0.05$ hyperthermia vs. normothermia. Means ± SD. **Hyperthermia vs. normothermia during acute hypertension. Each column represents the mean of 12 rats.

On the other hand, the influence of acute hypertension on BBB permeablity was first investigated in hyperthermia in this study. Acute hypertension is known to be associated with disturbances of the BBB (11,17). In these conditions there is a loss of autoregulation of cerebral blood flow. In the present study, adrenaline produced blood pressure elevations similar in amplitude in normothermic and mild hyperthermic animals. But brain level of Evans blue content decreased significantly from normothermic value when rats were made moderately hyperthermic by exposure to ambient heat for 20 min, before adrenaline induced acute hypertension. This reduction in the mean value for Evans blue dye in the rats exposed to ambient heat may reflect alterations in vesicular transport with mild hyperthermia. Williams et al. (27,28) have shown that the decreased entry of HRP and ^{14}C sucrose into the microvessel endothelium of hyperthermic rats is consistent with experimental evidence reporting hyperthermia-induced disruption of membrane function. But significant effects on body physiology and cellular function may begin at temperatures exceeding 40°C (28). The body temperature of the rats was 39°C in these experiments; therefore, it is difficult to say hyperthermia induced disruption of the membrane function. The detailed mechanism of the mild hyperthermia and mild hypertermia plus adrenaline induced hyeptension on the BBB are not known, but the results suggest some possible reasons for the protective effect of mild hyerthermia on BBB permeability during acute hypertension.

The first reason why the extravasation of Evans blue albumin is less pronounced in the brains of mild hyperthermic rats may be the duration of the mean arterial blood pressure during acute hypertension. Although there was no significant difference between normothermic and mild hyperthermic groups in maximal blood pressure attained, a significant difference was found in duration (Table 1). Besides the rate of increase in pressure, the duration of this increased pressure was the important factor in the disruption of the BBB during convulsions (10,20). Generally, the animals with shorter duration of maximal mean arterial blood pressure have less intense BBB permeability, in contrast to the animals with higher duration of maximal mean arterial blood pressure and increase in BBB permeability (20). Therefore, mean arterial blood pressure du-

ration observed in mild hyperthermic animals injected with adrenaline seems to be the parameter that determines the degree of BBB permeability because the duraiton of increased blood pressure was 3.7 ± 1.3 min in normothermic rats and 1.3 ± 0.4 min mild hyperthermic rats during adrenaline-induced hypertension. In conclusion, as pressure pulse (i.e., duration and maximal arterial blood pressure) is shorter under hyperthermic conditions, BBB is less disturbed in acute hypertension under hyperthermic conditions than normothermic conditions. Several other phenomena contribute to prevention of the BBB permeability in acute hypertension during mild hyperthermia. For example, sympathetic stimulation attenuates increases in cerebral blood flow during sudden increases in systemic arterial pressure and protects the BBB (2,23).

As a result we can say that moderate hyperthermia slightly increases BBB permeability, and normothermic rats are more prone to developed permeability disturbances than mild hyperthermic rats during acute arterial hypertension.

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