

Gender Difference in the Influence of Antioxidants on the Blood–Brain Barrier Permeability During Pentylenetetrazol-Induced Seizures in Hyperthermic Rat Pups

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Abstract Our purpose in this study was to investigate the protective effects of selenium and vitamin E on the blood–brain barrier (BBB) permeability in rats with convulsion under hyperthermic conditions. To eliminate the effect of sex on BBB, we performed our study on 4- to 5-week-old prepubertal rat pups. Evans-blue was used as a BBB tracer. Convulsions were induced by administration of i.p. pentylenetetrazol. In the selenium group, 4 ppm selenium was added to the drinking water for 4–5 weeks. Vitamin E was administered at 700 mg/kg ip. It was shown that the convulsions, both under normothermic and hyperthermic conditions, caused widespread increase in the BBB permeability ($p < 0.05$). In addition, a significant difference was observed among female and male rats ($f[1, 102] = 6.387, p < 0.05$). In convulsions under normothermic conditions, there was a further increase in the BBB permeability ($F[3, 102] = 43.534, p < 0.001$) and a greater increase of permeability in males compared to females ($F[1, 102] = 6.387, p < 0.05$). Selenium and vitamin E significantly decreased the BBB destruction caused by convulsions under hyperthermic conditions in males ($p < 0.05$). Treatment with selenium or vitamin E has beneficial effects on the BBB breakdown during convulsions. But gender differences are very important in BBB permeability under pathological conditions and antioxidant treatments.

Keywords Blood–brain barrier · Seizure · Selenium · Vitamin E

Introduction

The oxygen consumption and requirement increases when the body metabolism is increased [1, 2]. Increase in the oxygen consumption means increased free radical production. Hyperthermia and convulsions are important pathological conditions in which the metabolism is increased [3, 4]. In our study, in which we planned to investigate the effects

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of free radicals and antioxidants, selenium and vitamin E on the blood–brain barrier permeability in convulsions under hyperthermic conditions, we were looking for answers to the following questions: 1. To eliminate the effect of sex, and considering that previous studies were performed on adults, what are the effects of convulsions under hyperthermic conditions on blood–brain barrier in 4- to 5-week-old rats? 2. Does selenium, which is an important antioxidant and a cofactor of glutathione peroxidase, have a protective effect against the destruction of the blood–brain barrier permeability in rat pups fed with selenium-added mother milk? 3. Can high doses of vitamin E have a protective effect against free radicals produced in convulsions under hyperthermic conditions? A brief account of the results have appeared in an abstract form [5].

Materials and Methods

Wistar albino rat pups of both genders weighing 50–80 g were used in our study. This study was planned on eight groups. Experimental groups are: 1. control group ($n=12$), 2. convulsion group ($n=12$), 3. hyperthermia group ($n=12$), 4. hyperthermia + convulsion group ($n=12$), 5. Selenium + convulsion group ($n=12$), 6. selenium + convulsion + hyperthermia group ($n=12$), 7. vitamin E + convulsion group ($n=12$), and 8. vitamin E + convulsion + hyperthermia group ($n=12$). Evans-blue was used as a blood–brain barrier tracer: 4 ml/kg 2% Evans-blue was injected through the femoral vein under ether anesthesia [6]. Hyperthermia was achieved at a hot bath at 40–42°C. Body temperature of the rats reached 40°C within 20 min. Four parts per million of selenium ($\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$) was added to the mothers' drinking water and rat pups fed on their milk for 4–5 weeks. In previous studies, it was experimentally shown that the babies receive the selenium in the mother's milk both in man and experimental animals [7, 8]. Therefore, selenium for the rat pups was supplied by adding 4 ppm into the drinking water of the mothers. Vitamin E was injected intraperitoneally at 700 mg/kg. In all groups, 30 min after injection of Evans-blue, cerebral hemispheres of the rats were perfused. For this procedure thorax was dissected through the sternum and 0.9% NaCl was injected through the left ventricle at physiologic pressure, and the jugular veins and the atria were dissected. Perfusion was carried on until clear fluid drainage from the jugular vein was obtained. After the perfusion, the cerebrum was dissected and extravasation of Evans-blue was evaluated by gross examination. After this, the brain was divided into right and left hemispheres, cerebellum and brain stem parts. The wet weights of these parts were measured using preweighed aluminum foil. By adding 2.5 ml phosphate buffer, the brain samples were homogenized by a homogenizator; 2.5 ml 60% trichloroacetic acid was added to the tubes, mixed with a vortex and stored at +4°C for 30 min. After this period, the tubes were centrifuged at $1000 \times g$ for 30 min. The supernatant was carefully transferred to a measure and its absorbance was measured at 620-nm wavelength against phosphate buffer. By using the values measured at the spectrophotometer and Evans-blue absorbance amount regression curve, the amount was calculated on the computer per mg/100 g of brain tissue [9].

Statistical Analysis

Mean values of the measurements were assessed by the Student's *t* test and factorial ANOVA. The *p* values <0.05 were accepted as significant.

Results

In the control group, there was no extravasation of Evans-blue except in the circum-ventricular organs (area postrema, pineal, neurohypophysis, subformal organ, lamina terminalis) where the blood–brain barrier is absent. The amounts of Evans-blue detected in this group are indicated in Fig. 1. The intraperitoneal pentylenetetrazol-injected rats had generalized tonic clonic convulsions with hypersalivation within 20–30 s. The rats were examined for 20 min and the convulsions continued intermittently. In the convulsion group, there was bilateral extensive increase in the blood–brain barrier permeability, especially in the basal ganglia, cerebral hemispheres, and the cerebellum. In the hyperthermia group, there were ocular and nasal mucosal hemorrhages in the rats, which were kept in 40–42°C hot baths for 20 min. Only one male and one female rat had symmetrically increased permeability of the blood–brain barrier in the cerebral and cerebellar hemispheres. In other terms, hyperthermia alone did not result in increased permeability either in male or in female rats (Fig. 1).

In the hyperthermia + convulsion group, pentylenetetrazol-injected rats were transferred to hot baths and their convulsive activities and body temperatures were noted. The convulsions carried on intermittently while their body temperatures were 40±1.8°C. Hemorrhages were observed from the ocular and nasal mucosae. There was bilateral permeability increase in the basal ganglia symmetrically, right and left hemispheres, and cerebellar cortex of the male rats of this group (Fig. 1). In the female rats, there was asymmetrical permeability increase in the right and left hemispheres and the basal ganglia.

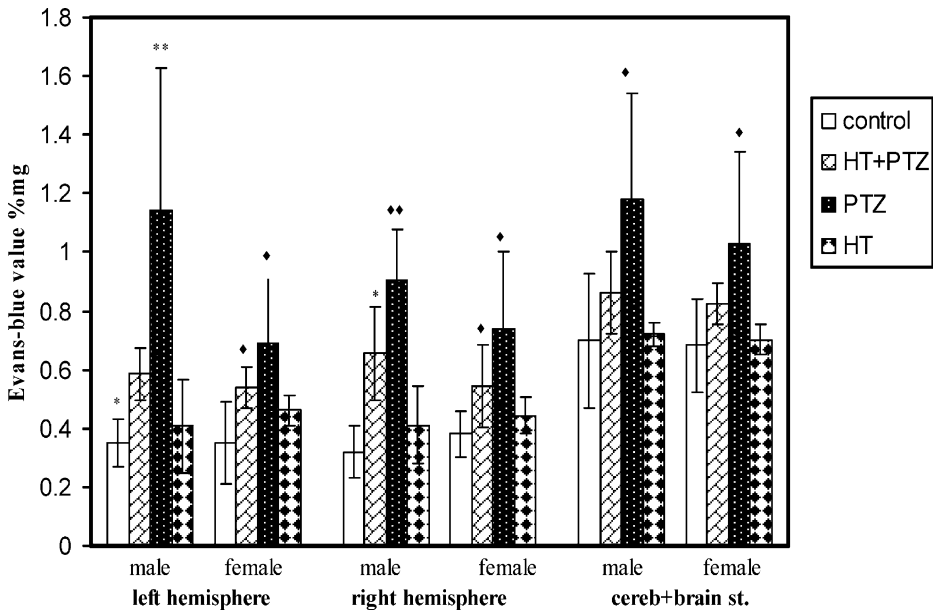


Fig. 1 Evans-blue content on the left and right hemispheres and cerebellum + brain stem in the control and experimental groups. HT: Hyperthermia, PTZ: Pentylenetetrazol. Left hemisphere: * $P=0.001$: control vs HT + PTZ in males; ** $P<0.01$: control vs PTZ in males; $\diamond P<0.05$: control vs other groups in females. Right hemisphere: * $P=0.001$: control vs HT + PTZ in males; ** $P<0.01$: control vs PTZ in males, $\diamond P<0.05$: control vs PTZ in males and females, cerebellum + brain stem: $\diamond P<0.05$: control vs PTZ in males and females

When these results were compared to those in the control group, the difference was significant for females ($p=0.001$), but not so significant for males ($p>0.05$).

As a result, selenium has been found to have different protective effects on different parts of brain ($F[1, 102]=35.758, p<0.001$). When sex difference is taken into consideration, selenium has a significant protective effect on the blood-brain barrier only in males ($F[1, 102]=6.387, p<0.05$). Depending on our results, we can conclude that selenium is protective in males but not in females (Fig. 2). In vitamin E + convulsive group, the acute effect of vitamin E on the blood-brain barrier was assessed. When the group under normothermic conditions was compared to the vitamin E group, it was observed that vitamin E has a significant protective effect on all brain areas in males. In females, the vitamin E has significant protective effect on the right and left hemispheres, but not on the brain stem region. When males and females were compared, it was revealed that vitamin E protects the barrier in males to a higher degree than in females ($F[1, 102]=6.387, p<0.05$). The Evans-blue values of the convulsive group under hyperthermic conditions are demonstrated in Fig. 3 to evaluate the effect of vitamin E. When Evans-blue values were compared to the males in the convulsive group under hyperthermic conditions but not given vitamin E, a significant difference was found in the right and left cerebral hemispheres, but not in the brain stem. According to this finding, the protective effect of vitamin E on blood-brain barrier shows a significant difference in various parts of brain ($F[2, 102]=35.758, p<0.001$). In females of the same group, vitamin E did not have a protective effect in any part

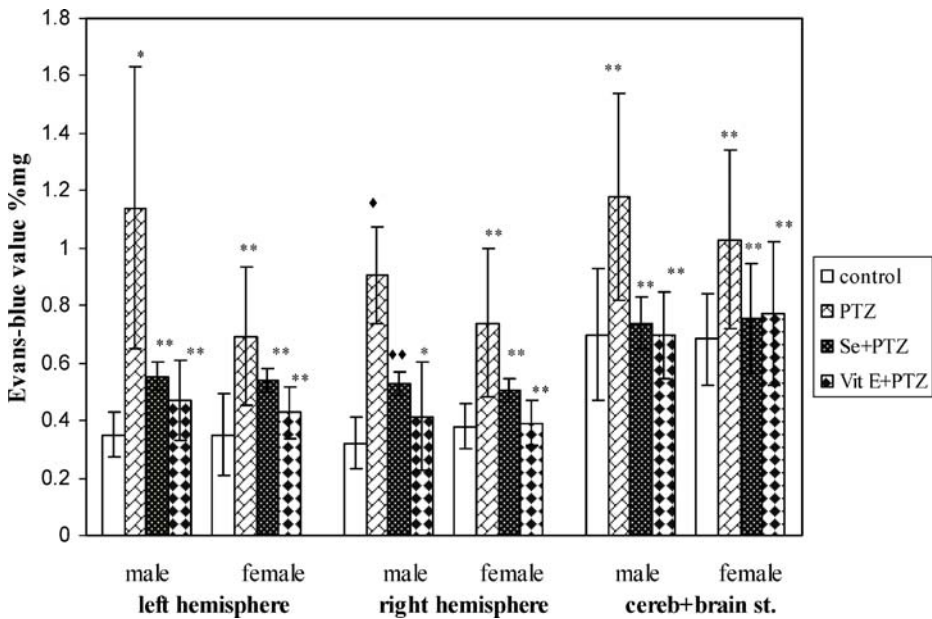


Fig. 2 Evans-blue content in the left, right hemispheres and cerebellum + brain stem in the control, convulsion, selenium and vitamin E-treated animals. PTZ: Pentylentetrazol; Se + PTZ: Selenium + Pentylentetrazol; E vit + PTZ: Vitamin E + Pentylentetrazol. Left hemisphere: * $P<0.01$: control vs PTZ in males; ** $P<0.05$: PTZ vs Se + PTZ in males, PTZ vs E vit + PTZ in males, control vs PTZ in females, PTZ vs Se + PTZ in females, PTZ vs E vit + PTZ in females. Right hemisphere: ♦ $P<0.001$: control vs PTZ in males; ** $P=0.001$: PTZ vs Se + PTZ in males; * $P<0.01$: PTZ vs E vit + PTZ in males, ** $P<0.05$: control vs PTZ in females, PTZ vs Se + PTZ in females, PTZ vs E vit + PTZ in females. Cerebellum + brain stem: ** $P<0.05$: control vs PTZ in males, PTZ vs Se + PTZ in males, PTZ vs E vit + PTZ in males, control vs PTZ in females, PTZ vs Se + PTZ in females, PTZ vs E vit + PTZ in females

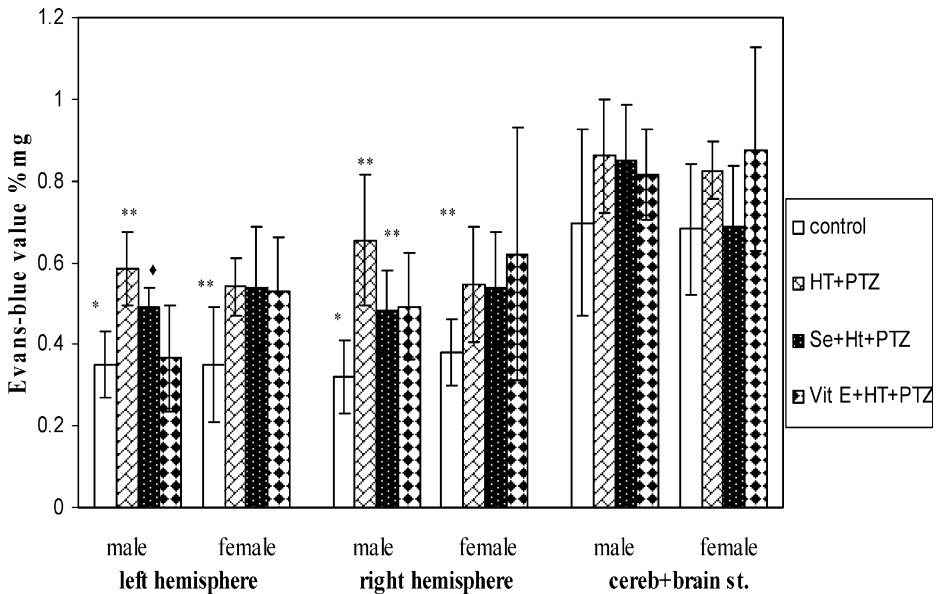


Fig. 3 Evans-blue content in the left and right hemispheres and cerebellum + brain stem in the control, hyperthermia, pentylenetetrazol, selenium, and vitamin-E-treated animals. PTZ: Pentylenetetrazol, HT + PTZ: Hyperthermia + Pentylenetetrazol, Se + HT + PTZ: Selenium + Hyperthermia + Pentylenetetrazol, Vit E + HT + PTZ: Vitamin E + Hyperthermia + Pentylenetetrazol. Left hemisphere: * $P=0.001$: control vs HT + PTZ in males, ** $P<0.05$: HT + PTZ vs Se + HT + PTZ in males, control vs HT + PTZ in females, ♦ $P<0.01$: HT + PTZ vs E vit + HT + PTZ in males. Right hemisphere: * $P=0.001$: control vs HT + PTZ in males, ** $P<0.05$: HT + PTZ vs Se + HT + PTZ in males, HT + PTZ vs E vit + HT + PTZ in males, control vs HT + PTZ in females

of the brain. As a result, when compared to females, it was deduced that vitamin E protects males significantly ($F[1, 102]=6.387, p<0.05$).

Discussion

In our study hyperthermia was used for two purposes. First, we imitated the febrile convulsions, which are very frequent clinical conditions, by this model. The second reason is that hyperthermia is an event that stimulates metabolism. Thus, the main consideration is that higher amounts of free radicals will be produced in hyperthermic convulsions because of the effect of both hyperthermia and convulsions. In our experiments, 4- to 5-week-old prepubertal rat pups were used. There are two reasons for this. First, in pathological conditions, the blood-brain barrier is destroyed differently in different sexes. By using ^{14}C -sucrose, Cragg and Philips [10] were the first to report that female rats have increased blood-brain barrier permeability. Oztas et al. [11] showed in their study with bicuculline that blood-brain barrier is destroyed differently in different sexes. Convulsions lead to greater breakdown of blood-brain barrier in females. The other reason for our use of rat pups is that febrile convulsions are encountered in children from 5 months to 5 years old. The frequency of febrile convulsions is 2–5% in overall childhood [12]. Similar to adult rats, the blood-brain barrier permeability increased in rat pups in convulsions under normothermic conditions. We hypothesized that convulsions under hyperthermic conditions

will lead to a higher increase in the blood–brain barrier permeability caused by induction of the metabolism and production of free radicals. But, as mentioned earlier, in contrast to this hypothesis, normothermic convulsions led to greater increase in blood–brain barrier permeability. If we consider the effects of blood pressure, this can be explained by the vasodilatation caused by hyperthermia. The clinical finding indicating this vasodilatation is hemorrhage from the ocular and nasal mucosae. The vasodilatation caused by hyperthermia may prevent the blood pressure increase and have a protective effect on the blood–brain barrier permeability. This hypothesis should be supported with further studies. One of the original findings in this study is, when compared to males, female rats have less destruction in blood–brain barrier permeability both in normothermic and hyperthermic convulsive groups. Whereas studies on adults show that females have a greater breakdown of blood–brain barrier [11]. In rat pups, the difference caused by sex cannot be linked to the sex steroids, as they are not secreted in prepuberty. It may be caused by the genetic background or gonadotropins encountered in the intrauterine period that is responsible. On the other hand, Ehrenbrink et al. [13] showed that antioxidant enzymes activities were different in female and male rats. These findings can be interpreted to support our different results between sexes. The effect of sex on the blood–brain barrier permeability is not well known. We think this field should be clarified with further studies. Our findings indicate that both selenium and vitamin E decrease the blood–brain barrier permeability, which is increased during convulsions, similar to the adult rats. Our results are in accordance with the studies of Wei et al. [14] and Oztas et al. [6] on adult rats. Wei et al. [14] showed that arachidonic acid increases the blood–brain barrier in their study with cats. They proposed that topical administration of arachidonate stimulates the free radicals, as this stimulatory effect was eliminated with antioxidants like superoxide dismutase and catalase. Oztas et al. [6] showed that administration of chronic selenium and acute vitamin E prevented the destruction of blood–brain barrier permeability caused by pentylene tetrazol in adult female rats. Their results are parallel to those in our study. Another original finding in this study is that selenium and vitamin E have protective effects in males but not at all in females. If sex would be considered as not having an effect in normothermic conditions, this difference, caused by sex, can be explained by the effect of hyperthermia on the blood–brain barrier and free radical production by some unknown mechanism in males and females. Maybe the free radical production has less importance in blood–brain barrier destruction in female rats. This may be the reason for our finding that vitamin E and selenium do not protect the blood–brain barrier in female rats. To explain these possibilities, new studies should be performed on the field, as free radicals are widely accepted as being responsible for many diseases like atherosclerosis [15], ischemia-reperfusion injury, aging [16], cancer [17], multiple sclerosis [18], Alzheimer disease, Huntington chorea [18–20], and schizophrenia [21]. For this reason we consider antioxidants to be important for the treatment and prognosis of many diseases.

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