Influence of Orchidectomy and Ovariectomy on the Blood-Brain Barrier Permeability During Bicuculline-Induced Seizures

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The changes in the permeability of the blood-brain barrier (BBB) during bicuculline-induced seizures were investigated in ovariectomized female and orchidectomized male rats. The rats were anesthetized with diethyl ether. Evans blue, which was used as a BBB tracer, was injected into femoral vein 5 min before administering bicuculline to induce grandmal seizures. Ten groups of rats were studied: Group I: control female; Group II: control male; Group III: intact female + bicuculline; Group IV: intact male + bicuculline; Group V: ovariectomized female; Group VI: orchidectomized male; Group VII: ovariectomized female + bicuculline; Group VIII: orchidectomized male + bicuculline (1.2 mg/kg, i.v.); Group IX: ovariectomized female + estrogen + bicuculline; Group X: orchidectomized male + estrogen + bicuculline. Adult male and female rats were orchidectomized and ovariectomized 3 weeks before the experiments, or sham operated under general anesthesia. During bicucculline-induced seizures, the mean arterial blood pressure increased significantly in both intact and ovariectomized and orchidectomized rats. BBB lesions were present in 80 percent of intact female rats and 50 percent of ovariectomized rats after bicuculline-induced seizures. This difference between intact and ovariectomized rats was found to be significant ($p < 0.01$). There was no statistically significant change in the BBB permeability between intact and orchidectomized rats after convulsion. Generating seizures in both ovariectomized and orchidectomised rats, after administrating of estrogen, did not lead to any significant alteration in BBB permeability. Our results suggest that the extravasation of Evans blue albumin was most pronounced in the brain of intact female rats when compared to ovariectomized rats after bicuculline-induced seizures. After administrating estrogen, the decreased BBB permeability values of ovariectomised rats could not reach the values in intact rats.

Key words: Evans blue – Sex hormones – Female – Male – Rat

Introduction

Sex steroid hormones in the central nervous system appear to play a significant role in the regulation of a number of physiological process (1,2). Estrogen in the circulating blood crosses the blood-brain barrier (BBB) relatively rapidly and is distributed to all parts of the brain (3). The mechanism by which estrogen modulates the permeability of cerebrovascular endothelium is poorly established. There are a few previous reports of male-female differences in BBB permeability in normal rats (4,5). Cragg and Phillips (5) indicated that the product of the permeability × vascular surface area of the BBB to ^{14}C-sucrose has a significant sex difference in an inbred strain of rats. Recently, we described that there was a sex related difference in the BBB permeability between male and female rats after bicuculline-induced seizures. The extravasation of Evans blue albumin was most pronounced in the brain of the female rats compared to male rats during seizures (6). Therefore, it seems that there may be a relationship between BBB permeability and sex hormones like estrogen and androgen (7).

We attempted to investigate the BBB permeability during bicuculline-induced status epilepticus in intact female and ovariectomized female rats, and intact male and orchidectomized male rats. A preliminary report of these findings has been published (8).

Materials and Methods

Experimental procedure

The experiments were performed on adult male and female Wistar rats weighing between 200 – 260 g. Males were bilaterally orchidectomized *via* a single incision along the midline of the scrotum. Sham orchidectomies received only the scrotal incision. Surgery was performed under diethyl ether anesthesia. Bilateral ovariectomy was performed on females through subumbilical incisions using standard procedure. The rats were ovariectomized or orchidectomized 2 to 3 weeks before experiments.

On the day of experiment, under dietyl ether anesthesia, polyethylene catheters were inserted into a femoral artery for recording mean arterial blood pressure (Ugo-Basil apparatus). The femoral vein was cannulated for intravenous drug injection. Body temperature was maintained at 37 °C by external

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Table 1 Mean arterial blood pressure (MABP) and degree of blood-brain barrier (BBB) breakdown for the various experimental groups

Experimental Groups	n	MABP (Mean Arterial Blood Pressure) mmHg		Degree of BBB Breakdown			
		Initial	Maximal	0	1+	2+	3+
Control female	6	109 ± 8	–	6	–	–	–
Control male	6	103 ± 12	–	6	–	–	–
Intact female + bic	20	106 ± 14	186 ± 12*	4	6	4	6
Intact male + bic	20	109 ± 11	183 ± 15*	9	5	6	–
Ovariectomized female	8	101 ± 11	–	8	–	–	–
Orchidectomized male	6	114 ± 9	–	6	–	–	–
Ovariectomized + bic	16	110 ± 13	188 ± 15	8	5	3	–
Orchidectomized + bic	10	99 ± 13	181 ± 13*	5	3	2	–
Ovariectomized + Estrogen + bic	5	100 ± 10	180 ± 15	2	2	1	–
Orchidectomized Estrogen + bic	5	109 ± 10	181 ± 11	3	2	–	–

* In comparison with initial value $p < 0.01$ (Means ± SD); n = number of animals; bic = bicuculline

heating. All experiments were carried out in the morning, between 9:00 and 11:00 a.m.

Ten groups of rats were studied. Group I: control female; Group II: control male; Group III: intact female + bicuculline; Group IV: intact male + bicuculline; Group V: ovariectomized female; Group VI: orchidectomized male; Group VII: ovariectomized female + bicuculline; Group VIII: orchidectomized male + bicuculline; Group IX: ovariectomized male + estrogen + bicuculline; Group X: orchidectomized male + estrogen + bicuculline.

Groups I, II, V and VI served as control for permeability of the BBB and received Evans blue only. Evans blue, which binds to serum albumin, was used as a BBB tracer and given intravenously at a dose of 4 ml of 2 % solution per kg body weight. In this dose, the dye binds to serum albumin. Evans blue albumin complex normally does not pass the BBB. When the barrier is disrupted during convulsion, the brain becomes stained with blue dye. In the third, fourth, seventh and eighth groups of rats, after recording initial blood pressures, Evans blue was injected intravenously and after 5 min later bicuculline (1.2 mg/kg) was rapidly administered in the intact, ovariectomized and orchidectomized animals. Seizures were generated two hours after the administration of 1000 ng/kg i.v. 17-estradiol (E_2) in experimental groups IX, and X following ovariectomy and orchidectomy. At the end of the experiments, i.e., approximately 20 min after drug injection, under diethyl ether anesthesia, all rats were killed by perfusion through the heart with saline to avoid artificial staining of the brain during removal.

Estimation of the degree of blood-brain barrier opening

The brains were removed and examined for Evans blue albumin extravasation, extent and intensity of the staining. Staining of each hemisphere and the coronal sections by Evans blue was graded as follows: grade 0, no staining; grade 1+, just noticeable staining; grade 2+, moderate blue staining; and grade 3+, dark blue staining (9,10). A quantitative estimation with spectrophotometer using homogenized brain to release the dye was also performed as described previously (11). Briefly, the brain was removed and divided at the midline. Each half cerebrum and cerebellum was placed in tared tubes which were immediately reweighed. They were homogenized with 5 ml of phsophate-buffered saline containing a 5 ml % solution of 1 N NaOH. The homogenized brain was centrifuged (10 000 rpm for 10 min) and a spectrophotometrical analysis at 620 nm of wavelength was performed to measure the amount of resolved dye.

Statistical analysis

Data are expressed as means ± SD, and statistical analysis was performed by the Student's t-test. Values of p less than 0.05 were considered significant. Bicuculline and Evans blue were obtained from Sigma Company.

Results

Epileptic seizure

In all rats, a single rapid intravenous administration of bicuculline resulted in immediate generalized tonic-clonic convulsions. Tonic convulsion was typically preceded by extension of the trunk-pelvis-tail, head nodding and myoclonic jerks. Typically, the tonic phase lasted approximately 12 s and was followed by generalized clonic activity. All animals had multiple tonic-clonic seizures with brief interictal periods. No marked difference in seizure pattern was observed in intact, ovariectomized and orchidectomized rats.

Mean arterial blood pressure before and after drug administration and the visual estimation of Evans blue albumin extravasation are presented in Table **1**. Bicuculline-induced grand mal seizures gave rise to an abrupt increase in blood pressure. Initial mean arterial blood pressure was 109 ± 8 mmHg in intact female and 101 ± 11 mmHg in ovariectomized female rats. Bicuculline caused a rapid increase of 80 mmHg in intact female and 78 mmHg in ovariectomized rats. The initial mean arterial blood pressure was 103 ± 12 mmHg in intact male, and 114 ± 9 mmHg in orchidectomized male. These rapidly increased to 188 ± 15 mmHg and 181 ± 13 mmHg respectively.

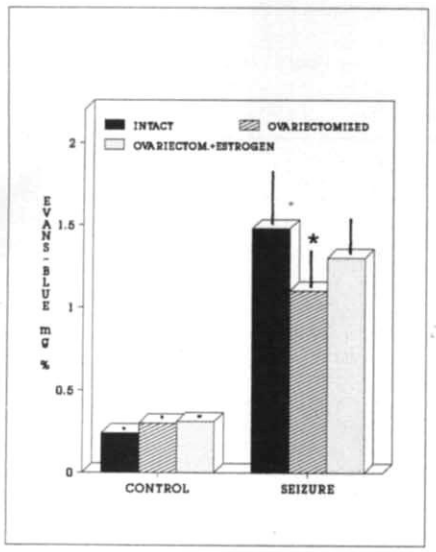

Fig. 1 Evans blue (mg%) content in the control and bicuculline-induced seizure in female rats. * $p < 0.01$ intact versus ovariectomized.

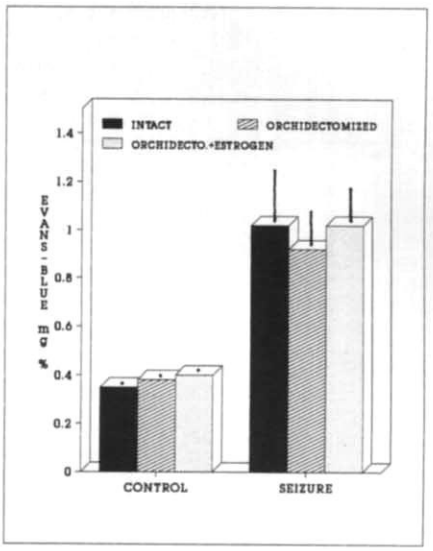

Fig. 2 Evans blue (mg%) content in the control and bicuculline-induced seizures in male rats.

Blood-brain barrier permeability changes

No Evans blue albumin extravasation was seen in the brain of control male and female, or in those animals orchidectomized and ovariectomized except in the pineal body, pituitary gland and choroid plexus-regions, in which capillaries are known to be leaky. Increased penetration of Evans blue albumin into the brain was observed in 11 of the 20 male rats and 16 of the 20 female rats convulsed by bicuculline. The extravasation of Evans blue albumin was most pronounced in the brains of female rats compared to male rats. The mean value for Evans blue dye in the whole brain was found to be 1.48 ± 0.3 mg % in the group consisting of the female rats and 1.0 ± 0.2mg % in the group of male rats during bicuculline-induced seizure. This difference between female and male rats was found to be statistically significant ($p < 0.01$). Evans blue content was compared in ovariectomized and intact rats after bicuculline-induced seizure, it was reduced significantly in ovariectomized rats, i.e., ovariectomized animals have shown less intense disruption of BBB permeability. The mean value for Evans blue dye was found to be 1.48 ± 0.3 mg % whole brain in intact female rats and 1.14 ± 0.2 mg % whole brain in ovariectomized animals after bicuculline-induced seizure (Fig. **1**). This difference between intact and ovariectomized animals was found to be significant ($p < 0.01$). Generating seizures after administration of estrogen in ovariectomized rats seemed to increase BBB permeability by 12 % when compared with ovariectomy, but could not bring it to intact values, and this increase was not significant. Quantity of Evans blue after the seizures was 1.14 ± 0.2, whereas this value became 1.30 ± 0.2 after the administration of estrogen and this difference was not significant ($p > 0.5$).

However, the extravasation of Evans blue was compared with orchidectomized male and intact rats after bicuculline-induced seizure, and there was no significant difference between them, i.e., the orchidectomized animals have shown that similar intense BBB disruption. The mean value for Evans blue dye was found to be 1.0 ± 0.3 mg % in the whole brain of intact rats and 0.92 ± 0.2 mg % in the whole brain of the orchidectomized animals after bicuculline-induced seizure ($p > 0.5$) (Fig. **2**). Administration of estrogen in orchidectomised rats, did not lead to any significant alteration on the BBB permeability (Fig. **2**).

Discussion

This is the first study to examine the effect of orchidectomy and ovariectomy on the BBB permeability during seizure. There are two new findings of the present study. First, disruption of BBB permeability during seizure is similar in orchidectomized male and intact male rats, and estrogen has no effect on the BBB permeability of orchidectomized rats. Second, in contrast to male rats, ovariectomy weakened the increase in BBB permeability during seizures, but administration of estrogen in ovariectomized rats only caused an insignificant increase of 12 % compared to intact animals. The mechanism by which estrogen modulates the permeability of cerebrovascular endothelium is poorly established, i.e., the detailed mechanism of the protective action of ovariectomy on the BBB is not known. These hormones may alter the BBB permeability through a wide variety of mechanisms. Estrogens may play a very important role in the regulation of the functions of various neurotransmitter in the central nervous system. It has been known for many years that there is a functional link between gonadal hormones and catecholamine metabolism and action in the brain (1). Estrogen entering anywhere in the brain may then be converted to catechol estrogen. Catechol estrogen has been found to be major metabolite of estrogen in human brain (12). It has also been shown that catechol estrogen inhibits the catecholamine metabolizing enzyme cathechol-o-methyltransferase (12,13). This effect would tend to raise brain catecholamine levels in the brain. Studies by Crowley et al. (14) have provided evidence that removal of gonads increases the synthesis and/or turnover of norepinephrine in the whole brain or in particular areas. There is evidence that microvascular function could be altered by neurogenic mechanisms; particularly, it has been shown that the central noradrenergic system can actually alter the microvascular function (15). If ovariectomy increases the level of brain norepinephrine, the effect of norepinephrine on vascular smooth muscle would be constrictive (16). Constricted vessels are less vulnerable to the insult, i.e., constriction of cerebral vessels has a prophylactic ef-

fect on the BBB (15). On the other hand, Zuckerman et al. (17) showed that estrogen increases in brain water content in rats, while Reid et al. (18) reported that a single injection of estrogen is capable of altering the permeability surface area product for water in the brain without disturbing cerebral blood flow. Decreasing of plasma estrogen level during ovariectomized rats may diminish the effect of estrogen on the BBB permeability, as progesterone and estrogen can cause benign cerebral intracranial edema and increase BBB permeability to water. The other steroids (dexamethasone and other synthetic glucocorticoids) are commonly used to decrease brain edema in patient with inflammatory or neoplastic cerebral lesions (19). The mechanism of this effect is not known, but may involve reduction in the permeability of the BBB (19). Alternatively, since it has been showed that endothelial cells contain estrogen receptor (20). It seems reasonable to suppose that observed actions of estrogen in the modulating of BBB permeability could be the hormone interaction with the receptor in the endothelial cells. Decreased estrogen level in the plasma may be the influence of these receptors and change in BBB permeability. However, inability of administration of estrogen to normalize decreased BBB in ovariectomy may suggest the influence of factors other than estrogen on BBB permeability, or mention can be made that estrogen administered 2 – 3 weeks after ovariectomy could not be adequately effective due to decreased number of estrogen receptors in ovariectomy.

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