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Blood–brain barrier permeability: regional alterations after acute and chronic administration of ethinyl estradiol

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In this study we measured the effect of acute and chronic estrogen treatment on cerebrovascular permeability to sucrose and inulin. Animals were subcutaneously injected once with 0.1 μg/rat of ethinyl estradiol or injected daily with the same drug dose for 3 weeks. Control rats received the same amount of arachis oil vehicle. Three weeks treatment but not the single injection of ethinyl estradiol produced significant increases in the cerebrovascular permeability–surface area product for sucrose and inulin in almost all brain regions.

The blood–brain (BBB) is a continuous cellular layer formed by brain capillary endothelial cells with tight junctions (zonulae occludens) and restricts the passage of polar compounds and macromolecules from blood into the brain interstitium [10, 13]. These restrictions in transport across the BBB serve, in part, to maintain homeostasis within the central nervous system (CNS); however, this function of the BBB may be impaired in various pathologic states. It has also been suggested that a number of lipophilic drugs, some of which are widely used local anesthetics, steroids or tranquilizers, alter membrane permeability [15].

In particular, steroids are of special interest since dexamethasone and other synthetic glucocorticoids have been used for several years in the treatment of cerebral edema associated with cerebral ischemia and brain injury [6, 12], while the other steroids (progesterone, estrogen) can cause benign cerebral intracranial edema and increase BBB permeability to water [14, 25]. It has also been shown that some steroids (dexamethasone) reduce experimentally induced increased cerebrovascular permeability, associated with hypertension [7, 20], convulsive seizure [18], tumor and cerebral infarction [5, 16]. In our previous studies [22, 24] we have also shown that steroid administration influences BBB permeability. We found that acute (single injection), subacute (3 days) and chronic (3 weeks) administrations of a synthetic glucocorticoid, dexamethasone, which is commonly used in clinical treatment for cerebral edema, markedly (30–50%) decreases the blood-to-brain transport of α-aminoisobutyric acid (AIB) and sucrose. As an extension of these studies, we investigated the effect of an estrogen hormone on permeability of cerebral capillaries by using two poorly penetrating uncharged test radiotracers of differing molecular weight and size. An abstract of this work has been published [23].

Female Sprague-Dawley rats (from IFFA Credo, France) weighing 200–250 g were used. Ethinyl estradiol (EE_2) (Sigma, St. Louis, MO, U.S.A.) was administered according to one of two schedules: (1) a single subcutaneous (s.c.) injection of 0.1 μg/rat (0.4–0.5 $\mu g \cdot kg^{-1}$) in arachis oil 20 h prior to the experiment, (2) a daily s.c. injection of 0.5 $\mu g \cdot kg^{-1}$ for 3 weeks. Control rats received either a single s.c. injection of the same amount of arachis oil vehicle or daily injection of vehicle for 3 weeks.

Cerebrovascular permeability was determined for two test radiotracers, [^{14}C]sucrose (340 Da, radius 5 Å) and [^{3}H]inulin (5500 Da, radius 15 Å). Details of animal preparation and calculations have been presented previously [19]. To summarize, under pentobarbital anesthesia (35 $mg \cdot kg^{-1}$), catheters filled with heparinized saline (0.9% w/v NaCl) were inserted into a femoral artery and vein. Temperature was monitored with a rectal temperature probe, and external heat lamps were utilized to maintain body temperature at 35–37°C. A total

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of 0.6 ml isotonic saline containing 5 μCi [^{14}C]sucrose (spec. act. 340 mCi/mmol) and 25 μCi [^{3}H]inulin (spec. act. 4.0 mCi/mmol) (Amersham International, U.K.) were injected intravenously into rats treated with EE_2 and into control animals that received arachis oil vehicle alone. Multiple blood samples (50–100 μl per sample) were taken from the femoral artery until the rats were killed by decapitation 10 min after injection of radiotracers. The brain was then quickly removed and dissected into 14 specific regions. Whole blood, arterial plasma and weighed regions of brain were prepared for counting as previously described [22]. The amount of each tracer in both blood and brain was measured using a liquid scintillation counter (Intertechnique SL 3000) enabling calculation of cerebrovascular permeability in specific brain regions.

Cerebrovascular permeability–surface area (PA) products for [^{14}C]sucrose and [^{3}H]inulin were calculated from the tissue and plasma radioactivity data using the following equation developed by Ohno et al. [9] as described previously [19]:

$$PA = \frac{C_{br}}{\int_0^T C_{plasma}\, dt} \quad (1)$$

where C_{br} is the parenchymal brain concentration of the tracer at the end of the experiment (dpm·g^{-1}), T is the duration of experiment (min) and C_{plasma} is the arterial plasma concentration (dpm·ml^{-1}). Parenchymal brain radioactivity was determined as measured brain radioactive concentration minus intravascular radioactivity. Intravascular radioactivity was calculated using regional sucrose and inulin blood volumes.

Regional cerebral blood volume was determined in control and EE_2-treated animals for sucrose and inulin space as (dpm·g^{-1} brain)/(dpm·ml^{-1} whole blood) at the time of death, 2 min after i.v. injection of both tracers. Radioactivity was determined in whole blood and brain samples as described above.

Mean values of brain regional cerebral blood volume for [^{14}C]sucrose in untreated control animals were between 1.06 and 3.08%. The inulin spaces were 1.26 and 2.76% as expected for mixed grey and white matter [4]. No detectable differences were found between sucrose and inulin spaces. Treatment with EE_2 did not have a significant effect in any of the regions studied.

Tables I and II list the mean PA values for [^{14}C]sucrose and [^{3}H]inulin as calculated by Eqn. 1 in vehicle-treated controls and rats treated with EE_2 killed 10 min after i.v. [^{14}C]sucrose and [^{3}H]inulin injections. The average 10-min [^{14}C]sucrose PAs were about 13.0 $S^{-1} \times 10^{-6}$, whereas similarly computed PAs for [^{3}H]inulin did not differ significantly from zero ($P<0.05$) in any brain region. The PA values for [^{14}C]sucrose and [^{3}H]inulin in brain regions of control rats given in Tables I and II are in general agreement with previous studies [9, 11, 19, 21]. The PA products for [^{14}C]sucrose and [^{3}H]inulin were significantly higher than control PAs in almost all brain regions in animals treated with EE_2 for 3 weeks ($P<0.05$ for all regions except olfactory bulb, medulla, pons and cerebellum). The PA values in many brain regions of rats injected s.c. with a single dose of 0.5 $\mu g \cdot kg^{-1}$ EE_2 20 h prior to decapitation were also elevated, but the alterations were not significant ($P<0.05$ for all regions except thalamus, superior colliculus, hippocampus and white matter).

TABLE I

REGIONAL PERMEABILITY–SURFACE AREA (PA) PRODUCTS FOR [^{14}C]SUCROSE IN CONTROL AND ETHINYL ESTRADIOL (EE_2)-TREATED RATS

Values are means ± S.E.M., $n=8$. The PA was calculated by Eqn. (1).

Brain regions	[^{14}C] sucrose PA ($S^{-1} \times 10^{-6}$)			
	Single injection		21 Days treatment	
	Control	EE_2-treated	Control	EE_2-treated
Olfactory bulb	19.3±2.3	22.2±2.2	18.2±1.4	20.2±2.0
Caudate nucleus	8.1±0.8	13.0±1.2*	10.2±0.7	14.8±1.2
Hippocampus	9.6±0.8	12.3±0.9*	11.2±0.8	14.7±1.8*
Frontal lobe	11.1±1.4	12.2±1.0	11.0±1.0	16.0±1.3*,**
Occipital lobe	10.5±0.7	11.5±0.8	9.5±0.8	16.3±1.4*,**
Thalamus	13.0±1.2	13.7±1.4	14.1±1.4	18.3±1.4**
Hypothalamus	13.7±1.4	16.0±1.4	13.5±1.0	19.8±1.7*
Superior colliculus	16.1±1.0	18.2±1.7	15.8±1.4	20.9±2.5
Inferior colliculus	16.0±1.0	18.2±1.1*	15.5±1.3	21.9±1.8*
Cerebellum	11.2±1.3	12.5±1.0	10.1±0.9	15.4±1.0*
Pons	13.5±1.9	14.8±1.5	11.2±0.8	14.7±1.8
Medulla	15.5±1.5	14.4±1.4	12.6±1.4	16.5±1.5
Midbrain	10.2±0.9	10.9±1.0	10.3±0.5	14.5±1.6*,**
Gray matter	11.6±0.7	13.1±0.9	11.4±0.7	14.5±1.1*
White matter (Corp. callosum)	10.3±1.0	18.2±2.0*	10.5±0.5	16.3±1.1*

*,**Significant differences ($P<0.05$) between value of control and estradiol-treated animals, and between single injection and 21-day-treated-animals (C), respectively.

Despite extensive clinical and experimental observations on the effect of glucocorticoids on cerebrovascular permeability in the treatment of cerebral edema, there are only a few experimental observations suggesting that estrogens may also be involved in the regulation of BBB permeability. In this study the 3-week treatment but not the single injection of EE_2 produced significant increases in PAs for sucrose and inulin in almost all brain regions. Zuckerman et al. [25] showed that EE_2 increases brain water content in rats, while Reid and co-workers [14]

TABLE II

REGIONAL PERMEABILITY–SURFACE AREA (PA) PRODUCTS FOR [^{3}H]INULIN IN CONTROL AND ETHINYL ESTRADIOL (EE_2)-TREATED RATS

Values are means ± S.E.M., $n=8$. The PA was calculated by Eqn. (1).

Brain regions	[^{3}H] inulin PA ($S^{-1} \times 10^{-6}$)			
	Single injection		21 Days treatment	
	Control	EE_2-treated	Control	EE_2-treated
Olfactory bulb	0.70±0.18	1.06±0.13	0.83±0.19	1.10±0.10
Caudate nucleus	0.54±0.15	0.76±0.11	0.44±0.10	0.92±0.08*
Hippocampus	0.57±0.14	0.86±0.06*	0.53±0.08	1.00±0.12*
Frontal lobe	0.51±0.20	0.84±0.10	0.45±0.09	0.95±0.16*
Occipital lobe	0.46±0.17	0.67±0.10	0.42±0.10	0.92±0.09*,**
Thalamus	0.56±0.16	0.99±0.07*	0.75±0.12	1.13±0.15*
Hypothalamus	0.94±0.26	1.13±0.09	0.59±0.20	1.35±0.14*,**
Superior colliculus	0.49±0.18	0.96±0.09*	0.61±0.20	1.06±0.14
Inferior colliculus	0.62±0.21	0.81±0.13	0.52±0.12	1.09±0.12*
Cerebellum	0.39±0.21	0.67±0.07	0.47±0.14	0.80±0.11
Pons	0.53±0.20	0.77±0.13	0.45±0.21	0.94±0.12
Medulla	0.71±0.24	0.91±0.14	0.56±0.22	0.94±0.13
Midbrain	0.29±0.14	0.65±0.09*	0.31±0.09	0.70±0.10*
Gray matter	0.45±0.22	0.65±0.11	0.40±0.07	0.78±0.13*
White matter (Corp. callosum)	0.38±0.16	0.88±0.17*	0.39±0.12	0.73±0.10*

*,**Significant differences ($P<0.05$) between value of control and estradiol-treated animals, and between single injection and 21-day-treated animals, respectively.

reported that a single injection of 0.1 μg estrogen is capable of altering the permeability surface area product for water in the brain without disturbing cerebral blood flow. These authors also concluded that the effects of estrogen are regionally variable and are more clearly seen in the cerebral cortex. In this respect, the results obtained in the present study on the regional alteration in BBB permeability are in good agreement with previously published reports [14]. The findings on the distribution of altered permeability may reflect the functional variety of the different brain regions. According to our observation, estrogen seems to act in numerous regions of the CNS to alter the cerebrovascular permeability. The sites or mechanisms by which estrogens modulate the permeability of cerebrovascular endothelium are poorly established. Several explanations and sites of action of the hormone are possible. Since it has been shown that steroids alter the phospholipid composition and permeability of several membrane systems [1, 2, 8, 17], a possible explanation for the observed alterations is that these lipophilic steroids may act non-specifically by a direct interaction at the level of cerebrovascular endothelial cell membranes resulting in altered membrane fluidity or other changes.

Alternatively, since it has been shown that endothelial cells contain estrogen receptors[3], it seems reasonable to suppose that observed actions of estrogen in the modulation of cerebrovascular permeability could be due to hormone interaction with the receptors in these cells. Definition of the mechanisms regulating permeability and the interplay between these proposed regulators awaits further study. In summary, our findings clearly demonstrate that, irrespective of the mechanisms involved, estrogen administration may alter the BBB permeability and that its effects are regionally variable. These experimental findings also suggest that similar changes in cerebrovascular permeability may be involved in the brain swelling and increased intracranial pressure seen clinically in benign intracranial hypertension.

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