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AGE-DEPENDENT ALTERATION IN REGIONAL CEREBROVASCULAR PERMEABILITY DURING DRUG-INDUCED EPILEPSY

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SUMMARY

Age-related changes in blood-brain barrier permeability were investigated during pentylenetetrazol-induced seizures in rats aged from 15 days to 120 days. Tracers such as [^{14}C]sucrose and [^{3}H]inulin which diffuse very slowly across the intact endothelium were simultaneously injected i.v. in rats treated with pentylenetetrazol (PTZ) or in control animals. Permeability-surface area products (PA) were determined in 9 brain regions. Pentylenetetrazol-induced seizures caused a significant increase in PA for both sucrose and inulin in all brain regions studied. Blood-brain barrier dysfunction was present only in animals in which the mean arterial blood pressure rose at seizure onset. Although increased blood-brain barrier permeability was found partly in similar areas in both young and adult rat brains, in adults the increase was the highest in the preoptic area, septum, colliculus inferior, hypothalamus and in the cerebellum while the increase was comparatively much smaller in the same areas of young brains. The increase in blood-brain barrier permeability was extremely high in the hippocampus, hypothalamus and cerebellum of 15-day-old rat brain and, was least affected in the corpus striatum and cerebral cortex in contrast to older rats. From the results obtained it may be concluded that the increased cerebrovascular permeability induced by pentylenetetrazol differs markedly in localization in young and adult rats. The age-dependent increased blood-brain barrier integrity is not over all dependent on variations in the blood pressure, but rather on progressive maturation of capillaries and changes in their internal structure, and local phenomena in neuronal activity during the seizures.

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INTRODUCTION

Various studies indicate that cerebrovascular permeability is increased in specific regions of the brain during repeated electroshock seizures [1,8] and drug-induced convulsions [10,11,16]. It has also been suggested that the increased permeability during seizures could be caused by a defective blood-brain barrier (BBB) with a subsequent alteration in the ionic environment of central neurons and glia resulting in neuronal damage [22]. It has been shown that the convulsive threshold as well as seizure patterns and incidence of convulsions vary considerably in immature, young and older animals [12,13,21]. Our previous studies with Evans blue-albumin [28] showed that the regional distribution of selective BBB opening induced by pentylenetetrazol (PTZ) differs markedly in developing and adult rats. In this study, the tracer (Evans blue) used, was linked to a large molecule (albumin) and the regional changes in BBB permeability were estimated by qualitative measurement of dye leakage only. Therefore, in these present experiments we used a BBB tracer technique which allows the precise localization and quantitative expression of changes in barrier permeability [15]. Thus, we investigated the regional pattern of BBB breakdown induced by systemic application of a convulsant drug in 15-, 30- and 120-day-old rats by measuring cerebrovascular permeability of two non-electrolyte radiotracers which differ in molecular size and weight (sucrose; 340 Da, radius 5 Å and inulin; 5500 Da, radius 15 Å) and which under normal conditions cross the BBB very slightly [18,25].

We conducted the present study (1) using the radiotracer technique to explore the possibility of BBB alterations during convulsions which are undetected through the use of large molecular weight tracers, such as Evans blue-albumin (EB) and horseradish peroxidase (HRP); (2) to compare the quantitative and qualitative data on regional distribution of BBB dysfunction during drug-induced seizures in young and older animals. In these experiments PTZ, which has a direct excitatory action [7], was used as seizure evoking agent. An abstract of this work has been published [27].

MATERIALS AND METHODS

Experimental procedure

The method and experimental protocol for the measurement of blood-brain barrier integrity are the same as those used previously [28] except the [^{14}C]sucrose and [^{3}H]inulin replaced Evans blue as the tracer. A total of 48 male Sprague–Dawley rats (from Iffa Credo, France) of different ages were used. Animals were studied in three groups with their randomly selected corresponding controls: 15-, 30-, and 120-day-old animals. Animals were anaesthetized with diethyl ether. Catheters filled with 100 IU heparin in isotonic saline (0.9% w/v NaCl) were inserted

into a femoral artery and vein for blood sampling, administration of radiotracer and continuous measuring of mean arterial blood pressure (MABP). The hind quarters of animals were immobilized in a loose-fitting plaster cast and the rats were allowed to recover. Temperature was monitored with a rectal thermometer probe and external heat lamps were utilized to maintain body temperature at 35–37°C.

At least 30 min or more after ether anaesthesia, when the animal was entirely conscious, a single injection of 80 mg kg^{-1} PTZ (Sigma Chemical Co., St Louis, MO) was administered intravenously. Mean arterial blood pressure was recorded by connecting the arterial catheter to a strain gauge transducer (Gould Statham, Gould Inc., USA).

Although the electrocorticogram (ECoG) was not recorded the behavioral characteristics of convulsions were observed and duration of convulsions was calculated in each individual animal for a period of 30 min.

Determination of regional permeability

Cerebrovascular permeability was determined simultaneously for both [^{14}C]sucrose (sp. act. 540 mCi/mmol) and [^{3}H]inulin (sp. act. 4 Ci/mmol). Both were obtained from Amersham International (U.K.). [^{14}C]Sucrose (1, 3 and 5 μCi) and [^{3}H]inulin (5, 15 and 25 μCi) were injected i.v. in rats receiving saline or in rats treated with PTZ at the age of 15, 30 and 120 days respectively. Blood samples (100–150 μl/sample), were taken periodically from the femoral artery until the rat was decapitated 10 min after injection of both radiotracers and were rapidly centrifuged. Immediately following decapitation, the brain was removed from the skull and placed on cold filter paper wetted with 0.9% NaCl. The olfactory bulbs which remained in the etmoid fossae were scooped out first. The brain then was hemisectioned at the midline and according to dissection procedure described by Chiuch et al. [2] dissected into anatomic regions. All tissue samples were placed in tared vials, and reweighed to determine their weights. Sample solubilization was accomplished by adding 1 ml of soluene-350 (Packard instrument, Downers Grove, IL). Whole blood samples were decolorized with hydrogen peroxide before counting. Finally, 10 ml of scintillation fluid was added to all tissue, plasma and blood samples. Counting was performed with an Intertechnique SL-3000 liquid scintillation spectrometer. All sample counts were approximately corrected for background and quenching.

Calculation

The permeability surface area product (PA) for [^{14}C]sucrose and [^{3}H]inulin in control and PTZ-treated brains was calculated from the tissue and plasma radioactivity data using the following equation developed by Ohno et al. [18]:

$$PA = \frac{C_{brain}(T)}{\int_0^T C_{plasma} dt} \qquad (1)$$

where the C_{brain} (dpm g^{-1}) equals the parenchymal brain concentration (i.e., total minus intravascular radioactivity), at time T, T is the duration of experiment (min) and C_{plasma} is the arterial plasma concentration (dpm ml^{-1}). Details of calculation for PA determination have been presented previously [22].

Regional cerebral blood volume

To explore the possibility that PTZ may have induced changes in the vascular compartment and to calculate the amount of tracer that crossed the capillaries unidirectionally during the experiment according to Eqn. 1, the amount of tracer within the vascular space (blood volumes) was measured in control and PTZ treated rats. Regional blood volumes were determined from [^{14}C]sucrose and [^{3}H]inulin space as the value (dpm g^{-1} brain)/(dpm ml^{-1} whole blood) $\times$ 100 at the time of death, 2 min after i.v. injection of both radiotracers. Radioactivity was determined in whole blood and brain samples as discussed. Therefore the study employed an additional pair of groups of animals (21 rats) similarly treated but decapitated 2 min after the tracer injection.

Statistical analysis

Statistical differences in [^{14}C]sucrose and [^{3}H]inulin PAs between brain regions of 15-, 30- and 120-day-old rats at 10 min after convulsions as well as between those regions and corresponding regions of untreated controls were performed by analysis of variance and Bonferoni t-statistics [14]. Significance was taken as $P < 0.05$.

RESULTS

All rats given a single rapid i.v. injection of 80 mg kg^{-1} PTZ showed immediate generalized tonic-clonic convulsions. Seizures were defined by a tonic phase (the extension of trunk-pelvis-tail and shaking of head) and followed by clonic jerks (clonic phase). The tonic phase lasted approximately 20 s followed by generalized clonic activity. Repeated bouts of tonic-clonic seizures were observed for 30 min until the end of experiments. No marked differences in seizure pattern were observed in rats aged from 15 to 120 days. These observed seizure patterns are in agreement with published information [12,21]. Administration of PTZ caused a rapid transient increase in the blood pressure of approximately 70 mmHg (40–110 mmHg) above the normal in all rats except in two adults. MABP decreased again after 1–3 min to normal range, about 86 and 101 mmHg in rats 15-, 30- and 120-day-old, respectively, although the convulsions persisted for 30 min.

Two-minute [^{14}C]sucrose and [^{3}H]inulin space in 15-, 30- and 120-day-old control rats, which were taken as the regional blood volume in order to calculate PA by means of Eqn. 1, showed no marked alteration after the seizures. This justifies the use of control [^{14}C]sucrose and [^{3}H]inulin space when calculating the respective PAs in PTZ-treated rat brain as well. The lowest spaces were measured for sucrose

and inulin in the white matter and caudate nucleus, and the highest value was found in olfactory bulbs. The spaces in remaining brain regions for sucrose ranged from 1.06–3.90%, 1.23–3.38% and 1.06–3.08% and for inulin ranged from 1.21–2.65%, 1.37–3.04% and 1.26–2.76% in 15-, 30- and 120-day-old rats, respectively. These measured values are in general agreement with literature values [15,22].

Figures 1A and B summarize the mean values of PAs for [^{14}C]sucrose and [^{3}H]inulin in brain regions of 15-, 30- and 120-day-old control and PTZ-injected rats. The average mean, 10 min [^{14}C]sucrose PAs in control were about 16.5, 14.2 and 12.3 $\times$ 10^{-6} s^{-1} in 15-, 30- and 120-day-old rats, respectively. The inulin PA was in fact indistinguishable from zero in each brain region of rats aged from 15 to 120 days.

The PA values for [^{14}C]sucrose and [^{3}H]inulin increased significantly ($P < 0.05$) in PTZ-treated rats. Although BBB dysfunction was found partly in similar areas in young and older rat brains the increase in BBB permeability in 120-day-old rats was significantly higher than permeability of corresponding regions in 30-day-old and still higher than in 15-day-old rat brains. The increase in PAs in most of the brain regions of 120-day-old rats was three- and two-fold higher than in those of 15- and 30-day-old rats, respectively. Similarly, in most of the brain regions of 30-day-old rats the PA values were markedly greater than in those of 15-day-old rats ($P < 0.05$).

In adults the increase in PAs was highest in the preoptic area, septum, olfactory bulb, inferior colliculus and hypothalamus while the increase was comparatively much lower in the same areas of young rat brains. In young rats, the increase in BBB permeability was surprisingly higher in the hippocampus and in contrast to older rats there was no marked alteration in permeability of the cerebrovascular endothelium in the corpus striatum and cerebral cortex.

DISCUSSION

In a number of studies, increase of arterial blood pressure that normally occurs during seizures has been suggested for the change in BBB permeability [11,16,19,24,26,28] when chemical convulsants such as PTZ or bicuculline, or electrical stimulation are used.

In these respects increase in systemic blood pressure was also observed in our study during seizures. In addition, we found the change in BBB permeability was not solely related to increases in blood pressure, because the blood pressure of most of the rats was back in the normal range at the time of injection of tracers or of sacrifice. Moreover, alteration of vascular permeability as a result of drug-induced acute hypertension was mainly found in posterior and occipital cortex and was never seen in deep brain areas [7,26]. As it can be seen in Figs. 1A and B the increased permeability of BBB was observed in most deep brain regions and distribution of increased permeability was different in young and adult rat brain. If the observed increased BBB permeability was only related to the blood pressure enhancing effect

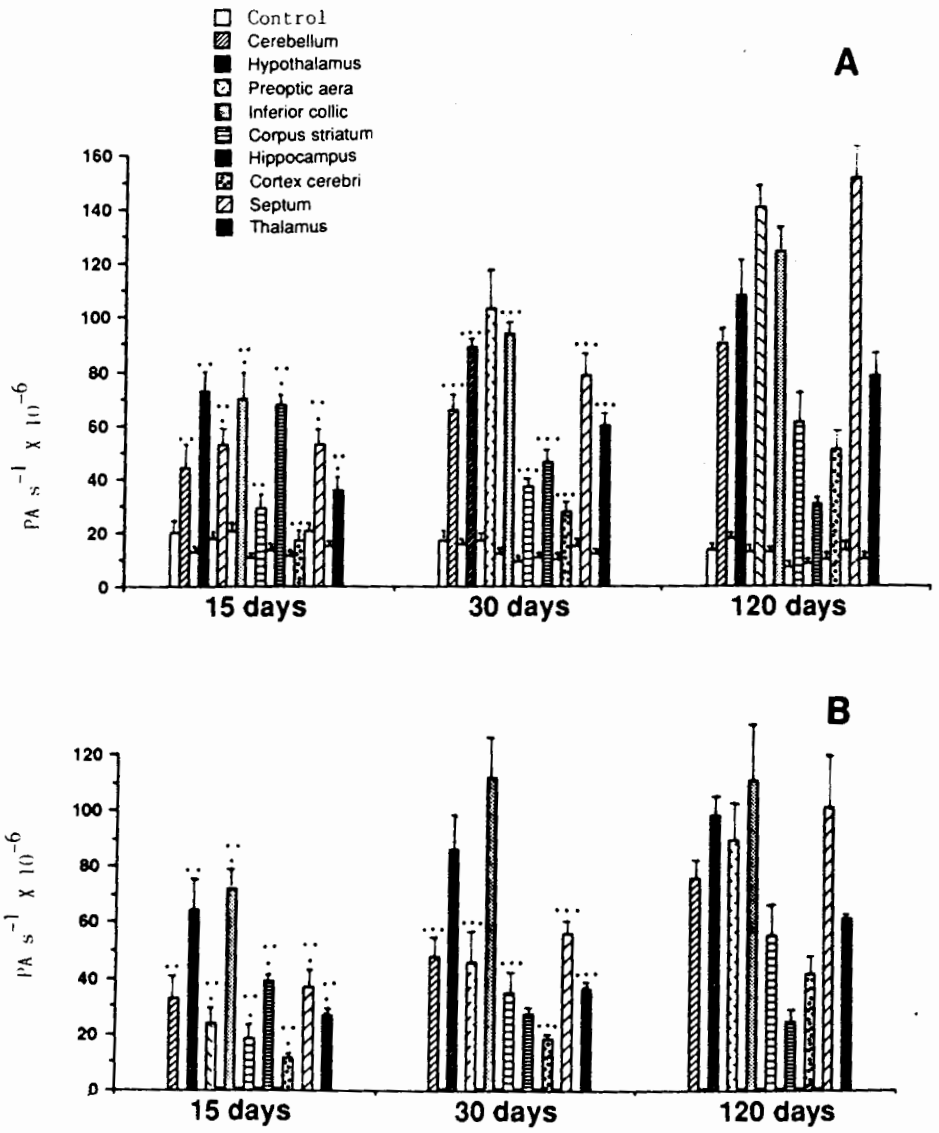

Fig. 1. Regional permeability-surface area products (ml g^{-1} s^{-1}) (PA) in selected brain regions for [^{14}C]sucrose (A) and [^{3}H]inulin (B) in control and PTZ-treated rats. Values are means ± S.E., n = 6–10 (n = 6 in control, n = 10 in PTZ-treated groups). The PA was calculated by Eqn. 1 from the integral of plasma concentration and C_{brain} (total minus intravascular radioactivity) when the animals were killed 10 min after i.v. injection of radiotracers. The radioactivity is measured in (dpm g^{-1}) of tissue and (dpm ml^{-1}) of plasma. PA for inulin was not measurable in any brain region of control rats. PTZ-induced seizures caused a significant and often dramatical increase in the PA for both sucrose and inulin in all brain regions studied. *Significant difference between values of 15- and 30-day-old rats. **Significant difference between values of 15- and 120-day-old rats. ***Significant difference between values of 30- and 120-day-old rats.

of PTZ, then increased BBB permeability should be found in similar areas which were observed in hypertension-induced BBB breakdown and also to a similar extent in corresponding regions of young and adult brain. Thus there seems to be no question that the increased blood pressure is not responsible for the regional variation in the vascular permeability of the young and adult rats observed in our experiments. This statement is applicable to our specific experiment only and we do not intend to make a general statement that changes in blood pressure do not influence regional cerebrovascular permeability.

The dependence of severity of BBB dysfunction and distribution pattern of increased permeability on age was also demonstrated in our experiments. The present knowledge of the mechanisms responsible for this age-dependent regional selective BBB breakdown in seizures induced by PTZ are still far from being clarified. In view of the earlier experiments [12,16,17,21] it seems plausible that morphological and physiological development of blood-brain barrier and certain postnatal changes in the central nervous system (CNS) are responsible for the variations in localization of BBB dysfunction in brain of rats of different age. However, it has been observed that different seizure patterns occur in response to drug-induced convulsions due to postnatal maturation of central synaptic pathways, development of blood-CNS barrier and postnatal biochemical maturation of CNS [21]. These certain chemical changes which occur in the growing rat may modify the convulsive response of animals to the convulsive drugs. It has been suggested that changes in enzyme activity, e.g., in activity of carbonic anhydrase which has low activity in the brain of newborn rat and cholinesterases which increase in activity with age, may interfere with the actions of convulsive drugs during the postnatal development of the CNS in young animals [5,15]. In addition it has recently been reported that mice become more sensitive to anticonvulsants as they become older, which again points to the importance of maturation of CNS correlated with seizure phenomena [17].

Therefore, it might be possible that PTZ stimulates the various undetermined neuromechanisms located at different levels of the central nervous system, and the neuromechanism can be activated on more areas as a function of age. Thus, it is not surprising to find the particularly extreme increase in BBB permeability in hippocampus and hypothalamus of young rats because cerebrovascular changes induced by epilepsy differ according to the structure involved [20]. It might be a reflexion of the active part played by these regions in the mechanism of epileptic seizures in young rats. Although the barrier mechanisms are also present in young brain [3,4,6,19], the mechanisms that respond to PTZ may not be fully developed.

Alternatively it might be possible that capillary permeability may differ between young and mature rats in the same region of the brain depending on their progressive maturation [3,6,23]. This maturation appears to be slow during the period from 8 to 16 days and rapid during the period from 16 to 21 days of postnatal life, thereafter the vessels exhibit the same pattern as in adult animals [17,23]. Since there is a lack of correlation between BBB dysfunction and the progressive maturation of BBB in

our seizure studies, developmental changes in capillary structure could not be an explanation for the seizure-induced alteration in BBB permeability.

In conclusion, the PTZ-induced cerebrovascular permeability increases differ markedly in localization in young and adult rats. The age-dependent regional distribution pattern of changes in permeability is not dependent on over-all variation in blood pressure but rather on local phenomena in neuronal activity occurring during the seizures. In certain brain areas the BBB seems to be more vulnerable to PTZ indicating that the sensitivity of the adult animals to the BBB mechanisms may depend on progressive maturation of capillaries and changes in their internal structure.

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