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Chapter 42

Unchanged blood-brain barrier permeability to GABA in experimental hepatic encephalopathy

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Summary

Blood-brain barrier permeability to the inhibitory neurotransmitter γ-aminobutyric acid (GABA) was studied in galactosamine-treated rats and in control rats by means of an arterial integral uptake technique.

Permeability to GABA was unaltered in brain cortex (frontal, parietal and occipital regions) as well as in mesencephalon and cerebellum in galactosamine rats as compared to controls. The finding that peripherally administered GABA does not penetrate the blood-brain barrier to any higher extent in hepatic encephalopathy provides evidence against one of the elements the GABA hypothesis.

Zusammenfassung

Knudsen und Poulsen (S 28-U): Keine Permeabilitätsänderung der Blut-Hirn-Schranke für GABA bei experimenteller hepatischer Enzephalopathie. An Ratten wurde ohne und mit Zufuhr von Galaktosamin eine Methode, die Aufnahmequoten aus Arterien integriert, eingesetzt, um die Durchlässigkeit der Blut-Hirn-Schranke für den hemmenden Neurotransmitter gamma-Aminobuttersäure (GABA) zu untersuchen. Bezüglich der Permeabilität für GABA resultierten zwischen den Galaktosamin-behandelten Ratten und den Kontrolltieren in frotalen, parietalen und okzipitalen Arealen der Hirnrinde wie auch im Mittelhirn und Kleinhirn keine Unterschiede. Der Befund, daß peripher zugeführte GABA bei hepatischer Enze-

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phalopathie die Blut-Hirn-Schranke keineswegs in erhöhtem Maße passiert, widerspricht zumindest einem Teil der GABA-Hypothese.

I. Introduction

In spite of extensive research into the pathogenesis of hepatic encephalopathy (HE), a basic understanding has not been reached. As recently reviewed by Hoyumpa [1], the GABA theory has, among other hypotheses, been at the centre of interest for a considerable time. Originally proposed by Schafer and Jones [2] and partly supported in later studies [3, 4], the neuroinhibitory substance GABA might, due to increased serum levels, increased blood-brain barrier (BBB) permeability and an increased number of GABA binding sites in the brain, be responsible for the cerebral disturbances. It is, however, generally accepted that the BBA is almost completely impermeable for GABA [5 – 7] and marked disturbances in BBB permeability even in early stages of HE must then be postulated. The rapid peripheral and central degradation of GABA by GABA-transaminase has until now precluded the use of radiolabelled GABA for the study of BBB transport in HE. In the present study, GABA degradation is inhibited by gammavinyl-GABA and by surgical occlusion of the hepatic artery and the portal vein.

The purpose of the present study was to investigate whether GABA permeates the BBB to a higher degree in galactosamine-induced HE than in normal rats.

II. Materials and methods

Age- and weight-matched male Wistar rats were randomly allocated into two groups: liver-insufficient rats and control rats. Twenty-six hours before the experiment liver damage was induced by injection of galactosamine (Sigma) into a tail vein at a dosage of 1 g/kg (0.5 g/ml). The control animals received the same volume of saline. The degree of liver insufficiency was estimated by antipyrine clearance [8]. In vivo metabolism of ^{3}H-labelled GABA during the experiment was inhibited by injection of γ-vinyl GABA (Merrel Dow) i.p. 11.5 h prior to investigation. Two minutes prior to the tracer experiment described below, hepatic metabolism of GABA was eliminated by ligation of the hepatic artery and the portal vein.

Permeability-surfae area (PS) in different brain regions was determined by means of an arterial integral uptake technique described earlier [9, 10]. Two μCi of ^{3}H-labelled GABA (Amersham) was given as a bolus injection followed by infusion of 8 μCi [^{3}H]GABA.

For determination of the cerebral vascular space 0.5 mCi of ^{113m}In (Amersham) was dissolved in 1 ml of rat plasma and injected 3 min before decapitation. ^{113m}In binds to plasma transferrin, which does not leave the intravascular space.

The rats were anaesthetized with halothane and tracheotomized. Catheters were inserted into both femoral arteries and veins and relaxation was achieved with suxamethonium. Arterial blood pressure was registered continuously. The tracer circulation time was 30 min, after which the rats were decapitated. The brain was removed

and placed on an ice-cooled table and tissue samples (10 – 130 mg) were taken from the cortex of frontal, parietal and occipital regions and from the midbrain and cerebellum.

III. Results

Twenty-six hours following galactosamine injection, the rats showed clear clinical signs of encephalopathy, such as drowsiness, ataxia and, in most instances, absent grasping reflexes. In the given dose, galactosamine was lethal between 48 and 72 h after i.v. injection. The mean reduction in antipyrine clearance compared to control rats was 41%.

Blood-brain barrier permeability to GABA in terms of PS products was in most brain regions lower in galactosamine-treated rats as compared to control rats (Table 1). No significant differences between rats with HE and control rats in the examined brain regions were found.

IV. Discussion

In the present study, the BBB permeability to GABA is unchanged in galactosamine encephalopathy. An increase in permeability with a concomitant decrease in the surface area (e.g., closing of the cerebral capillaries) in galactosamine encephalopathy would lead to unchanged PS products as compared to controls. This appears less likely and would not change GABA brain influx per weight unit. If systemic GABA causes the cerebral changes in HE, an increased GABA permeability should occur even before the neurological symptoms apear, which was the reason for examining the rats early, i.e. 26 h, after galactosamine administration.

In experimental studies using the indicator dilution method in dogs with biliary cirrhosis or porta-caval anastomosis, no alteration in the transport of GABA was demonstrable [11]. The possibility cannot be excluded, however, that this less sensitive method for measuring brain uptake of slowly permeating substances could

TABLE 1 Blood-brain barrier permeability of GABA in galactosamine-treated rats (GAL, $n=7$) and in control rats (CON, $n=9$)

	GAL	CON
Frontal cortex	2.12 ± 0.44	2.32 ± 0.49
Parietal cortex	2.44 ± 0.47	2.60 ± 0.49
Occipital cortex	2.32 ± 0.33	2.62 ± 0.42
Mesencephalon	2.32 ± 0.60	2.22 ± 0.37
Cerebellum	3.13 ± 0.91	2.68 ± 0.47

Values are individual permeability surface area products ± SD, the unit being 10^{-5} $cm^3 \cdot g^{-1} \cdot s^{-1}$.

conceal small changes in the extraction. In other experimental studies, no changes in brain GABA content were detected [12–14]. Increased brain uptake indexes (BUI) for inulin, D-sucrose and L-glucose have been reported in galactosamine animals [15, 16] and in hepatectomized rats [17]. In another study, increased BUIs for inulin and L-glucose could not be demonstrated in rats with a porto-caval anastomosis [18]. The BUI method is, however, not ideal for the evaluation of the BBB passage of low-permeable substances such as sucrose and inulin. If the measured BUI is not corrected for intravascular tracer content, it will predominantly be influenced by changes in cerebral blood flow and volume and consequently BBB permeability changes could be obscured. Furthermore, the BUI method does not take into account factors such as different water permeability, reflux and recirculation.

Quantitative autoradiographic studies in galactosamine-treated rabbits have revealed increased permeability for AIB (which is a non-metabolizable isomer of GABA) in grey matter preceding neurological symptoms [19]. AIB is usually considered to compete for the same transport carrier as other small neutral amino acids and the transport might also be influenced by changes in plasma levels of NH_3^+ or large neutral amino acids, since a cooperation between the two neutral amino acid carrier transport systems exists. The increased BBB permeability found in this study might thus be directly related to amino acid transport, and not a sign of generally disturbed permeability of the BBB.

In the present study, the first part of the GABA hypothesis, i.e. that increased peripheral GABA levels lead to increased brain influx, is not confirmed. However, the possibility that GABA somehow plays a role in the development of HE cannot be excluded. In that case, alterations in receptor density [3] or in the activity of GABA-catabolic enzymes [20] seem to be more likely explanations.

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