

# Blood-brain barrier permeability in galactosamine-induced hepatic encephalopathy

## No evidence for increased GABA-transport

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### Summary

Blood-brain barrier permeability to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), to sucrose and to sodium was studied in rats with galactosamine-induced liver damage and hepatic encephalopathy by means of an arterial integral uptake technique. Permeability to GABA was unaltered in all examined brain regions ( $2.47 \pm 0.25 \cdot 10^{-5} \text{ cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$ , mean  $\pm$  S.D.) as compared to control rats ( $2.49 \pm 0.19 \cdot 10^{-5} \text{ cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$ ). The permeability to sucrose (galactosamine  $0.25 \pm 0.02$  vs. controls  $0.24 \pm 0.02 \cdot 10^{-5} \text{ cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$ ) and to sodium (galactosamine  $5.33 \pm 0.04$  vs. controls  $5.40 \pm 0.05 \cdot 10^{-5} \text{ cm}^3 \cdot \text{s}^{-1} \cdot \text{g}^{-1}$ ) was also unchanged in hepatic encephalopathy. At the time of investigation mean liver function measured by antipyrine clearance was reduced from 0.39 in control rats to 0.23 ml/min/100 g body wt. in galactosamine-treated animals. The present study does not support the suggestion that peripheral GABA penetrates the blood-brain barrier to any higher extent in hepatic encephalopathy. This provides evidence against at least part of the GABA-hypothesis. Furthermore, an unspecified increased blood-brain barrier permeability in hepatic encephalopathy, as measured by sucrose and sodium uptake, was not found. It is concluded that the GABA-theory requires further careful reevaluation.

### Introduction

For a considerable time the GABA-theory has been one of the most widely accepted explanations for the pathogenesis of hepatic encephalopathy (HE) [1]. The theory was originally proposed by Schafer

and Jones [2] and has been partly supported in later studies [3,4]. According to the theory, the neuroinhibitory substance GABA, due to increased serum levels, increased blood-brain barrier (BBB) permeability and an increased number of GABA binding sites in the brain, is responsible for the cerebral disturbances

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in HE. It is, however, generally accepted that GABA is almost completely BBB-impermeable [5-7], and significant disturbances in BBB permeability, even in early stages of HE, must then be postulated.

Low transfer across the BBB is probably partly explained by the low lipid solubility of GABA, and partly due to inactivation by its primary catabolic enzyme 2-oxoglutarate aminotransferase (GABA-T) [8] present in high concentrations in cerebral endothelial cells [9]. Until now, the rapid ubiquitous metabolism of GABA by the GABA-transaminase has precluded the use of radiolabeled GABA for the study of BBB transport in HE. In the present study, GABA metabolism was abolished by  $\gamma$ -vinyl-GABA inhibition and by occlusion of the hepatic artery and the portal vein.

The primary purpose of the present study was to investigate whether GABA permeates the BBB to a higher degree in galactosamine liver insufficiency, so that increased GABA levels in the brain could cause hepatic encephalopathy. Furthermore, we examined whether a general increase in BBB permeability is present in galactosamine liver insufficiency.

## Materials and Methods

Age- and weight (250-350 g)-matched male Wistar rats were randomly allocated into two groups: liver-insufficient rats and control rats. The criteria of the institution for the care and use of laboratory animals were fulfilled in this study.

The hepatotoxin galactosamine hydrochloride was chosen to induce HE [10,11], since most studies supporting the GABA-theory have involved its use [3,4,12]. Twenty-six hours before the experiment liver damage was induced by injection into a tail vein of galactosamine (Sigma, St. Louis, MO) in a dosage of 1 g/kg (0.5 g/ml saline). The control animals received an identical volume of saline. Prior to the experiments the animals had a neurological examination and presence of encephalopathy was noted. The degree of liver insufficiency was estimated by antipyrine clearance [13], p-bilirubin and p-SGPT. Metabo-

lism of isotopically labeled GABA during the experiment was inhibited by injection of 1.25 g/kg body wt.  $\gamma$ -vinyl-GABA (Merrel Dow, Strasbourg, France) i.p. 12 h prior to investigation.

Permeability-surface area (PS) in different brain regions was determined by means of an arterial integral uptake technique [14,15].

### Animal preparation

The rats were anaesthetized with halothane and tracheotomized. Arterial blood gas concentrations were measured during the experiments with conventional microelectrodes (Radiometer, Copenhagen) and respiration was maintained at normocapnia ( $\text{CO}_2$  tension: 36-40 mm Hg). Catheters were inserted into both femoral arteries and veins and the animals were relaxed with suxamethonium. Arterial blood pressure was recorded continuously. Body temperature was maintained constant at  $37.0 \pm 0.5$  °C by means of a thermostat-controlled heat lamp. Two minutes prior to the tracer experiment the abdominal wall was opened and the hepatic artery and the portal vein were ligated in both groups. After the operation, 100 IE heparin was injected i.v.

### Arterial integral technique for determination of PS

A saline solution containing  $10 \mu\text{Ci}$  [ $^3\text{H}$ ]GABA (50 Ci/mmol, Amersham, U.K.),  $5 \mu\text{Ci}$  [ $^{14}\text{C}$ ]sucrose (10 mCi/mmol, Amersham) and  $100 \mu\text{Ci}$   $^{24}\text{Na}$  (1 mCi/ml, Risø, Roskilde, Denmark) per ml was given into a femoral vein as a 0.2 ml bolus followed by a continuous infusion at a rate of 0.027 ml/min. The arterial plasma concentration-time curve integral was calculated as  $C_{\text{pi}} \cdot T$ .

$C_{\text{pi}}$  was determined on one plasma sample obtained by continuous collection of blood at a constant rate of 0.027 ml/min into a 1-ml syringe mounted to an electrical syringe pump. In order to correct for intravascular tracer content, the cerebral vascular space was determined by injection of 0.5 mCi of  $^{113\text{m}}\text{In}$  (Amersham, U.K.) dissolved in 1 ml of rat plasma injected 3 min before decapitation.  $^{113\text{m}}\text{In}$  binds to plasma transferrin, which does not leave the intravascular space. At the end of the experimental period, 30 min after the start and immediately before

decapitation, a final blood sample was taken for determination of the blood tracer concentration ( $C_i$ ).

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.

#### Tissue preparation

The tracer content in weighed tissue samples (10–130 mg) and in the blood samples (40  $\mu$ l of plasma from the arterial integral blood sample, 20  $\mu$ l of the final blood sample) were counted by gamma-spectrometry and corrected for decay and spillover. Tissues were dissolved in 1 ml of Soluene 350<sup>®</sup> (Packard, Downers Grove, IL), decolorized with hydrogen peroxide (35%) and neutralized with hydrochloric acid before addition of the scintillation fluid (Optifluor<sup>®</sup>, Packard). After at least 10 days  $^{24}\text{Na}^+$  ( $t_{0.5} = 12.4$  h) and  $^{113\text{m}}\text{In}$  ( $t_{0.5} = 1.5$  h) had decayed to insignificant activity, making it possible to count beta emission which was corrected for spillover and quenching (Tricarb 4530, Packard).

#### Calculations and statistics

The amount of tracer taken up by the brain following the intravenous injection is determined by the differential equation

$$\frac{dC_b}{dt} = \text{PS} \cdot (C_p - C_b/V_e) \quad (1)$$

where  $C_p$  = plasma tracer concentration (cpm/ml),  $C_b$  = brain tracer concentration (cpm/mg), PS = per-

TABLE 1  
LIVER TESTS

Values are given as means and ranges.

	HE	Controls
Antipyrine clearance (ml/min/100 g body wt.)	0.231 (0.090–0.290)	0.391 (0.290–0.50)
Bilirubin ( $\mu\text{mol/l}$ )	32 (22–40)	4 (1–13)
SGPT (U/l)	8780 (2450–15 000)	15 (<5–39)

meability surface area product and  $V_e$  = tracer distribution volume in the brain.  $C_b/V_e$  is negligible as compared to  $C_p$  because of the relatively short circulation time and because of the maintained tracer plasma levels obtained by the constant infusion.

By integrating Eqn. 1 and introducing the correction factor for intravascular tracer amount, the following expression is obtained:

$$\text{PS} = \frac{C_b(t) - C_i(t)}{C_p(t) dt} \quad (2)$$

where  $C_i$  = intravascular tracer content and  $t$  = experimental time (30 min).

$C_p$  signifies the mean cerebral capillary tracer concentration which depends upon the arterial tracer concentration, the cerebral blood flow, and upon the brain extraction. However, for substances such as GABA, sucrose and sodium with a very low brain extraction, the mean capillary concentration can for all purposes be considered equal to the arterial concen-

TABLE 2  
BLOOD-BRAIN BARRIER PERMEABILITY OF GABA, SUCROSE AND SODIUM IN GALACTOSAMINE-TREATED RATS (GAL) AND IN CONTROL RATS (CON)

Values are individual permeability surface area products  $\pm$  S.D., the unit being  $10^{-5} \text{ cm}^2 \cdot \text{g}^{-1} \cdot \text{s}^{-1}$ .

	GABA		Sucrose		Sodium	
	GAL, $n = 7$	CON, $n = 9$	GAL, $n = 7$	CON, $n = 9$	GAL, $n = 7$	CON, $n = 9$
Frontal cortex	2.12 $\pm$ 0.44	2.32 $\pm$ 0.49	0.24 $\pm$ 0.06	0.29 $\pm$ 0.05	4.34 $\pm$ 0.56	3.83 $\pm$ 0.34
Parietal cortex	2.44 $\pm$ 0.47	2.60 $\pm$ 0.49	0.16 $\pm$ 0.08	0.17 $\pm$ 0.03	5.20 $\pm$ 0.85	5.37 $\pm$ 0.80
Occipital cortex	2.32 $\pm$ 0.33	2.62 $\pm$ 0.42	0.32 $\pm$ 0.04	0.29 $\pm$ 0.04	6.34 $\pm$ 0.99	6.43 $\pm$ 1.18
Mesencephalon	2.32 $\pm$ 0.60	2.22 $\pm$ 0.37	0.29 $\pm$ 0.05	0.19 $\pm$ 0.05	4.54 $\pm$ 0.77	5.83 $\pm$ 1.25
Cerebellum	3.13 $\pm$ 0.91	2.68 $\pm$ 0.47	0.24 $\pm$ 0.07	0.23 $\pm$ 0.08	5.20 $\pm$ 0.98	5.53 $\pm$ 1.50

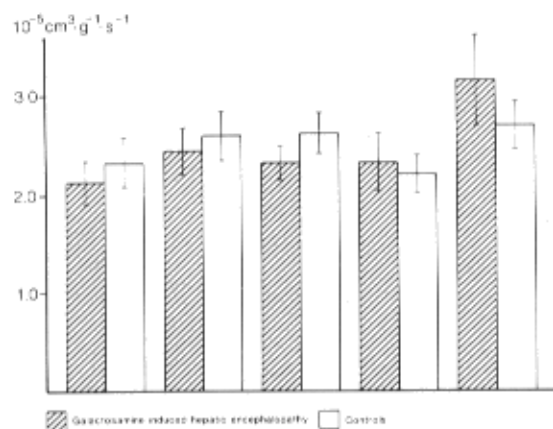


Fig. 1. PS products for GABA in the frontal, parietal and occipital cortices and for mesencephalon and cerebellum in HE and in controls.

tration. Changes in the cerebral blood flow within physiological limits are thus without any significance [16]. Values of PS were compared with the Student *t*-test using a 5% limit of significance.

## Results

Twenty-six hours following galactosamine injection, the rats showed manifest clinical signs of encephalopathy (drowsiness, ataxia) and had, in most

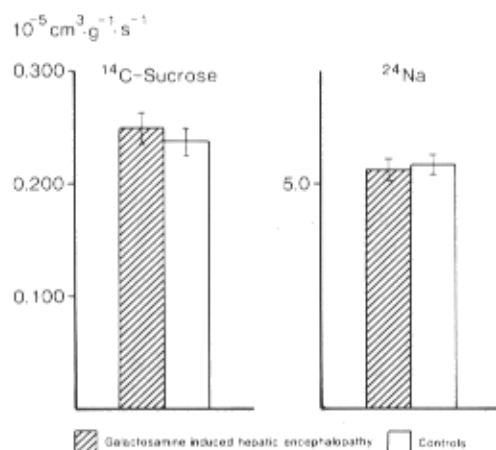


Fig. 2. Average PS products for sucrose and sodium in HE and in controls.

instances, absent grasping reflexes. Mean body weight was 337 g in the control group and 313 g in the galactosamine-treated group. The results of the liver tests are shown in Table 1; antipyrine clearance in average decreased by 41%, bilirubin increased to 8-times normal values and SGPT values were nearly 600-times increased. Fig. 1 shows PS products for GABA for each of the examined brain regions; in no regions were there any statistically significant differences between HE and control rats. Average PS products for sucrose and sodium in the two groups were also not statistically significantly different (Fig. 2). PS values for all three tracers of each brain region are listed in Table 2.

## Discussion

In the present study BBB permeability to GABA was unchanged in galactosamine-induced encephalopathy. An unchanged permeability coefficient and unchanged capillary surface area as well as an increase in permeability with a concomitant decreased surface area (e.g., closing of cerebral capillaries) would lead to unchanged PS products. The latter possibility appears less likely and would, in any instance, not change the fact that brain GABA influx per weight unit is the same in the two groups. The pretreatment with  $\gamma$ -vinyl-GABA irreversibly inhibits the action of GABA-transaminase [17] and avoids metabolism of GABA during the tracer experiment. This treatment is probably without significance in the evaluation of the permeability, since treatment with various other less specific compounds known to inhibit endothelial GABA-T does not change permeation of radiolabeled GABA into the brain [18].

Our results on the BBB permeability in galactosamine-induced HE are in agreement with the recent work of Lo et al. [19] showing no alterations in the BBB permeability for sucrose and methylaminoisobutyric acid, also during later stages of galactosamine-induced hepatic failure. The unchanged BBB permeability to  $^{24}\text{Na}^+$  and to sucrose ( $M_r$  314) in HE in the present study is in keeping with studies in portacaval anastomosis-operated rats with HE who had

unchanged permeability for  $^{22}\text{Na}^+$  and  $^{51}\text{Cr-EDTA}$  ( $M_r$  341) using up to 30 min circulation time of the tracers [20]. In experimental studies using the indicator dilution method in dogs with biliary cirrhosis or portacaval anastomosis, no alterations in the transport of GABA or sucrose was demonstrable [21]. Very marked increases in the BBB permeability should, however, occur before any changes would be detectable in the measurement of the transcapillary escape during one single passage. In other experimental studies, no changes in brain GABA content have been detectable [22–24].

The obtained results are in conflict with other studies on the general permeability of the BBB in HE. For determination of BBB permeability in experimental studies, the brain uptake index (BUI) technique [25] has most commonly been used. Increased BUI values for inulin, D-sucrose and L-glucose have been reported in animals with galactosamine-induced HE [26,27] and in hepatectomized rats [28]. Increased BUI values for inulin and L-glucose were, however, not observed in rats with portacaval anastomosis [29]. Due to the short circulation time, the BUI method is unsuitable for the evaluation of the BBB passage of low-permeability substances such as sucrose and inulin, since the transcapillary escape of tracer will be negligible during the capillary passage. If the measured BUI is not corrected for intravascular tracer content, it will predominantly reflect changes in cerebral blood volume and therefore be insensitive to changes in BBB permeability. Quantitative autoradiographic studies have, preceding the

neurological symptoms in galactosamine-treated rabbits, shown increased permeability for aminoisobutyrate (which is a non-metabolizable isomer of GABA) in gray matter [12]. Aminoisobutyrate is considered to compete for the same transport carrier as other small neutral amino acids so the transport might be influenced by changes in other small or large neutral amino acids, since a cooperation between the two neutral amino acid carrier transport systems exists [30]. The increased BBB permeability found in that particular study might thus be directly related to the amino acid transport, and hence not a sign of generally disturbed 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, it cannot be excluded that GABA somehow plays a role in the development of HE. If so, cerebral alterations in receptor density [3] or in the activity of GABA-catabolic enzymes [24] seem to be more likely explanations. We conclude that the GABA theory for the explanation of pathogenesis in HE requires careful reevaluation.

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