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BRAIN PROTEIN SYNTHESIS IN RATS WITH PORTACAVAL SHUNTS

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Effects of portacaval shunts on the brain protein synthesis were studied in rats 7, 10 and 13 weeks after the surgery. We evaluated the incorporation of $[^3H]$-glycine with the usage of liquid scintillation. In comparison to the controls, the labelled amino acid was incorporated less effectively in all the examined brain regions throughout the experimental period. The lowest values were observed 10 weeks after the portacaval shunt and the drop was most distinct in the cerebellum (47%). The least profound changes occurred within 7 weeks after the surgery and the results were statistically significant only in the frontal cortex and the brain stem. Thirteen weeks after the shunting the incorporation of $[^3H]$-glycine was moderately decreased (the drop ranged between 12% and 18%) but all the differences were statistically significant. It seems that the inhibition of the brain protein synthesis may contribute to the pathogenesis of the portal-systemic encephalopathy.

K e y   w o r d s: *protein synthesis, portacaval shunt, hepatic encephalopathy.*

INTRODUCTION

After a portacaval shunt most portal blood can bypass the liver and reach directly the inferior vena cava. Such a condition may also be due to portal hypertension — as it was observed in many cases of cirrhosis of the liver. Portacaval shunting can be performed to avoid lethal complications of the portal hypertension including bleeding from oesophageal varices. Recently, such operations have also proved useful in the treatment of metabolic disorders such as homozygous familial hypercholesterolemia (1, 2) or the diseases in which glycogene storing occurs (3, 4).

Apart from the apparent advantages connected with a therapeutic portacaval shunt, some undesirable effects may arise. These include disturbances in the liver and central circulation, morphological and biochemical changes within the liver and other organs and given due time the developments of the chronic hepatic encephalopathy.

Although several hypotheses have been presented (5—9) the pathogenesis of the chronic hepatic encephalopathy still remains unclear. Ammonia is proved to be the main pathogenic agent of hepatic encephalopathy connected with subacute and chronic liver diseases (10,11). What's more, pathologic changes of the brain noted in hepatic encephalopathy may be interpreted as ammonia-induced gliopathy (12—14). The ammonia effect on CNS is said to cause such disordes of protein synthesis in the brain which may be one of the factors affecting development and course of hepatic encephalopathy. Although effects of the portacaval shunts on the brain protein synthesis have already been studied the results are not unequivocal. In this study, we report on the protein biosynthesis in brain of rats 7, 10 and 13 weeks after the portacaval shunting.

MATERIALS AND METHODS

Adult Wistar rats weighing between 220 and 250 g were used in the experiment. During the experimental period the animals were kept under the conditions of temperature 21—23°C, relative humidity 60—70% and the day-night regime 12/12 h, with standard chow and water *ad libitum*. After a week long adaptation, the total of 24 rats were divided into 4 groups of 6 individuals each. The animals in three experimental groups were subjected to ether anaesthesia and portacaval shunting was performed by end to side anastomosis after Lee and Fisher (15). Novafil 10/0 continuous sutures produced by Davis and Geck were used. The controls were ether-anasthetized and sham-operated with laparotomy, isolation of the portal vein from the surrounding tissues and its microclamping for 15 minutes being performed. The period approximated the average time required for the portacaval shunting in the experimentals.

Protein synthesis in brain was evaluated 7, 10 and 13 weeks after the surgery. The experimentals and the controls received a single intraperitoneal injection of labelled [^{3}H]-glycine (with specific activity 140 mCi/mM, produced by Amersham) in a dose of 0,25 μCi/g.b.w. Four hours after the exposure the animals were killed by decapitation and left to drain of blood. From skinned and fascia removed heads, cerebra were isolated and placed on glass plates in ice bath (the salt -ice mixture in 1:1 ratio).

With the meninges and blood vessels removed, cerebra were divided into the frontal cortex, the corpus striatum, the thalamus with hypothalamus, the cerebellum and the brain stem. Thus obtained sections were weighted, placed in scitillation plastic vials (Zinnsser Analytic, Cat. No. 3071401) and treated with 1 ml tissue solubilizator Soluene-350 (Packard, Cat. No. 6003038). The samples were incubated at 40°C and, with the tissues completely dissolved, immersed in 10 ml scintillation fluid Dimilume-30 (Packard, Cat. No. 6003037). The samples were counted 3 times in 1 minute in a scintillometer, Intertechnique SL-30. The results were expressed as the number of disintegrations per minute (dpm) for 100 mg tissue. Tables and figures were provided. Statistically significant differences were calculated according to Student's t-test with $p < 0,05$.

RESULTS

In comparison to the controls, [^{3}H]-glycine was incorporated at a slower rate in all the examined brain regions 7, 10 and 13 weeks after the portacaval shunt. The radioactivity was least decreased 7 weeks after the shunting; only in
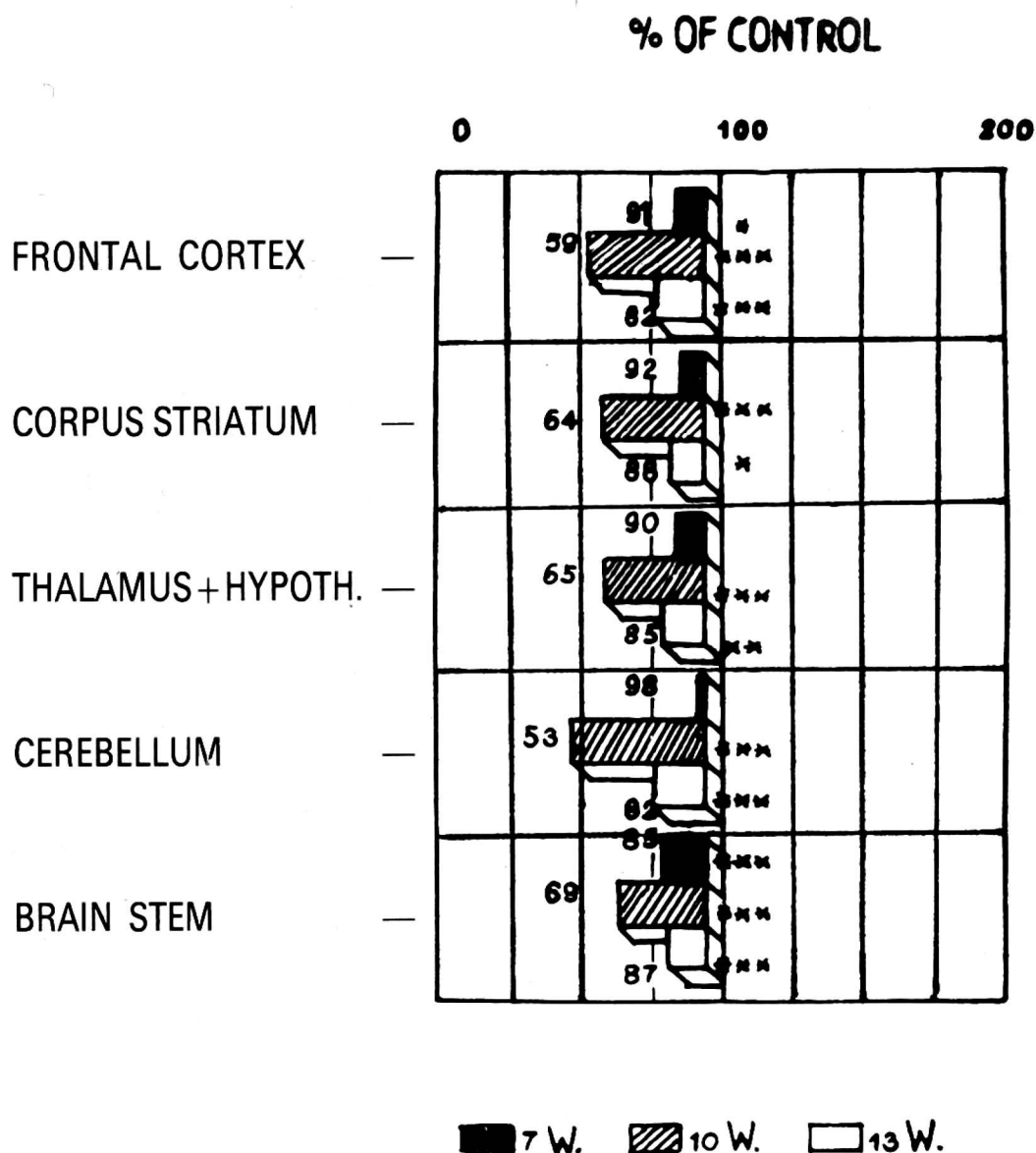
Fig. 1. [³H]-glycine incorporation in brains of rats 7, 10 and 13 weeks after the portacaval shunt expressed as the percentage of the control values (the controls taken as 100%). 7 W, 10 W, 13 W — the experimental groups 7, 10 and 13 weeks after the portacaval shunt. The asterikses indicate statistically significant results: *p < 0,05, **p < 0,01 and ***p < 0,001.

the frontal cortex and the brain stem the results were statistically significant (p < 0,05 and p < 0,001, respectively) (*Fig. 1.*) In relation to the controls, the most drastic reduction in [³H]-glycine incorporation occured in the brains of rats 10 weeks after the surgery (*Fig. 1*); it was ewident in all the examined brain regions being most severe in the cerebellum and least manifested in the brain stem (47% and 31% respectively). All the differences were statistically significant in this experimental group (p < 0,001). Finally, 13 weeks after the shunting, [³H]-glycine incorporation was moderately diminished when compared to that in the controls (by 12% to 18%). However, all the differences remained statistically significant (*Fig. 1*). The labelled glycine incorporation rates were compared in the experimentals 7 and 10 weeks after the portacaval shunting. In the latter group the values of the radioactivity were lower in all the examined brain regions and all the differences were statistically significant
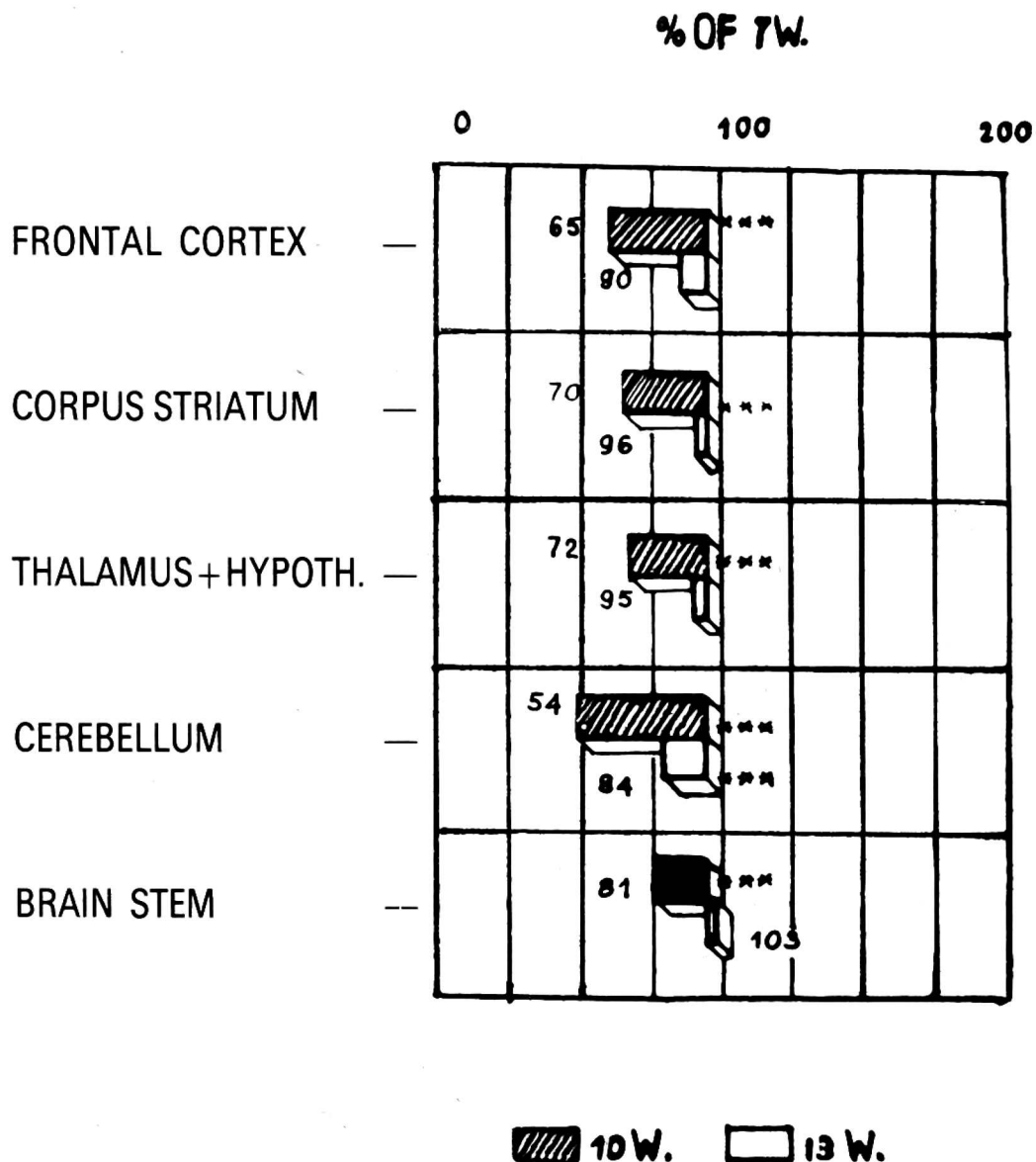
Fig. 2. [³H]-glycine incorporation in brains of rats 10 and 13 weeks after the portacaval shunt expressed as the percentage of the values for the rats 7 weeks after the shunt (the value for this experimental group taken as 100%). Abbrevations and asterixes as in *Fig. 1.*

(*Fig. 2*). When, in turn, the comparison was drawn between the experimental groups containing the rats 10 and 13 weeks after the surgery, elevated levels of the labelled glycine were found in the latter group. The values ranged from 55% to 27% in the cerebellum and the brain stem, respectively (*Fig. 3*).

DISCUSSION

We assume that the incorporation of [³H]-glycine into the brain proteins indicates the level of the brain protein synthesis. Accordingly, on the basis of our results, we can conclude that portacaval shunts inhibit the protein biosynthesis in all the examined brain regions throughout the experimental period. The dynamics of the observed processes sugested that 10 weeks after
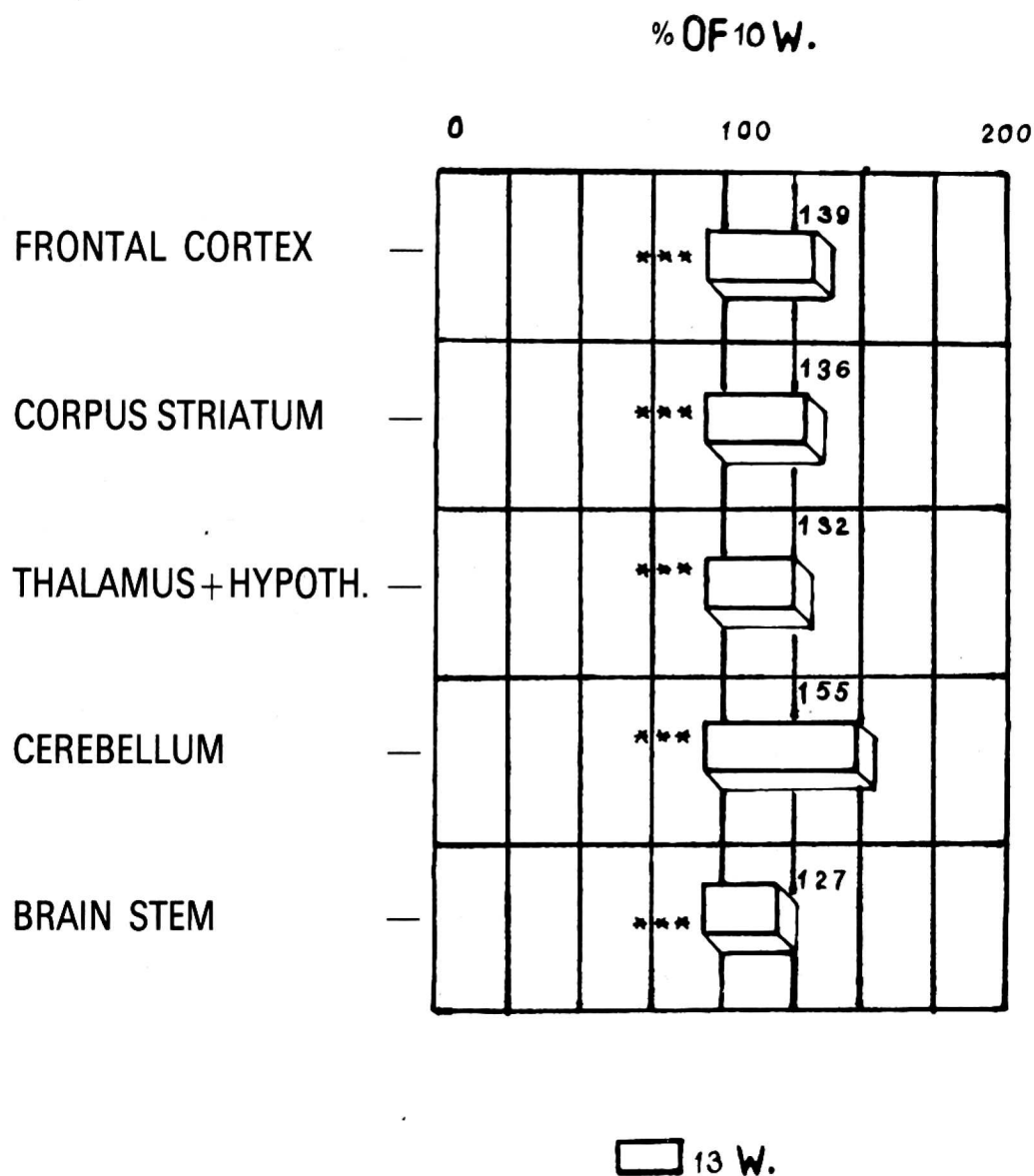
% OF 10 W.

Fig. 3. [³H]-glycine incorporation in brains of rats 13 weeks after the portacaval shunt expressed as the percentage of the values for the rats 10 weeks after the shunt (the value for this experimental group taken as 100%). Other abbrevations and asterixes as in *Fig. 1.*

Table 1. [³H]-glycine incorporation in brains of rats after the portacaval shunt

Region	The controls	The experimentals — period after the shunt		
		7 weeks	10 weeks	13 weeks
Frontal cortex	4268 ± 168	3888 ± 317	2513 ± 81	3499 ± 311
Corpus striatum	5275 ± 530	4834 ± 242	3402 ± 161	4620 ± 154
Thalamus and hypothalamus	4451 ± 414	4006 ± 202	2875 ± 232	3805 ± 174
Cerebellum	4210 ± 42	4131 ± 177	2245 ± 100	3469 ± 40
Brain stem	4349 ± 124	3704 ± 112	2992 ± 23	3797 ± 105

The values are expressed in disintegrations for 100 mg of the tissue in 1 min. (mean values ± SEM). The control and 3 experimental groups consisted of 6 animals each.

the surgery the suppression is most severe. However, having examined only three experimental periods, we can not exclude the possibility that there is a certain point at which the values of [^{3}H]-glycine incorporation are actually lower.

Slower rates of protein biosynthesis were also observed by Wasterlain et al., who reported the 50% decrease 8 weeks after the portacaval shunting in rats (16).

In our experiments the lowest values of [^{3}H]-glycine incorporation were observed within the tenth week after the shunting but the reduction was never as high as 50% in any of the examined brain regions. Our results are in contradiction with the findings of Cremer et al. (17) and those of Dunlop et al. (18), who did not detect any changes in the protein biosynthesis in brain after the portacaval shunts.

Our report on the suppresion of the brain protein synthesis after the portacaval shunting agrees well with that presented by Hindfelt et al., who observed the decreased over — all protein content and albumine levels in brains of rats 3,8 and 16 weeks after the portacaval shunt (19) and Brun et al. who described lower levels of proteins in brains of the patients who died of hepatic encephalopathy (20). The underlying mechanism are not fully understood so far but they may include also the inhibition of the brain protein synthesis. As the water content does not change in brain after the portacaval shunting (21), the cerebral oedema is the least likely cause of disturbances in the protein metabolism in brain.

On the basis of our results it is impossible to determine whether the inhibition of the protein synthesis takes place mainly in the glia or in the nervous cells. If astrocytes are the main site, the protein biosynthesis must cease completely there because the glial cells are relatively less engaged in the over-all brain protein synthesis. On the other hand most of the pathologic changes of the brain noticed in experimental models of hepatic encephalopathy occur in astrocytes. They are manifested in: astrocytic proliferation, Alzheimer cells II, abnormal astrocytic nuclei, hyperplasia and hypertrophy of astrocytes (12, 14, 22, 23).

However, when we compare our results with Albrecht's report on the increased incorporation of the radioactive protein precursors into the astrocytes in brains subjected to the experimental hepatic encephalopathy (24), we may assume that the suppression of the labelled glycine incorporation in our experiments involved mainly the nervous cells.

Neither can we define, on the basis of our results, what biochemical mechanism are responsible for in the inhibition of the protein synthesis in brain. The pertinent literature brings reports on the possible effects of the ammonia (11, 16), tryptophan (25) and many other factors. The role of ammonia in the pathogenesis of many disordes seen in hepatic encephalopathy

seems to be particularly noteworthy. There are known to be several mechanisms interpreting neurotoxic ammonia action. They include: modification of blood-brain barrier transport, alterations of cerebral energy metabolism, direct actions on the neuronal membrane and decreased synthesis of releasable glutamate resulting in impaired glutamatergic neurotransmission (10). Moreover, it has been reported that the portacaval shunting may result in decreased rates of permeability, especially that of basic amino acids, through the blood-brain barrier. (26, 27). This may also suppress the processes of the protein biosynthesis in brain.

When our results are correlated with a close relation between the hyperammonia and the inhibition of the protein synthesis in brain (16) and with the fact, that the levels of proteins in brain are diminished during encephalopapthy (20), it is possible to suggest that the inhibition of the brain protein synthesis may contribute to the pathogenesis of the portal-systemic encephalopathy.

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