CHANGES IN THE SUBCOMMISSURAL ORGAN OF RATS ON A DIET FREE FROM OR CONTAINING AN EXCESS OF SODIUM

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The subcommissural organ of rats exhibits high glutamate dehydrogenase (GDH), glycerol-3-phosphate dehydrogenase (G-3-PDH), $NADH_2$ dehydrogenase ($NADH_2$-DH) and acid phosphatase (AP) activity, very low succinate dehydrogenase (SDH) and glycerophosphate dehydrogenase activity, and a high glycogen content. Absence of sodium in the diet of the experimental animals led to an increase in GDH, G-3-PDH, $NADH_2$-DH, and AP activity and to a decrease in the glycogen content. Changes of the opposite order were observed in animals receiving an excess of sodium in the diet.

The subcommissural organ (SCO), which is composed of specific ependyma, is now distinguished as part of the single circumventricular system of the brain and it has recently been investigated [18]. Neither the function of the SCO in mammals nor its metabolic features have yet been adequately explained. The organ is known to have a high glycogen content [17] and to exhibit activity of several enzymes: acid and alkaline phosphatases, nonspecific esterase [11, 19], glusoce-6-phosphate dehydrogenase, lactate dehydrogenase, and $NADH_2$- and $NADPH_2$-dehydrogenases [12]. The suggestion has been made that metabolism of the SCO in amphibians is specifically connected with the secretion of the mucopolysaccharide-protein complex [4], whose secretion into the cerebrospinal fluid has been connected [6-8, 15] with fluctuations in the water and mineral balance of the body.

It was therefore decided to make a histochemical investigation of the SCO in rats at times of extreme variation in its hypothetical function, i.e., in experimentally induced hyponatremia and hypernatremia, and also to seek specific tests to shed light on the metabolism of the SCO and to objectively reflect changes in its functional state.

EXPERIMENTAL METHOD

The experimental animals were 72 noninbred male albino rats weighing 150-170 g, divided into three groups. The animals of group 1 (Control), were kept on a synthetic diet in accordance with Eigenstein's formula [5]. They were given tap water to drink. The animals of group 2 were kept on the same diet but they drank 2.5% sodium chloride solution. Salt was completely excluded from the synthetic diet of the animals of group 3, and they drank distilled water. Animals were sacrificed on the 1st, 3rd, 14th, 21st and 28th days after the beginning of the experiment. The subcommissural organs taken from two animals of each group (six pieces altogether) were mounted in a single block with solid carbon dioxide. Several series of sagittal sections were cut from this block, and seven histochemical tests were carried out on each of them: for glutamate dehydrogenase (GDH), glycerol-3-phosphate dehydrogenase (G-3-PDH) [16], succinate dehydrogenase (SDH), glycerophosphate dehydrogenase (GPDH) [13], $NADH_2$ dehydrogenase ($NADH_2$-DH), acid phosphatase (AP) [2], and glycogen (the PAS reaction). The histochemical reactions in the sections were assessed visually. The level of activity of these enzymes varied along the antero-posterior axis of the organ (see Fig. 1). For this reason, the assessment and comparative study of histochemical

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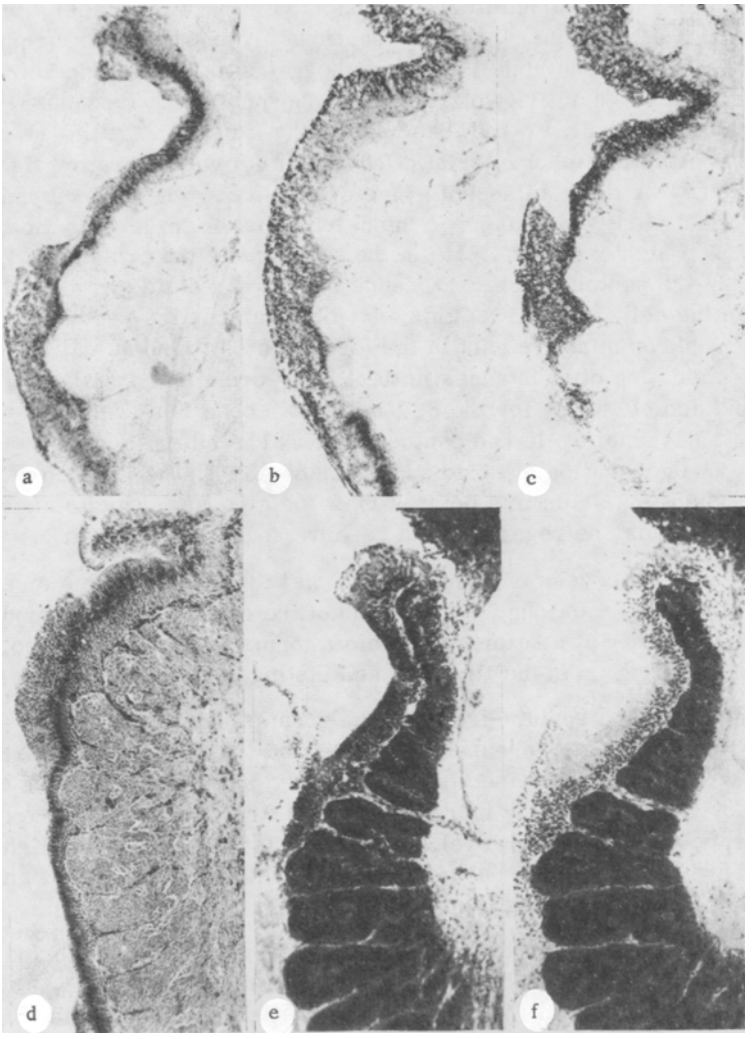

Fig. 1. Subcommissural organ of an intact rat: a) high activity of glutamate dehydrogenase; b) of glycerol-3-phosphate dehydrogenase; c) of $NADH_2$ dehydrogenase; d) of acid phosphatase; e) high glycogen content; f) control for glycogen after treatment with amylase, 150 ×.

reactions in frontal sections of the SCO are extremely difficult and may lead to erroneous conclusions. To obtain an adequate idea of the effect of various conditions on the intensity of the histochemical test, it is better to use sagittal sections through the organ.

EXPERIMENTAL RESULTS

The SCO of the control rats showed high GDH, G-3-PDH, $NADH_2$-DH, and AP activity and a high content of glycogen (Fig. 1). Activity of SDH and GPDH in the SCO of the control rats was very low and was unchanged when the animals were in a state of hypo- and hypernatremia. High G-3-PDH activity suggests that intensive glycolysis and synthesis of lipids take place in the SCO cells. The basis for this assumption is the direction of the reaction catalyzed by this enzyme: reduction of the di-hydroxyacetone phosphate formed in the Embden–Meyerhof cycle into α-glycerophosphate, a structural component of lipids. According to Diederen [4] intensive glycolysis can be the source of fructose-6-phosphate, a precursor of the mucopolysaccharide component of the secretion of the SCO cells; by addition of an NH_2-group from glutamine fructose-6-phosphate takes part in the synthesis of hexosamines. The high $NADH_2$-DH activity does not clash with the view that there is a high intensity of glycolysis in the SCO cells. This enzyme is located not only in the mitochondria but also in the hyaloplasm of the cells. High $NADH_2$-DH

activity in the SCO cells can be assumed to be connected with the high intensity of the oxidation-reduction reactions taking place in the hyaloplasm. These reactions are not necessarily connected with the respiratory chain of the mitochondria. The high AP activity suggests high synthetic activity in the SCO cells. Other evidence of this is the high activity of glucose-6-phosphate dehydrogenase, which participates in the initial stages of the glucose oxidation cycle, reported in the literature [12]. The high intensity of glucose oxidation in the pentose cycle is a characteristic feature of actively secreting tissues in general [3]. During oxidation by this route $NADPH_2$, an essential factor in fatty synthesis,is formed. The fatty acids are incorporated into phospholipids, which undergo intensive metabolism in secreting tissues [9]. Precursors of amino acids and RNA, which can participate in the synthesis of the protein component of the CRO cell secretion, are formed in the pentose cycle. Evidence of active protein synthesis is given by the discovery of high GDH activity in the cells of the SCO: this enzyme catalyzes the synthesis of glutamic acid which, through transamination, plays an active part in the formation of amino acids. The low SDH and GPDH activities suggest that oxidation of substances in the Krebs' cycle and oxidation of lipids are not of primary importance in the provision of energy for the SCL cells. Glucose is evidently metabolized in this organ mainly by the Embden–Meyerhof cycle and pentose cycle. The latter is not concerned with the production of energy in the cell. The generation of large quantities of ATP in the Embden– Meyerhof cycle requires a high consumption of glucose. From this point of view the glycogen, which is normally found in large quantities in the SCO cells, may be regarded as a reserve source of glucose.

In animals consuming an excess of sodium in the diet for three weeks a small decrease in GDH, G-3-PDH, and $NADH_2$-DH activity was found. By the end of the experiment the normal activity of these enzymes was restored. AP activity at all times was below normal. The glycogen content in the SCO was considerably increased starting from the first day and lasting until the end of the experiment.

In the animals consuming a sodium-free diet the greatest changes affected AP activity and the glycogen content. The AP activity was increased after 24 h and it remained high throughout the experiment. The glycogen content was slightly increased on the first day, and during the next 27 days it fell sharply below normal. GDH activity was slightly increased for the first 7 days of the experiment, while on the 14th day it was slightly below the control level, and on the 21st and 28th days slightly above normal. G-3-PDH activity was above normal for 28 days. $NADH_2$-DH activity showed no significant change at the beginning of the experiment, and starting from the 14th day it was above normal.

These results showing changes in the activity of the various enzymes during hypo- and hypernatremia demonstrate that the absence of sodium in the diet consumed by the animals was accompanied by stimulation, while excessive consumption of sodium was accompanied by slight inhibition of the SCO. It is striking that in the experimental animals the most marked changes were found in AP activity and in the glycogen content. AP catalyzes phosphorylation processes which are essential not only in synthesis, but also for the transport of substances through membranes. The possibility cannot be ruled out that in this case the level of AP activity may reflect processes connected with the synthesis and liberation of secretion. This hypothesis is supported by results obtained by Markina [1] who showed that absence of sodium in the diet of rats is accompanied by an increase in the production of secretion and its liberation from SCO cells, whereas an excess of sodium leads to accumulation of secretion.

In the present experiments a decrease in the glycogen content was constantly observed in the period of stimulation of function (sodium-free diet), and an increase in its content was observed during inhibition of function. These results apparently confirm the earlier hypothesis that glycogen can be stored in the SCO as a source of glucose. When the demand for glucose rises quickly (sodium-free diet) the reserves of accumulated glycogen are utilized, and conversely, during inhibition of function glycogen again accumulates in the organ.

It can be concluded from these experiments that histochemical tests for AP and glycogen can provide very useful information concerning the functional activity of the SCO.

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