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EFFECT OF EXPERIMENTAL UREMIA AND INJECTION OF EXOGENOUS PARATHYROID HORMONE ON AXOSPINOUS SYNAPSES IN HIPPOCAMPAL AREA CA_3

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The encephalopathy which patients with chronic renal failure (CRF) develop is due to a combination of pathogenetic factors which exert a toxic influence on the nervous system. A leading role in this situation is ascribed to parathyroid hormone (PTH), although the mechanism of its toxic action is not yet clear [11]. Elevation of the blood PTH level is accompanied by an increase in the calcium concentration in the brain, its accumulation being greatest in the neocortex and hypothalamus [10], and characteristic changes also are found in the EEG [11]. Experiments have shown that PTH is present in the cerebrospinal fluid, where its concentration is equal to one-third of that in the blood serum [6].

The aim of this investigation was to study ultrastructural morphometric parameters of axospinous synapses in area CA_3 of the hippocampus (HC), analysis of which enables changes in the efficiency of the axospinous synapses in this part of the brain to be characterized in response to injection of exogenous PTH and during the development of experimental uremia, when the conditions are created for accumulation of endogenous PTH in the blood [10]. The reason why area CA_3 of HC was chosen was not only the absence of experimental data enabling the efficiency of synapses in this part of the brain to be assessed in uremia, but also the ability of area CA_3 of HC to exert modulating influences on activity of hypothalamic nuclei involved in the regulation of water and electrolyte metabolism [2].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200 g. To create experimental uremia a model of subtotal nephrectomy was used [12]; the development of uremia was monitored by biochemical tests of the blood and urine, using standardized techniques. Exogenous PTH was administered by intraperitoneal injections in a dose of 50

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TABLE 1. Changes in Ultrastructural Morphometric Parameters of Axospinous Synapses in Area CA_3 of HC in Rats at Different Stages of Development of Experimental Uremia and under the Influence of PTH and EGTA

Time after subtotal nephrectomy, procedure	Morphometric parameter						
	1	2	3	4	5	6	7
2 weeks	NC	NC	I	NC	D	I	NC
1 month	D	D	NC	NC	NC	NC	NC
2 months	D	D	NC	D	D	I	NC
3 months	D	D	NC	D	D	NC	NC
Intraperitoneal injection of PTH	I	D	NC	D	D	I	NC
EGTA applied to surviving brain slices*	D	I	NC	D	I	—	I

Legend. 1) LS, 2) DH, 3) DN, 4) LAZ, 5) PSC, 6) DSA, 7) SA-PSM, D) decrease and I) increase at $p \leq 0.005$, NC) no change. *) By method [5].

U/kg for 14 days. The electron-microscopic study of axospinous synapses was conducted on pieces of brain tissue from area CA_3 of HC. Fixation of the material, embedding, and subsequent statistical analysis of the results did not differ in principle from methods used to study the effect of EGTA on surviving slices of the albino mouse sensomotor cortex [5]. Morphometric parameters of the axospinous synapses, reflecting their synaptic activity, were studied by the use of electron micrographs of axospinous synapses in area CA_3 of HC, obtained under a magnification of 30,000× [4, 7-9]. The greatest lengths of the spines (LS), the greatest diameters of the heads of the spines (DH), the least diameters of the necks (DN), the lengths of the active zones (LAZ), the thickness of the postsynaptic condensation (PSC), the greatest total diameters of the cisterns of the spinous apparatuses (DSA), and the least distances from the cisterns of the spinous apparatuses to the postsynaptic membranes (SA-PSM). On the basis of the measurements histograms of distribution of the various parameters were plotted. Histograms of distribution were subjected to statistical analysis relative to the control experiments by the Kolmogorov method of statistical comparison for small samples [3]. The number of spines analyzed for each position of the experiment and control was not less than 60.

EXPERIMENTAL RESULTS

Changes in the morphometric parameters of axospinous synapses in area CA_3 of HC compared with the results of the control experiments are illustrated by the composite Table, drawn up for each experimental position analyzed.

After intraperitoneal injection of PTH there were significant changes in LS, DH, LAZ, PSC, and DSA (Table 1). Since the mean dimensions of LS and DH in the control corresponded to the most effective parameters for synaptic activation, changes in their values under the influence of PTH may indicate a decrease as a result of a change in the cable properties of the spines, even when DN was unchanged [3, 7]. A similar suggestion also is possible in the case of a decrease in PSC. Conversely, compaction of LAZ and enlargement of DSA are regarded as the result of depolarization of synapses during activation [8, 9]. Superposition of the electrical effects during corresponding ultrastructural reactions evidently not only reveals their complex character, but likewise does not rule out the possibility of a preferential reduction of synaptic activity as a result of significant changes in LX, DH, and PSC. Reduction of LAZ, taking place simultaneously with a decrease in PSC, likewise is not an independent parameter of synaptic activation [1, 7, 8]. Lowering of synaptic activity also is confirmed by qualitative ultrastructural changes: grouping of synaptic vesicles in the presynapse, a decrease in PSC (Fig. 1). Similar changes have been described at the qualitative level in the sensomotor cortex also after injection of PTH [1]. A significant increase in DSA can be interpreted as evidence of the release of Ca^{2+} from cisterns of the spinous apparatus [9]. The experimental data ob-

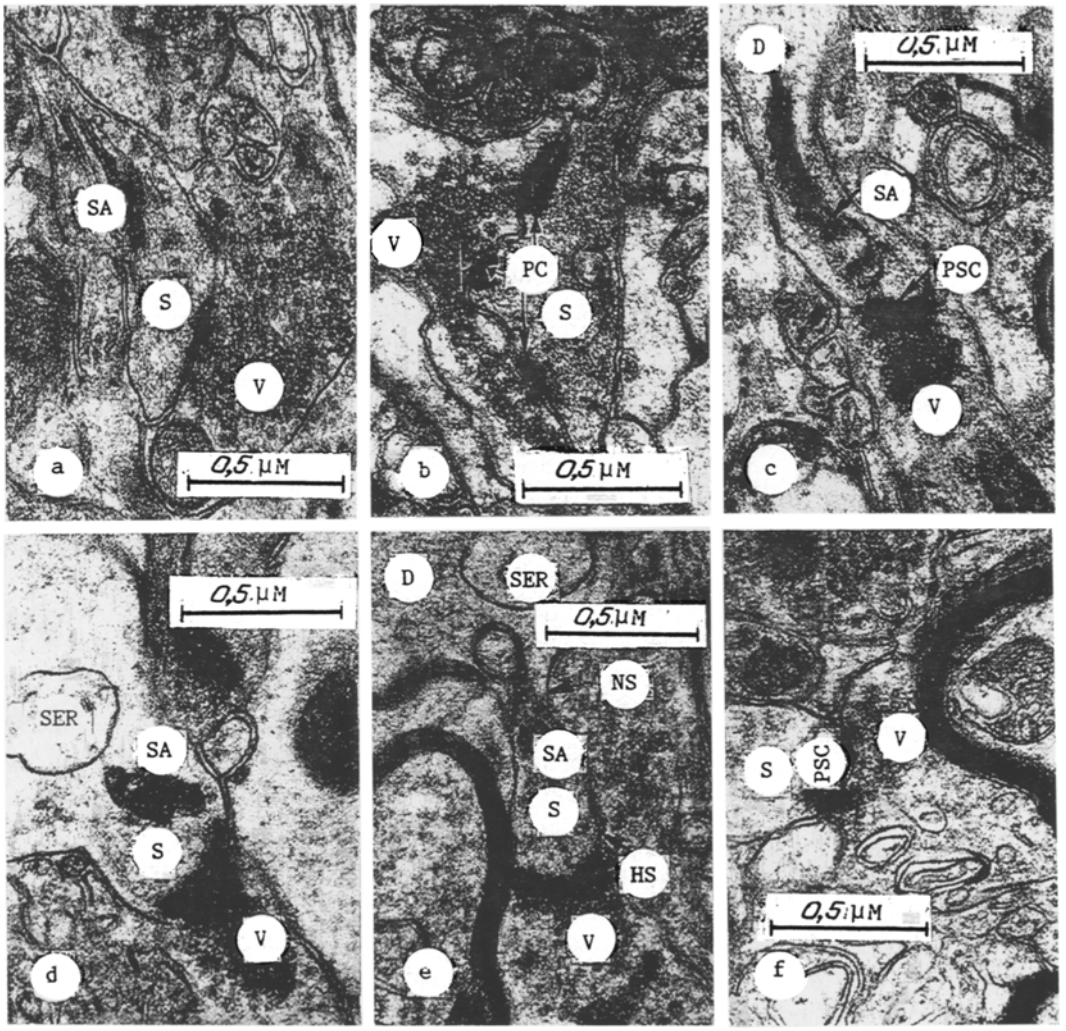

Fig. 1. Ultrastructural organization of axospinous synapses in area CA_3 of hippocampus under standard experimental conditions (a) and 2 weeks (b), 2 months (c), and 3 months (d) after beginning of development of experimental uremia, and also following intraperitoneal injection of exogenous PTH (e) and application of EGTA to surviving slices of the mouse sensomotor cortex (f): photomicrographs from materials of a previous study [5]. D) Dendrite, S) spine, V) vesicles, PSC) postsynaptic condensation, PC) perforated contact, SA) spinous apparatus, SER) cisterns of smooth endoplasmic reticulum, NS) neck of spine, HS) head of spine.

tained under the influence of PTH are particularly interesting when compared with results obtained under the influence of EGTA which, as we know, binds Ca^{2+} into a chelated complex [5]. The trend of most analogous morphometric parameters of the axospinous synapses showed opposite tendencies under the influence of PTH and EGTA, and, which attracts particular attention, the effects of administration of exogenous PTH and the effects of experimental uremia 2 and 3 months after subtotal nephrectomy did not differ greatly in principle (Table 1; Fig. 1). On this basis it can be suggested that both under the influence of exogenous PTH and in experimental uremia calcium accumulates in the spinous synapses of area CA_3 of HC. Moreover, the spinous apparatus is evidently a labile ultrastructure in these processes [4, 5].

Comparisons of morphometric parameters of synapses in HC with the degree of uremia also tend to support the predominant role of PTH in the development of structural changes in the synapses under the particular experimental conditions specified. For instance, in the group of animals tested 2 months after the operation, in which the character of the changes in the synapses agreed largely with that observed after injection of exogenous PTH, the parameters of nitrogen metabolism were within normal limits (the urea level did not exceed 5.9 ± 0.35 mM), whereas

the blood PTH concentration was significantly higher than physiologically normal values (the PTH level reached 192.0 pg/liter, compared with the normal level of 50-90 pg/liter). Conversely, the parameters of nitrogen metabolism in the group of animals 1 month after nephrectomy corresponded to a moderate degree of uremia (blood urea 18.2 ± 1.7 mM), and the PTH concentration corresponded to the upper limit of normal (90 pg/liter). Essentially, at this stage most of the morphometric parameters were unchanged, evidence that no marked Ca^{2+} accumulation had taken place.

In the earlier stages (2 weeks after the operation) changes in the morphometric parameters relative to normal were significant, but did not show any distinct trend (Table 1). The state of the morphometric parameters 1 month after nephrectomy, as the nearest to the normal state, can be interpreted as being connected with the presence of compensatory processes in the CNS at the hippocampal level.

Our results thus confirm a possible decrease in synaptic efficiency of the axospinous synapses in area CA_3 of HC both during the action of PTH injected intraperitoneally, and during experimental uremia as a result of a significant change in morphometric parameters reflecting the cable properties of the spine and activity of their synaptic membranes [4, 5, 7-9]. The decrease in efficiency was due to accumulation of Ca^{2+} in the cytoplasm of the spine and it probably correlated with the blood PTH level. It can be tentatively suggested that one mechanism of calcium accumulation is by the redistribution of Ca^{2+} between the spinous apparatus and the cytoplasm of the spine.

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