normal conditions or in AA. This state of affairs raises doubts about the data given by some investigators on the membrane-stabilizing properties of vitamin E [13]. Meanwhile these results, which demonstrate that vitamin E has the properties of a true antiarthritic agent (capable of inhibiting generalization of the pathologic process), can with full justification be linked with the antioxidant properties of TPA. It will be evident that LPO processes play a far more important role in the pathogenesis of rheumatoid arthritis and its experimental model (AA) than has hitherto been considered. Considering the abundant data in the literature on the role of LPO in other forms of pathological processes [3, 4], it can be tentatively suggested that the intensification of LPO, leading to injury to cell membrane structures, is an initial factor in the development of various pathological forms.

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EFFECT OF ACTH ON RATE OF ^{32}P-ORTHOPHOSPHATE UPTAKE INTO SYNAPTOSOMAL PHOSPHOINOSITIDES OF THE ISCHEMIC RAT BRAIN

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The close attention paid by many investigators to phosphoinositides (PI) is due both to the high metabolic activity of these phospholipids and their sensitivity to changes in the functional state of the body [2], and also to the important role which they play in synaptic transmission [5, 10]. There is much evidence of the action of certain neuromodulators and hormones in PI metabolism in nerve tissue, and also on the possible regulatory role of these phospholipids in phosphoprotein phosphorylation under the influence of ACTH [7, 8]. PI metabolism in nerve tissue in pathology has been studied mainly at the tissue level. It has been shown, for example, that cerebral ischemia in rats caused marked changes in metabolism of

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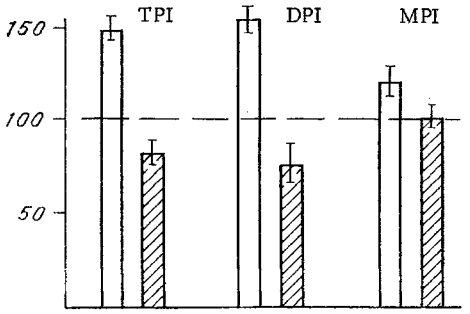

Fig. 1. Effect of ACTH on rate of uptake of ^{32}P-orthophosphate (in %) into synaptosomal PI of normal and ischemic rat brain. Here and in Figs. 2 and 3: unshaded columns — PI of normal brain; shaded — of ischemic brain.

poly-PI (PPI) [3], one result of which may be the development of irreversible changes in nerve tissue membranes, which lie at the basis of postischemic brain pathology. To study processes taking place on membranes, synaptosomes can serve as a convenient model, for during isolation they largely preserve both their structure and their ability to respond by a change in metabolism to electrical stimulation and to depolarizing influence, such as an increase in the K^+ concentration in the incubation medium [1, 4].

The aim of this investigation was to study PI metabolism in rat brain synaptosomes under normal conditions, under the influence of ACTH, and during depolarization of the synaptosomes, and also to compare it with the metabolic response of synaptosomes obtained from the ischemic rat brain.

EXPERIMENTAL METHOD

The brain together with the brain stem (without the cerebellum) of male Wistar albino rats weighing 180-220 g was used. Synaptosomes were obtained by low-speed gradient centrifugation of the total mitochondrial fraction [6]. The synaptosomes were preincubated for 30 min at 32°C in an iso-osmotic medium of the following composition: NaCl, 124 mM; KCl, 5 mM; $MgCl_2$, 10 mM; glucose 10 mM; EDTA 3 mM; Tris-HCl (pH 7.4) 26 mM, containing 1.85 MBq of ^{32}P-orthophosphate. ACTH (final concentration 25 μM) was added in a volume of 0.15 ml. Stimulation lasted 1 min. The reaction was stopped by adding a mixture of chloroform, methanol, and concentrated HCl (200:100:1) in a volume equal to 10 times that of the incubation medium. PI were extracted by mixing for 30 min and washed with 1 N HCl. The film formed on the phase boundary between chloroform and water-methanol was taken for determination of its protein content. The chloroform extract containing PI was evaporated in a current of nitrogen, dissolved in a mixture of chloroform, methanol, and water (75:25:2), and applied to formol-treated paper and chromatographs. Spots corresponding to individual PI were cut out and placed in a scintillation counter for recording their radioactivity. After mineralization of the paper the inorganic phosphate (P_i) content was determined. The rate of incorporation of ^{32}P-orthophosphate was expressed as the ratio of specific radioactivity of phosphorus of PI (in cpm/mg P_i) to the protein content. Depolarization of the synaptosomes was produced by addition of KCl to the incubation medium for 1 min up to a final concentration of 80 mM. Cerebral ischemia was induced by the application of ligatures to the common carotid arteries for 90 min.

EXPERIMENTAL RESULTS

PI, especially di- and tri-PI (DPI and TPI, respectively) of the brain have a high turnover rate of phosphate groups. During incubation of synaptosomes for 30 min with labeled phosphate, most of the radioactivity found in the phospholipids was accounted for by PPI: about 40% in TPI, 45% in DPI, and 5% in phosphatidylinositol (MPI). Under the influence of ACTH, incorporation of labeled phosphates into all PI fractions of synaptosomes obtained from normal animals was stimulated by 40-45% (Fig. 1). No such stimulation under the influence of ACTH was found in synaptosomes obtained from the ischemic rat brain. On the contrary, not only was the rate of incorporation of ^{32}P-orthophosphate into TPI not increased by ACTH, as under normal conditions, but it was reduced by 20%. In DPI and MPI it did not differ significantly from the rate of phosphate uptake without stimulation. These are data in the literature on changes in PPI metabolism in the membrane fraction containing protein kinase B-50 under the influence of ACTH. The authors cited consider that ACTH has a modulating action on activity of protein kinase B-50 in DPI-kinase activity, which is dependent on it, and on the process of stimulation of TPI metabolism bound with it [7, 8]. Disturbance of the mechanism of stimulation of turnover of the phosphate group of PI under the influence of ACTH in synaptosomes of the ischemic brain may be connected with disturbances at the level of phosphoprotein phosphorylation, due possibly to a decrease in the ATP [11] and cAMP [9] concen-

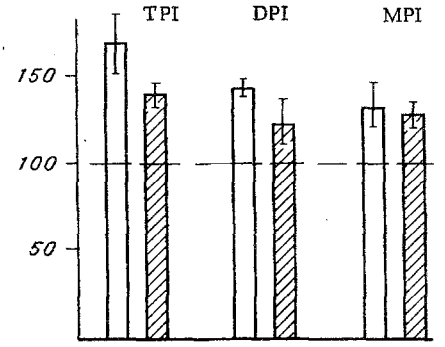

Fig. 2

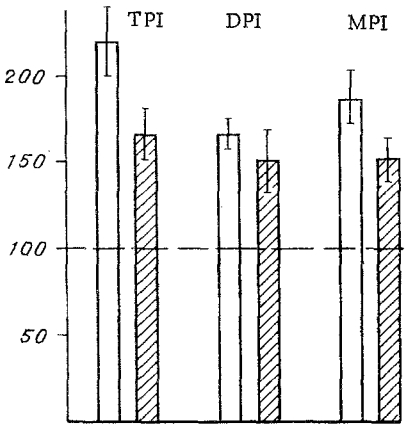

Fig. 3

Fig. 2. Effect of depolarization (increase in K^+ concentration in incubation medium) on rate of incorporation of labeled phosphate into PI (in % of control).

Fig. 3. Combined effect of depolarization and ACTH on rate of incorporation of labeled phosphate into PI (in % of control).

tions at this period of ischemia. A decrease in the ATP concentration also took place during depolarization, which leads to activation of protein phosphorylation with the aid of cAMP-dependent protein kinase [1].

In this connection it was interesting to study PI metabolism during depolarization of synaptosomes caused by the addition of K^+ to the incubation medium. It was shown (Fig. 2) that an increase in the K^+ concentration in the medium stimulates the rate of incorporation of labeled orthophosphate into all PI of synaptosomes of normal rats by 30-70%. Stimulation of incorporation of labeled phosphate into PI also was found in the synaptosomes of the ischemic rat, although by a lesser degree than in normal animals. Data on the combined action of depolarization and ACTH are shown in Fig. 3. Increased incorporation of labeled phosphate into PI in response to stimulation by ACTH was found in synaptosomes of normal animals against the background of depolarization; summation of these two influences may have occurred, and was most marked fo TPI and MPI. Against the background of depolarization, the effect of stimulation of the PI metabolism by the action of ACTH, observed in the synaptosomes of normal animals and disturbed during ischemia, was restored in the synaptosomes of ischemic rats.

During ischemia the modulating effect of ACTH on PI metabolism of the synaptosomes is thus disturbed. Under the influence of complex changes in the synaptosomal membranes during depolarization the stimulating effect of ACTH on the rate of incorporation of labeled orthophosphate into PI is restored. It may be that the absence of a stimulating effect of ACTH on PI metabolism in synaptosomes of the ischemic brain and subsequent restoration of this effect during depolarization of the synaptosomes are not simply the result of a reduced concentration of the energetically important phosphate (ATP), but constitute a more complex chain of events, maintaining the high reliability of the receptor mechanism in pathology.

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