EFFECT OF FACTORS CAUSING EXCITATION AND INHIBITION ON POLYPHOSPHOINOSITIDE METABOLISM OF THE RAT LIVER

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The rate of incorporation of radioactive phosphate into the polyphosphoinositide (PPI) fraction is about 10 times greater than into other phospholipids (PL) of the normal rat liver. Rapid postmortem changes in the PPI content in the liver were found. During exposure to the various factors used, the only PL fraction in which changes were found was the PPI fraction. Changes in the content and also in the intensity of metabolism of PPI in the liver were similar in direction during exposure to these factors to those in the brain. The PPI fractions are the most labile PL fractions in the liver.

KEY WORDS: polyphosphoinositides; phospholipids; rat liver; excitation and inhibition of the nervous system.

Until recently it was considered that di- and triphosphoinositides (DPI and TPI), combined under the general term of "polyphosphoinositides" (PPI), occur only in nerve tissue. However, other animal tissues also are known to contain PPI, although in much smaller amounts [7, 8, 13]. Practically all data on the unique properties of PPI have been obtained by the study of nerve tissue. The PPI fraction of nerve tissue has been shown to have a high metabolic rate of its phosphate groups [10, 14], rapid postmortem breakdown [7], and changes in their content in vivo during exposure of the organism to certain factors [4-6] and in vitro under the influence of metabolic inhibitors [2]. Information about PPI metabolism obtained in other than nerve tissues is extremely scanty. All that is known is that PPI in all tissues studied have a high metabolic rate of their phosphate groups [12].

The object of this investigation was to study the metabolism of the PPI fractions in the rat liver in vivo during exposure to physiological and pharmacological agents: excitatory (electrical stimulation of the skin, amphetamine) and inhibitory (hypoxia, hypoglycemia, thiopental). Other acid phospholipids (PL) were studied for comparison – phosphatidylserine (PS), monophosphoinositides (MPI), and also the total PL fraction including all PL present in liver tissue.

EXPERIMENTAL METHOD

Adult male Wistar albino rats were used. Electrical stimulation of the skin was applied to rats kept in individual cages by connecting a voltage of 25-40 V to metal rods in their walls for 30 sec every 5 min. Amphetamine was injected subcutaneously in a dose of 15 mg/kg. As a result of these procedures the animals were brought into a state of marked excitation and they became aggressive. A state of hypoxic hypoxia was induced in the rats by placing them in a gas-flow chamber through which a mixture of 5% O_2 +95% N_2 was passed. Insulin hypoglycemia was induced in the animals by injecting insulin subcutaneously in a dose of 80 units/kg. A state of narcotic sleep was induced by injecting thiopental sodium subcutaneously in a dose of 150 mg/kg. As a result of these last three procedures the animals were inhibited, especially those in deep thiopental sleep.

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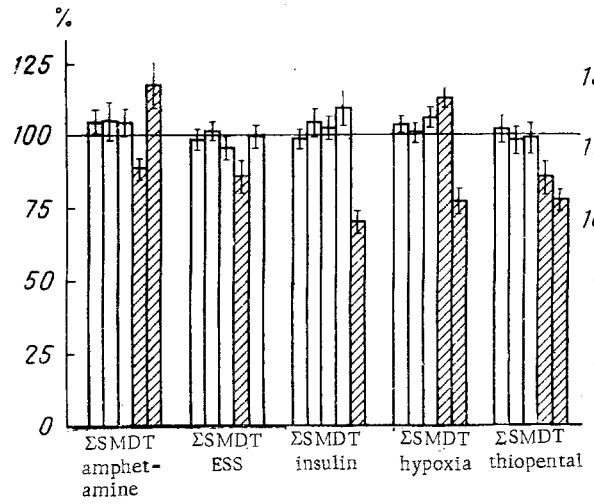

Fig. 1

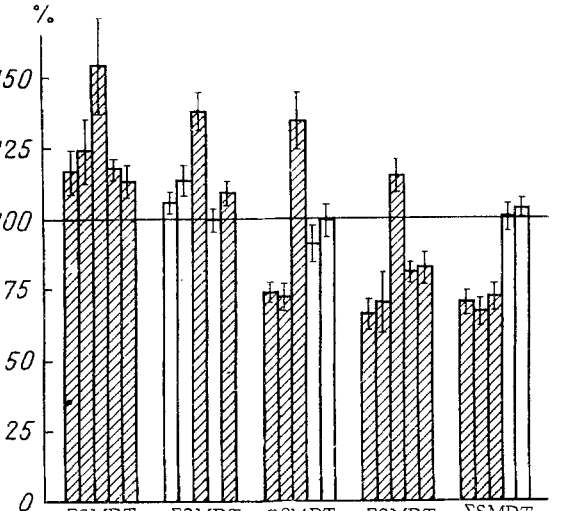

Fig. 2

Fig. 1. Changes in PL content (% of control) in rat liver during various procedures: ESS) electrical stimulation of skin; Σ) total phospholipids; S) phosphatidylserines; M) monophosphoinositides; D) diphosphoinositides; T) triphosphoinositides. Shaded columns represent values differing significantly ($P < 0.05$) from control. Errors of arithmetic mean are shown; n = 15.20.

Fig. 2. Changes in RSR of phospholipids (% of control) in rat liver after various procedures. Legend as in Fig. 1.

TABLE 1. Content and Intensity of Metabolism of PL from Rat Liver (M ± m)

Phospholipids	Content (μg P_i/g wet wt. of tissue)	RSR
Total PL	1340±40 (32)	2,91±0,09 (32)
PS	58,7±1,8 (22)	1,04±0,05 (22)
MPI	78,9±1,6 (22)	1,29±0,07 (22)
DPI	6,35±0,17 (26)	24,8±2,0 (26)
TPI	7,70±0,46 (26)	37,3±1,0 (26)

Legend. Exposure with P^{32} for 60 min; numbers in parentheses show number of experiments.

To study the intensity of metabolism of the PL phosphate groups, $Na_2HP^{32}O_4$ was injected subcutaneously into the animals in a dose of 5 Ci/g immediately before the procedure. The animals were anesthetized with ether 60 min after injection of the isotope (and at other periods also in some experiments), laparotomy was performed, and a piece of liver tissue weighing about 1 g was excised and ground in a mortar with 3 ml methanol. About 20 sec elapsed between taking the liver tissue and grinding it in methanol. The suspension of liver tissue with 6 ml of a chloroform–methanol–concentrated HCl mixture (100 : 100 : 0.6) was transferred quantitatively into centrifuge tubes. After centrifugation, the tissue residue was extracted a further four times, each time with 8 ml of the same mixture (200 : 100 : 1) for 10 min. The first three extractions were carried out at 20°C and the last two at 37°C. The methods of washing off the combined extract and concentrating it and the procedure of chromatographic fractionation of PL on paper treated with formalin, to one sheet of which 0.5 ml of the extract was applied, were described by the writers in detail earlier [1].

For each PL fraction the content of inorganic phosphorus (P_i) was determined in μg/g wet weight of tissue and the relative specific radioactivity (RSR) was calculated as the ratio (× 100) of the specific radioactivity (in counts/min/μg P_i) of the PL fraction to the specific radioactivity of P_i of the liver tissue.

EXPERIMENTAL RESULTS

Preliminary experiments showed that the PPI fractions of the liver undergo rapid postmortem changes, as a result of which the content of DPI and TPI falls. Accordingly, fixation of the liver tissue after its removal from the body must be carried out as quickly as possible.

The study of P^{32} incorporation into PL with time showed that during the first 60 min after injection of the isotope there was a sharp increase in the values of RSR of both PPI fractions, followed by a very slight decrease in RSR of DPI and a small increase in RSR of TPI during the next 3 h. The specific radioactivity of phosphorus of PS, MPI, and total PL was always lower than the corresponding values for the PPI fraction during the period of investigation (4 h), and it increased uniformly and in a straight line. The period of incorporation of P^{32}-orthophosphate of 1 h can thus be used for a simultaneous study of the in-

tensity of metabolism of all PL fractions of the liver. Data for the content and RSR values of PL of the rat liver under normal conditions 60 min after injection of P^{32} are given in Table 1. Compared with the brain, the rat liver contains only one-quarter as much PPI, although for equal exposures with P^{32} they have a higher RSR.

Metabolism of PPI and the other PL of rat liver in the pharmacologically induced states (amphetamine excitation and thiopental sleep) was investigated at two different times – after 30 min and 1 h. Changes in the content and intensity of metabolism of PL at both these times were found to be qualitatively the same, but quantitatively they were less marked after 30 min, evidence of the absence of phasic changes in PL metabolism during the first hour of exposure to the drugs. During the study of PL metabolism in the liver after the other procedures, only one time interval was therefore used, namely 1 h.

In the liver, as in the brain also, under the influence of the various procedures changes took place (Fig. 1) in the content of DPI and TPI; as a rule the changes were less sharp than in the brain [4, 5]. The direction of the changes in the content of PPI fractions in the liver tissue coincided with the direction of the changes in the content of these fractions in the brain tissue under corresponding conditions, with one exception – DPI during thiopental sleep. In amphetamine excitation the TPI content in the rat liver rose, whereas after the procedures causing inhibition (hypoxia, hypoglycemia, thiopental sleep) it fell. The DPI content in the liver changed without following any clearly defined pattern, as was observed earlier for the brain.

Changes in the intensity of PL metabolism in the liver (Fig. 2) were less marked than in the brain, especially for DPI and TPI. Changes in RSR in the brain were consistent: in response to excitation the intensity of metabolism of the PL fractions increased, whereas in response to inhibition it fell [3, 4]. All that can be said about the liver is that during excitation of the CNS, RSR of PL mainly increased, whereas during inhibition it mainly decreased. No change was found in RSR of several of the PL fractions in the liver during electrical stimulation of the skin, hypoglycemia, and thiopental sleep, and a particularly interesting result was the very distinctive changes in RSR of MPI. During excitation of the animals under the influence of amphetamine and electrical stimulation of the skin the intensity of MPI metabolism rose sharply, both compared with the metabolism of the other PL fractions in the liver and compared with changes in MPI metabolism in the brain under the same conditions of excitation. Moreover, even during the action of inhibitory factors (injection of insulin, hypoxia) the intensity of MPI metabolism increased. The increase in the intensity of MPI metabolism in the liver during excitation of the animal following injection of amphetamine or electrical stimulation of the skin can be explained on the basis of data in the literature [9, 11] pointing to specific stimulation of MPI metabolism by cholinergic and adrenergic agents, which are known to be synthesized intensively in the body under conditions of excitation. The deficiency of oxygen and glucose in hypoxia and hypoglycemia probably acts as a stressor on the animals, as a result of which foci of excitation or restlessness may arise in the CNS; these foci may specifically stimulate MPI metabolism in a corresponding manner in the liver, and only thiopental anesthesia inhibits these foci, so that the intensity of MPI metabolism falls.

The PPI of the liver thus possess all the features of lability that were found initially during the study of PPI in the brain. PPI metabolism in the liver under normal and excitatory conditions strongly resembles PPI metabolism in the rat brain. These extremely different tissues thus differ only quantitatively in their PPI metabolism.

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