DYNAMICS OF CHANGES IN THE SPECIFIC AND THE RELATIVE SPECIFIC RADIOACTIVITY OF PHOSPHORUS IN VARIOUS PHOSPHOLIPID FRACTIONS OF THE RAT'S BRAIN IN TIME

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The results of the study of the total phospholipids (PL) of brain tissue have shown that their content and metabolism in the various structures of the nervous system and the individual subcellular elements of nerve tissue differ and vary in the course of individual development. It has also been shown that the PL metabolism is intimately connected with the functional state of the nervous system and changes in the pathological conditions of the organism[2].

The intensity of the PL metabolism is most frequently determined from the rate of incorporation of radioactive phosphate. The measure of the intensity of metabolism which is adopted is the specific radioactivity (SR) usually expressed in pulses/min/μg lipid phosphorus. Radioactive phosphate, when introduced into the body, enters the tissues, including the brain tissue, continuously. Over a certain period of time there is a gradual increase in the SR of inorganic phosphate (IP) of the tissue, the magnitude of which depends on the dose of isotope administered, the volume velocity of the blood flow, the permeability of the blood vessel walls, and so on. Since the IP of the tissue is the source of the phosphate groups of PL, with a constant rate of synthesis of PL, their SR will be greater the higher the SR of the tissue IP. Accordingly, a more realistic idea of the rate of incorporation of administered radioactive phosphate into PL may be obtained from the relative specific radioactivity (RSR) of the PL, i.e., the ratio between the SR of phosphorus of PL and the SR of the tissue IP, conventionally regarded as the precursor of the phosphate groups of PL.

Analysis of the changes in the SR and RSR of the individual PL in time is essential for solving at least two problems. The study of the dynamics of SR may shed light on the genetic link between the individual members of the PL group if relationships characteristic of the products of synthesis and of their precursors can be found between them [11]. Knowledge of the dynamics of the RSR is of great practical importance when choosing a suitable time interval for the study of the intensity of metabolism of individual members of the PL group, characterized by different rates of metabolism, both separately and simultaneously. For the simultaneous study, this interval must lie on that part of the curve showing the change in RSR of PL in time at which a relatively linear increase in the RSR of all the studied phospholipids takes place.

The object of the present investigation was to study the SR and the RSR of the total PL and their individual fractions in the cerebral hemispheres of rats at various time intervals after administration of radioactive phosphate.

EXPERIMENTAL METHOD

Adult male Wistar albino rats weighing 180-250 g were given a subcutaneous injection of $Na_2HP^{32}O_4$ in a dose of 5 μCi/g body weight. The animals of the various groups (6 rats in each group) were decapitated 30 min and 1, 2, 4, 8, 24, 48, 72, and 120 h after injection of the isotope. The cerebral hemispheres were carefully freed from meninges and blood vessels and the blood was washed out with physiological saline. The PL were extracted from the moist brain tissue by a 2:1 mixture of chloroform and methanol and fractionated on a silica-gel column by graded elution with mixtures of chloroform and methanol with an increasing concentration of methanol [1].

Certain modifications were introduced into this method. The PL were applied to the column in smaller doses, so that columns of small diameter containing 2 g silica gel were used. The charge remained as before – 0.8 μg lipid phosphorus/g silica gel. Preliminary experiments were carried out to determine the volume of eluent required to elute each PL fraction, and these were subsequently collected in flasks of appropriate capacity. After the

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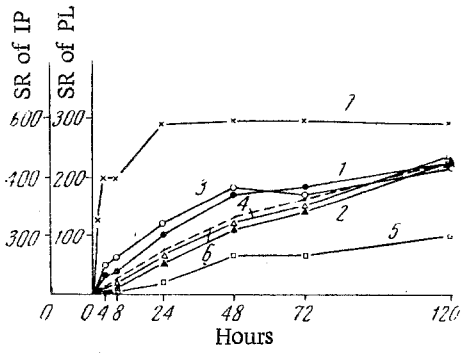

Fig. 1. Specific radioactivity of phosphorus of total phospholipids, of their individual fractions, and of the inorganic phosphate of the cerebral hemispheres of rats at various intervals after injection of P^{32}: 1) SR of fraction 1; 2) SR of fraction 2; 3) SR of fraction 3; 4) SR of fraction 4; 5) SR of fraction 5; 6) SR of total PL; 7) SR of inorganic phosphate.

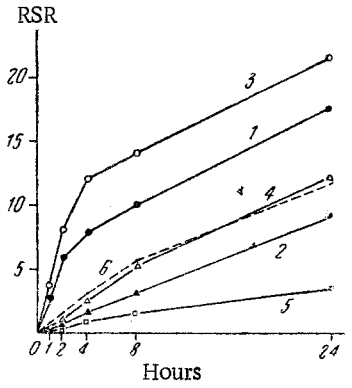

Fig. 2. Relative specific radioactivity of total phospholipids and individual phospholipid fractions of the cerebral hemispheres of rats at different times after injection of P^{32}. Legend as in Fig. 1.

collection of each fraction, two control tests were carried out, each on 1 ml of eluate. Absence of phosphorus in these tests was evidence of the completeness of separation of the fraction.

Five PL fractions were obtained: fraction 1, a mixture of phosphatidic acid (PA) and polyglycerophosphatides (PGP); fraction 2, aminophosphatides, a mixture of phosphatidylethanolamine (PEA) and phosphatidylserine (PS); fraction 3, phosphoinositides (PI); fraction 4, lecithins (phosphatidylcholines, PC); and fraction 5, sphyngomyelins (SPM). The content of total PL and their individual fractions was determined in μg phosphorus/g moist tissue. The SR of PL, SR of IP, and RSR of PL were calculated. Depending on the phosphorus content and its SR and RSR 2 h after injection of P^{32}, these fractions were identified by fractions obtained beforehand on large columns [1].

EXPERIMENTAL RESULTS

The results of determination of the SR of PL at various times after injection of P^{32} (from 30 min to 120 h) are given in Fig. 1. The total PL and all the fractions showed a characteristic increase in the SR throughout the period of time investigated after injection of the isotope. However, the dynamics of the changes in the SR of the phosphorus differed from one fraction to another. Fractions 1 and 3 were characterized by an irregular increase in SR, taking place comparatively quickly in the first hours, slowing to some degree 4 h after injection, and becoming negligible after 48 h. The increase in the SR of fractions 2 and 4 was slower and more regular. In the first hours after injection of the isotope, their SR was much lower than the SR of fractions 1 and 3. However, the lowering of the rate of increase of the SR of fractions 1 and 3, accompanied by the maintenance of a more constant rate of growth of the SR of fractions 2 and 4 had the result that after 72 h all four curves came together, and thereafter practically merged. The curve of the SR of fraction 5 was much below all the other curves throughout the period of investigation. The SR of the IP of the brain tissue rose during the 24 h after injection of the radioactive phosphate and thereafter remained unchanged.

The results of these experiments completely confirmed those obtained by other workers [6,7] who studied the incorporation of P^{32} into the total PL of the brain and who showed that the increase in the radioactivity of the PL takes place for approximately 200 h after administration.

During the study of the SR of the individual fraction 2 h after injection of the isotope, the following order of arrangement of the fractions was found [1] depending on the degree of decrease in their SR: PI, PA + PGP, PC, PEA + PS, SPM. The results of the present experiment showed that this order was definitely maintained for 48 h, but starting from 72 h the differences in the values of the SR of fractions 1, 2, 3, and 4 became negligible.

Several authors have studied the relative values of the SR of the individual phospholipids of the brain and their changes in time [3-5,8,10]. The results of these investigations are difficult to compare with the present findings, because different methods were used for fractionating the PL and, consequently, the composition of the fractions differed slightly. In addition, the investigations were carried out at different intervals after injection of the radioactive phosphate, and most frequently not more than two or three intervals were studied in the individual investigations. However, even in these circumstances, it is clear that the relative values of the SR of the various PL fractions obtained by these authors were in general similar to those now described: the phospholipids undergoing the most rapid metabolism were PA and PI; the lowest SR was found in the case of SPM. As regards the values of their SR, PC, PEA, and PS occupied an intermediate position. With an increase in the length of the time interval after injection of the isotope, these differences gradually disappeared [5], and this also agreed with the present findings.

The presence of a kink in the curves of the SR of fractions 1 and 3 was a sign of the nonhomogeneity of these fractions. In fact, according to reports in the literature, fraction 3 (PI) includes mono-, di-, and triphosphoinositides, differing not only in the number of phosphate groups in their molecule, but also in their metabolic activity; tri- and diphosphoinositides are characterized by a higher rate of phosphorus metabolism than monophosphoinositides [8]. Fraction 1 also was a mixture of components characterized by different intensities of metabolism (PA and PGP).

Fraction 2 is known to consist of at least two components, PEA and PS. The regularity of the course of the curve of the SR of this fraction in time indicates the approximately identical level of metabolic activity of the two components of this fraction. The similarity between the SR curves of fractions 2 and 4 evidently reflects the genetic closeness and the common pathways of biosynthesis of these phospholipids (PC, PEA, PS). The results obtained do not, however, confirm the view that aminophosphatides are precursors of PC, at least in respect to phosphate groups; throughout the period of investigation, the SR of fraction 2 was rather lower than or equal to the SR of fraction 4. Meanwhile this does not justify the complete rejection of the possibility that PC may be formed from aminophosphatides, because in some conditions it is not impossible that this process may take place by multistage methylation of PEA [9].

The low level of the SR of SPM, so sharply different from the SR of all the other phospholipids, and persisting throughout the period of investigation, may possibly be accounted for by peculiarities in the localization of SPM in nerve tissue, for it is concentrated mainly in the myelin sheath [2].

The RSR curves of the total PL and their individual fractions during the 24 h after injection of the isotope are given in Fig. 2. From 24 to 120 h the SR of the IP of the brain tissue remained unchanged and, consequently, at these periods the course of the RSR curves of all fractions completely reproduced the curves of the change in their SR.

The linear increase in the RSR of fraction 1 took place only during the first 2 h after injection of the isotope. After 2 h a marked change in direction of the curve was observed. A distinct break in the curve of fraction 3 was observed after 4 h. The RSR of the remaining fractions increased in a relatively linear fashion throughout the period of investigation (120 h).

The results obtained showing the changes in the RSR of PL in time showed that the study of the intensity of metabolism of the phosphate groups of PC, PEA, PS, and SPM is possible at any interval after injection of radioactive phosphate, at least for 120 h. The intensity of the metabolism of PC + PGP and PI may be studied only during an interval of not more than 2-4 h after injection of the isotope.

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