DYNAMICS OF INCORPORATION OF LABELED ORTHOPHOSPHATE INTO PHOSPHOLIPIDS OF A BRAIN MINCE AT DIFFERENT TEMPERATURES

S. V. Gasteva and T. E. Raize

UDC 612.015.32:547.953

The rate of incorporation of P^{32} into phospholipids of a rat brain tissue mince is directly dependent on the incubation temperature of the tissue (22-32°C). The character of the curves showing specific radioactivity of phospholipids as a function of incubation time within this temperature range is similar.

Investigations of the intensity of metabolism of brain phospholipids (PL) undertaken on rats by Chetverikov et al. [1, 2] showed that depression of this metabolism in hypoxia is not the direct consequence of oxygen lack in the nerve tissue cells, but is largely dependent on the lowering of body temperature which invariably accompanies hypoxia. To determine the actual role of the temperature factor in regulation of the intensity of brain PL metabolism in hypoxia, it is necessary to have a simpler system than the brain of an intact animal, subjected to complex regulatory influences of other systems of the body. Isolated brain tissue can be used as such a simple system.

The object of the present investigation was to study the dynamics of incorporation of labeled orthophosphate into PL of a rat brain mice when incubated at different temperatures and under a normal partial pressure of oxygen.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on adult Wistar albino rats. The animals were decapitated and the cerebral hemispheres carefully stripped of meninges and vascular plexuses and washed with physiological saline to remove blood. The tissue mince, in a weight of about 400 mg, was placed in the small container of a Warburg apparatus containing 2.5 ml Krebs–Ringer phosphate medium with glucose, pH 7.4, at a specified temperature (37, 32, 27, and 22°C). Radioactive phosphate ($Na_2HP^{32}O_4$) was added to the medium; the specific radioactivity (SR) of the medium was about 5×10^3 pulses/min/μg inorganic phosphorus.

After centrifugation, the incubation medium was decanted and the tissue residue washed 9 times with physiological saline. Lipids were extracted from the tissue three times with a 2:1 chloroform–methanol mixture by Folch's method. The pooled extracts were washed twice with 0.05 M KH_2PO_4 solution and 6 times with 0.29 M NaCl solution. Control experiments showed that after washing in this manner, contamination of the samples with radioactive phosphate was negligible.

The content of lipid phosphorus (after mineralization) and the radioactivity were determined in each sample, and its SR (i. e., the number of pulses/min/μg PL phosphorus) was calculated. In each form of the experiment (different times of incubation and temperature of medium), from 8 to 20 determinations were made.

Laboratory of Regulation of Brain Metabolism, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 9, pp. 31-33, September, 1970. Original article submitted June 10, 1968.

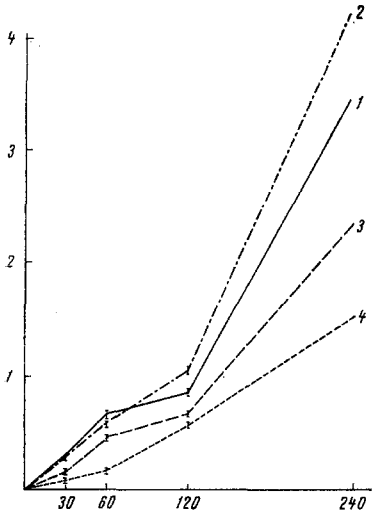

Fig. 1. Dynamics of increase in specific radioactivity of phospholipids when incubated at different temperature. 1) Incubation at 37°; 2) at 32°; 3) at 27°; 4) at 22°. Abscissa, incubation time (in min); ordinate, SR (pulses/min/μg phosphorus).

The dynamics of increase in SR of PL phosphorus of brain tissue with time, for different incubation medium temperatures, is shown in Fig. 1, from which it is clear that SR rises approximately uniformly with time during the first 2 h of incubation. Thereafter, at all temperatures, between 2 and 4 h (and somewhat sooner at 22°) the increase in SR of PL of the brain mince becomes much faster. This type of curve is a deviation from the normal course of an enzymic reaction [5]. It was therefore postulated that during comparatively prolonged incubation of the tissue, destruction of cells or denaturation of proteins takes place, possibly leading to an increase in the ability of the tissue to incorporate labeled orthophosphate. To test this hypothesis experiments were carried out in which radioactive phosphate was added after incubation for 3 h in a nonradioactive medium, after which incubation was continued for a further 1 h in the presence of P^{32}. It might be expected that if denaturation changes take place in the tissue during its incubation, PL of the samples after preincubation for 3 h would have an SR value much higher than the SR of samples incubated for only 1 h. However, the results showed that samples after preincubation had an SR of 0.394 ± 0.022, i. e., even lower than samples incubated for 1 h, whose SR value was 0.491 ± 0.001 ($P < 0.001$). This indicates that the increase in rate of incorporation of P^{32} into brain PL was not due to changes in the tissue during incubation.

It may be supposed that during comparatively long incubation, catabolic processes in the brain tissue are intensified; breakdown of PL or other phosphorus-containing compounds leads to the accumulation of considerable quantities of substances which can act as precursors of phospholipids during their synthesis and which are responsible for the faster formation of newly synthesized PL. When incubation for 4 h takes place in the presence of radioactive phosphate, phosphorus-containing precursors with comparatively high SR accumulate, and this must inevitably lead to an increase in SR of products of PL synthesis. In the case of preincubation in nonradioactive medium, on the other hand, this increased quantity of precursors has zero radioactivity, and it increases only during incubation for 1 h with P^{32}. This low SR of the PL precursors, together with possible disturbance of the normal course of metabolic processes in brain tissue during such comparatively long periods of incubation, evidently are responsible for the lower SR values of PL in the case of preincubation of brain tissue in nonradioactive medium.

The more rapid increase in SR of PL of the brain mince observed during comparatively long periods of incubation of brain tissue in radioactive medium is thus more likely to be the result of an increase in the mass of comparatively intensively labeled PL precursors and of the associated acceleration of PL synthesis than the result of degradation changes in the tissues leading to increased sorption of orthophosphate. Facts have been reported in the literature indicating a faster incorporation of labeled precursors into tissue PL after incubation for several hours [4, 7], although no attempt has been made to explain this phenomenon.

The curves given in Fig. 1 show that within the temperature range from 22 to 32° there is a direct relationship between SR of brain PL and the temperature for all times of incubation. The relationship between the curves showing the increase in SR of brain PL at incubation temperatures of 32 and 37° is a special case. Whereas in the early periods of incubation SR of PL was slightly lower at 32° than at 37° (after incubation for 1 h it was 91% of SR at 37°; $P < 0.05$), in the later periods of incubation this relationship was reversed, and after 4 h, SR of PL at 32° was much higher than SR of PL at 37°.

These results do not agree with those obtained by other workers [6], who showed that the rate of incorporation of P^{32} into PL of the sympathetic ganglia at 33° is 50-70% (depending on the PL fraction) of the corresponding values at 37°, when incubation continued for 4 h. However, the figures given by these workers have a considerable scatter, and in the writer's view no definite conclusions can be drawn from them.

The relationship between SR curves of PL of brain mince at temperatures of 32 and 37° obtained in the present experiments can evidently be explained on the basis of the known dual character of the effect of temperature on enzyme systems. On the one hand, with elevation of the temperature within certain limits, the velocity of enzyme reactions is increased, and this evidently was observed in the present experiments during short periods of incubation. On the other hand, with elevation of the temperature denaturation of the enzymes takes place more rapidly, so that during incubation for longer periods, a lower temperature (in this case 32°) may prove to be closer to the optimum for their action than a higher temperature, corresponding to the normal body temperature of rats. In recognition of this dual effect of temperature on enzymes, the Commission on Enzymes [3] recommends that, wherever possible, temperatures should be kept lower than 37°, high enough to secure an acceptable level of activity for recording but, at the same time, low enough to avoid thermal denaturation of the enzymes.

The results of the present experiments thus indicate that within the temperature range from 22 to 32° the rate of incorporation of inorganic phosphate into PL of rat brain tissue is directly dependent on the incubation temperature of the tissue; the character of the curves showing SR of PL as a function of incubation time is similar within this temperature range.

LITERATURE CITED

1. S. V. Gasteva and D. A. Chetverikov, Dokl. Akad. Nauk SSSR, 165, No. 3, 714 (1965).
2. D. A. Chetverikov, in: Biochemistry and Function of the Nervous System [in Russian], Leningrad (1967), p. 160.
3. The Classification and Nomenclature of Enzymes [in Russian], Moscow (1962).
4. P. A. Clayton and C. E. Rowe, Biochem. J., 101, 674 (1966).
5. M. Dixon and E. Webb, Enzymes [Russian translation], Moscow (1961).
6. M. G. Larrabee, J. P. Klingman, and W. S. Leicht, J. Neurochem., 10, 549 (1963).
7. S. S. Tsao and W. E. Cornatzer, Lipds, 2, 424 (1967).