EFFECT OF TEMPERATURE AND OXYGEN CONCENTRATION IN THE INCUBATION MEDIUM ON PHOSPHOLIPID METABOLISM IN ISOLATED RAT-BRAIN TISSUE

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The effect of different incubation temperatures (37, 32, 27, and 22°C) and different oxygen concentrations in the medium (21, 8, and 5%) on the rate of incorporation of P^{32}-orthophosphate into phospholipids of minced rat-brain tissue was studied. At 37°C the relative specific radioactivity (RSR) of the phospholipid phosphorus was directly dependent on the O_2 concentration in the medium. When the temperature was lowered to 27°C, the sensitivity of phospholipid metabolism to a lowered O_2 concentration in the medium was reduced. At 27°C the RSR of phospholipid phosphorus was independent of the O_2 concentration in the medium, and in atmospheres containing 8 and 5% O_2 the highest phospholipid RSR was observed at 27°C. It is postulated that a moderately lowered temperature has a protective effect on the phospholipid metabolism of brain tissue in a medium with low O_2 concentration.

Investigations of the intensity of phospholipid metabolism in the brain [1, 3] have shown that inhibition of the metabolism of these compounds in vivo during hypoxia is a direct consequence, not so much of oxygen lack in the nerve tissue, as of the lowered body temperature which goes with hypoxia. To determine the relative role of each of the factors acting on the body in a state of hypoxia (oxygen lack and hypothermia) it was necessary to use a simpler system than the brain of the intact animal, which is exposed to the influence of complex regulatory mechanisms. Isolated brain tissue makes a suitable simple model for the purpose [4-8].

The object of this investigation was to make a separate study of the effects of temperature and the oxygen concentration in the medium on incorporation of labeled inorganic orthophosphate into the phospholipids of a rat-brain mince.

EXPERIMENTAL METHOD

Experiments were carried out on adult female Wistar albino rats. The animals were decapitated, the meninges and large blood vessels were removed from the cerebral hemispheres, and the brain was washed free from blood with physiological saline. The minced tissue (about 100 mg) was placed in the small container of a Warburg apparatus containing 2.5 ml Krebs–Ringer phosphate medium with glucose at pH 7.4. Radioactive phosphate ($Na_2HP^{32}O_4$) was added to the medium to give a specific radioactivity (SR) of the medium of about 5×10^3 pulses/min/μg orthophosphate phosphorus. The material was incubated for 1 h in air (21% O_2) and in gas mixtures (8% O_2 + 92% N_2 and 5% O_2 + 95% N_2) at temperatures of 37, 32, 27, and 22°C. In the experiments to study the effect of hypoxia on phospholipid metabolism the gas mixtures of appropriate composition were blown through the small containers of the Warburg apparatus for 15 min under a pressure of about 100 mm water. Control experiments using nitrogen showed that satisfactory ventilation is thereby achieved, for under these conditions respiration was absent and incorporation of P^{32} into phospholipids was negligibly small. The methods of extracting lipids from nerve tissue and of removing all traces of inorganic phosphate from the extract were described earlier [2].

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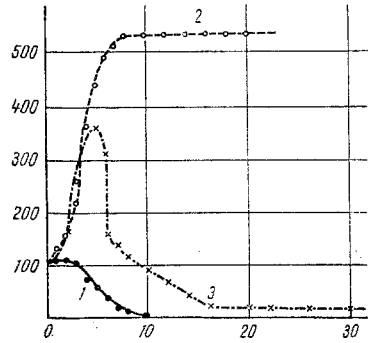

Fig. 1. RSR of phospholipid phosphorus of rat-brain tissue as a function of incubation temperature at different partial pressures of oxygen. Abscissa, temperature of incubation medium; ordinate, RSR of phospholipid phosphorus; values given are M ± m. Continuous line) air (21% O_2); dots and dashes) mixture with 8% O_2; dashes) mixture with 5% O_2.

The content of lipid phosphorus of brain tissue (after mineralization) and the radioactivity were determined in each sample and its SR was calculated (in pulses/min per μg phosphorus); the relative SR, i.e., the ratio between SR of the phospholipid phosphorus and SR of the inorganic phosphorus of the incubation medium (multiplied by 10^5), also was calculated. With each combination of temperature and gas mixture chosen 14-34 experiments were carried out. The results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

The relationship between the relative SR (RSR) of the phospholipid phosphorus of the brain tissue and the incubation temperature and composition of the atmosphere in the small containers is shown in Fig. 1. The character of the change in intensity of phospholipid metabolism under hypoxic conditions clearly depends on temperature. At 37°C there was a fairly sharp decrease (to 44% of the level in air) in P^{32} incorporation into phospholipids with a decrease in the O_2 concentration from 21% (pO_2 = 159 mm Hg) to 8% (pO_2 = 62 mm Hg). When the O_2 concentration in the atmosphere was lowered to 5% (pO_2 = 38 mm Hg), the phospholipid metabolism remained at the same level as in the mixture with 8% O_2.

At an incubation temperature of 32°C a progressive decrease was observed in the rate of incorporation of P^{32} into the brain-tissue phospholipids with a decrease in the O_2 concentration from 21 to 5% At 5% O_2 the RSR of the phospholipids was 45% of its value in air. At 27°C there was virtually no difference in the values of RSR of the brain-tissue phospholipids in all three gas mixtures tested. At 22°C, just as at 32°C, there was the same clear, progressive fall in the rate of incorporation of P^{32} into the brain phospholipids with a decrease in the O_2 concentration in the medium from 21 to 5%. The relative SR of the phospholipids at 5% O_2 was 46% of its value in air at 22°C.

These results indicate that a fall in temperature of the incubation medium is accompanied by changes in the metabolic response of the phospholipids to a decrease in the O_2 concentration in the medium. With a fall in temperature from 37 to 27°C hypoxia affected the decrease in sensitivity of phospholipid metabolism of the isolated brain tissue. It will be clear from Fig. 1 that during hypoxia there was an appreciable shift of the temperature optimum of P^{32} incorporation into the brain phospholipids toward lower temperatures: 27°C was the temperature at which the incorporation of P^{32} into the brain-tissue phospholipids in a medium with a lowered partial pressure of oxygen took place at the fastest rate, which was virtually identical in an atmosphere of air and after a sharp decrease in the O_2 concentration.

The results of this investigation show that, by contrast with the picture observed in the intact organism, the phospholipid metabolism of isolated brain tissue is affected by changes both in the O_2 concentration in the atmosphere and in the temperature of incubation. In the intact organism, even when exposed to a very marked decrease in the O_2 concentration in the inspired air, despite the considerable hypoxemia the cells of the nervous tissue obtain the greatest possible O_2 supply. Naturally, under tissue culture conditions, no such protective reactions can take place, and a deficiency of O_2 in the incubation atmosphere directly affects the intensity of oxidative processes and, consequently, of various processes of biosynthesis in the brain tissue. The essential point is that the combined effect of the simultaneous action of both factors – a fall in temperature and in the O_2 concentration – was less than the effect of a decrease in the oxygen concentration in the medium alone. For instance, lowering the O_2 concentration from 21 to 8% at a temperature of 37°C lowered the RSR of the phospholipids from 26.6 ± 1.4 to 11.8 ± 0.6. The relative SR of the phospholipids in medium containing 8% O_2 at 32°C was 13.6 ± 0.6. Consequently, a moderately reduced temperature decreases the degree of depression of phospholipid metabolism induced by a low O_2 concentration in the medium.

Despite the difference in character of the response of phospholipid metabolism to a decrease in the partial O_2 pressure in the surrounding medium at different temperatures in the isolated brain tissue and in the brain of the intact animal, there is nevertheless a certain similarity as regards the protective effect of

hypothermia on phospholipid metabolism in the brain tissue under hypoxic conditions. However, this effect is manifested only within a certain range of hypothermic states.

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