Ca^{++} CONTENT IN LIVER AND BRAIN MITOCHONDRIA OF HYPOPARATHYROID RATS

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The fall in blood and CSF levels of Ca^{++} and its deposition in bones and in the form of metastatic calcification in the tissues in hypoparathyroidism suggest the possibility of Ca^{++} redistribution and accumulation in other calcium depots of the body also. The most important of these depots are mitochondria, which play the role of buffer systems regulating the Ca^{++} level in the cytoplasm and, correspondingly, the course of cellular reactions triggered by these ions.

The object of this investigation was to study changes in the Ca^{++}-accumulating capacity of liver and brain mitochondria in parathyroid hormone deficiency. Besides characteristics of oxidative phosphorylation activity, the Ca^{++}-accumulating capacity of the mitochondria, which the writers studied previously [5], is regarded as an essential parameter of the functional state of these organelles.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats weighing 150-180 g. Hypoparathyroidism was induced by electrical coagulation of the parathyroid glands and the degree of hypoparathyroidism which resulted was determined by measuring changes in the serum Ca^{++} concentration.

Mitochondria were isolated from the liver and brain by the method in [4], 5, 12, and 30 days after the operation. Mitochondria isolated from the liver and brain of rats undergoing a mock operation served as the control. The degree of purity and structural integrity of the mitochondria was verified electron-microscopically.

The Ca^{++} content in the mitochondria was determined with the aid of the fluorescent probe chlortetracycline, which has high affinity for intramitochondrial calcium. The method is based on measurement of the intensity of slowly rising fluorescence of the complex formed between Ca^{++} and chlortetracycline [12]. Usually the Ca^{++}-accumulating capacity of the mitochondria is studied by the chlortetracycline method *in vitro* in incubation medium containing respiration substrate, ADP, and Ca^{++}. During energy-dependent Ca^{++} accumulation by mitochondria the concentration of the Ca^{++}-chlortetracycline complex in them rises, and this is accompanied by an increase in the total quantum yield of fluorescence. However, considering that mitochondria are the main calcium depot, in the case of parathyroprival hypocalcemia, it could be more informative not to study Ca^{++}-accumulating capacity of the mitochondria *in vitro*, but to determine the level of Ca^{++} accumulated by these organelles *in vivo*. For this purpose the technique described above was modified. The modification consisted of excluding Ca^{++}, respiration substrate, and ADP from the incubation medium. Under these conditions transfer of calcium from the bound into the ionized state does not take place [8] and, for that reason, determination of the maximal value of slowly rising fluorescence provides an estimate of the quantity of only stable endogenous Ca^{++} [12]. The medium in which the mitochondria were suspended contained EDTA, which prevented any appearance of fluorescence due to chlortetracycline with Ca^{++} adsorbed on the outer mitochondrial membrane, but did not affect fluorescence of the intramitochondrial complex, for the mitochondrial membrane is impermeable for EDTA [12].

The intensity of fluorescence was measured on a Hitachi MPF-4 (Japan) spectrofluorometer. Fluorescence was excited by light with wavelength 380 nm and the intensity of fluores-

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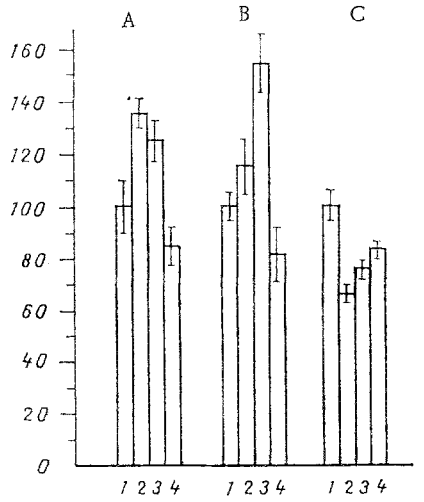

Fig. 1. Ca^{++} concentration (intensity of chlortetracycline fluorescence) in liver (A) and brain (B) mitochondria and in blood serum (C) at different times after partial parathyroidectomy. 1) Control; 2-4) 5th, 12th, and 30th days after operation respectively. Ordinate, Ca^{++} concentration (in % of control).

cence was measured at 540 nm. Mitochondria were added to the measuring cuvette at the rate of 1.2 mg protein to 1 ml incubation medium containing 0.25 M sucrose, 0.01M Tris-HCl, and 10 μM chlortetracycline, pH 7.4. The protein concentration was determined as in [15].

EXPERIMENTAL RESULTS

The investigation showed that in the early stages of hypoparathyroidism (5th-12th day, the period of maximal fall of the blood Ca^{++} level) the intensity of chlortetracycline fluorescence in the mitochondria rises appreciably. Although the total quantum yield of chlortetracycline fluorescence in the mitochondria depends both on the properties of the mitochondrial membranes, especially on their hydrophobicity, and on the Ca^{++} content [1], the changes which were found were associated mainly with changes in the Ca^{++} level in these organelles. The grounds for this conclusion are results of previous investigations [3, 9], which demonstrate an increase in hydrophilicity of mitochondrial membranes during the period of marked hypoparathyroidism, which is known [1] to reduce the intensity of fluorescence of the Ca^{++}-chlortetracycline complex. The increase in the intensity of chlortetracycline fluorescence in liver and brain mitochondria of hypoparathyroid rats was thus the result of an increase in the Ca^{++} level (Fig. 1). Changes in the liver reached a maximum on the 5th day after the operation, and amounted to 35% of the control, whereas in the brain the maximum occurred rather later — after 12 days, and amounted to 54%. In the late stages of hypoparathyroidism (30 days after the operation) the intramitochondrial calcium content in the liver did not differ significantly from normal, whereas in the brain it was a little below the control level. It must be pointed out that changes in the Ca^{++}-accumulating capacity of the mitochondria in hypoparathyroidism correlate with the degree of manifestation of parathyroid hypofunction, reflected in values of the serum Ca^{++} concentration (Fig. 1). The increase in the Ca^{++} concentration in the mitochondria of the hypoparathyroid rats could be the result either of activation of processes facilitating entry of Ca^{++} into the mitochondria or of inhibition of its outflow. Analysis of all the data indicates that both mechanisms may be present. The fact that the action of parathyroid hormone on mitochondria is effected through cyclic AMP [7] and that both these substances promote outflow of Ca^{++} from mitochondria [11] suggests that elevation of the intramitochondrial Ca^{++} level in hypoparathyroidism may be the result of a deficiency of both parathyroid hormone and cyclic AMP. This hypothesis is confirmed by investigations [10, 14] which demonstrated an increase in the cyclic AMP content in the body in the presence of an excess of parathyroid hormone. Support for the other mechanism is given by an investigation [13] in which it was shown by an isotope method that in chronic hypoparathyroidism the transfer of ^{45}Ca into liver mitochondria *in vitro* is intensified.

On the other hand, any de-energization of mitochondria leads to a decrease in their Ca^{++}-accumulating capacity [6]. Although the results of the writers' previous investigation [5] revealed a decrease in efficiency of phosphorylation during hypoparathyroidism, they

showed that synthesis of high-energy compounds is not affected under these circumstances, since the degree of increase in the rate of respiration was greater than that of the decrease in ADP/O. Such a disproportionate change in the parameters of mitochondrial respiration and phosphorylation may even be evidence of excessive synthesis of high-energy compounds [2]. Additional energization of mitochondria in hypoparathyroidism may perhaps also be accompanied by an increase in their Ca^{++}-accumulating capacity. The tendency for the intramitochondrial Ca^{++} level in the brain to fall, which is observed on the 30th day after the operation, can perhaps also be explained from this point of view. At this period, as the results of a study of oxidative phosphorylation parameters showed [5], there is no compensatory increase in the rate of respiration in Chance's 3rd metabolic state, whereas ADP/O remains low. In the liver mitochondria, on the other hand, toward the 30th day after the operation, normalization of the parameters of both oxidative phosphorylation and Ca^{++} concentration is observed.

In experimental hypoparathyroidism the Ca^{++} concentration thus rises in the liver and brain mitochondria and correlates with the degree of manifestation of parathyroid hypofunction.

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