THE INFLUENCE OF SODIUM AMYTAL ON THE PROCESSES OF OXIDATIVE PHOSPHORYLATION

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In 1951, Brody and Bain [1] showed that different barbiturates—Thiopental, amobarbital, pentobarbital—in conditions of experiments in vitro, have an "uncoupling" effect on the processes of oxidative phosphorylation in suspensions of mitochondria, isolated from cerebral and hepatic cells. The authors noted that the concentrations of barbiturates, exerting an "uncoupling" effect, approximated to those which are formed in the tissues of the organism upon introduction of narcotic doses of barbiturates, assuming equal distribution. Brody and Bain postulate that uncoupling of the processes of coupled phosphorylation in the brain lies at the basis of the narcotic effect of the barbiturates.

In a study of the processes of oxidative phosphorylation, (P : O) in particulate tissue suspensions isolated from the cardiac, renal and hepatic cells of normally fed rabbits and of starving rabbits [2], we attempted to apply amytal narcosis for the purpose of immobilizing the animals and obtaining the possibility of a living tissue extract for the subsequent work. In this, we, as did Brody and Bain, discovered inhibition of the coupled phosphorylation ("uncoupling") in the particulate tissue suspension from the organs of the animals, subjected to amytal narcosis introduced intravenously. Therefore, it was necessary to immediately abandon administration of sodium amytal as a narcotic medium in the experiments mentioned.

At the same time another fact attracted attention. I. I. Niselovskaya, simultaneously conducting experiments on the study of oxidative phosphorylation in the same rabbits subjected to amytal narcosis, but unlike us, not using the suspension of a particulate tissue, but extracts from the heart and kidneys, did not find any sign of uncoupling in her experiments (i. e., reduction of the P : O ratio on account of a reduction in the level of phosphorylation). In the literature, there are also references to the absence of the influence of barbiturates on the processes of oxidative phosphorylation in the hemogenates from the organs [3]. Thus, sodium amytal (and very likely other barbiturates), introduced intraveneously, exert an uncoupling effect on the processes of oxidative phosphorylation in the particulate tissue system, and do not influence these processes in extracts obtained from the same organs.

One of the possible causes of such a difference in the effect of sodium amytal may be the non-uniform distribution of the introduced barbiturate in the cells between their different structural elements, namely, concentration of barbiturate in the large granules.

Desirous of making clear the correctness of such a hypothesis, we decided to compare the effect of sodium amytal introduced intravenously on the processes of oxidative phosphorylation (P : O), with determination of its concentration in the systems studied.

EXPERIMENTAL METHODS

The experiments were conducted on rabbits. Sodium amytal was introduced into the vein in the ear of

the animal in the form of a 5% solution, 50 mg per 1 kg body weight. 20 minutes later, the abdominal cavity of the narcotized rabbit was dissected out, and the kidney swiftly extracted.

The suspension of the cortical substance of the kidney was pulverized in a cool mortar with three times its weight of isotonic sucrose solution with a phosphate buffer (pH-7.6), and centrifuged at 1500 revs. per min for 3 minutes and the resulting supernate recentrifuged for 30 minutes (2500 revs. per minute). The residue obtained was washed with a 3-5 ml isotonic sucrose solution, and finally suspended in that solution ("large granules"). The whole procedure took place in a room at a temperature of 0-4°. Microscopic examination of the suspensions showed that they consisted of mitochondria with a mixture of other granules.

In order to determine the oxygen intake, the suspension of the granules was transferred to Warburg vessels. The centrifugate, separated from the residue after 30 minutes of centrifugation, was placed in another vessel.

The composition of the incubated mixture was : suspension of granules, centrifugate, in some experiments an extract—1 ml phosphate buffer pH-7.6, with a final concentration of M/ 200, glutamic acid, M/ 100, glucose, 0.0025 M, $MgCl_2$, 0.005 M, NaF, 0.025 M, AT -0.0015M; Total volume, 2.0ml.

In certain experiments, to the incubated mixture was added 0.2 ml of a preparation of active yeast hexokinase (Fraction III) [4].

The mixture, after a period of equilibration was incubated in the Warburg apparatus for 20 minutes under aerobic conditions at a temperature of 30°. The value of the combined phosphorus was determined by the difference between the content of the inorganic phosphorus in the trichloracetic filtrate of the control test (not incubated), and the experimental one [5]. In addition, determination of the albumin content in the trichloracetic residue of the tests after incubation was carried out according to the biuret reaction [6]. Colorimetry was conducted with a photoelectrometer.

Determination of the sodium amytal in the suspension of the granules and centrifugate was made by spectrophotometry according to the method of Goldsmith, Lamprecht and Helmreich [8]. A conversion was made on the results obtained to 1 mg of albumin of the studied test.

EXPERIMENTAL RESULTS

The investigations confirmed the correctness of the hypothesis regarding the unequal distribution of the sodium amytal introduced intravenously between the various structural formations of renal tissue.

The findings in Table 1 show that the sharp degree of uncoupling (P:O-0.7) was observed only in suspensions from particulate renal tissue where the concentration of sodium amytal (6.0 μg/mg of albumin) was on average twice as high as in the centrifugate. A comparatively low concentration of sodium amytal in the centrifugate did not influence the processes of oxidative phosphorylation (P:O-2.7).

The comparison of the intensity of the processes of oxidative phosphorylation in the granules and centrifugates from the renal tissue with sodium amytal contained in them seemed to give an exhaustive answer to the question raised. However, analysis of the results of the individual experiments makes one doubt whether the concentration of sodium amytal in the granules and centrifugates in our experiments was the only single factor determining the level of coupled phosphorylation in these systems. In experiments Nos. 14 and 15, the concentration of sodium amytal in the centrifugate, was equal respectively to 4.4 and 4.9 μg/mg of albumin; sodium amytal in this concentration did not have a perceptible influence on the processes of oxidative phosphorylation(P:O - 2.7 and 2.3). At the same time, an almost similar concentration of barbiturates in the granules in experiment No. 12 (4.6 μg/mg of albumin) produced uncoupling (P:O-0.8). Thus establishment of the inequality of distribution of the introduced sodium amytal between the granules and the rest of the cytoplasm has so far been unable to explain fully the differences in the flow of the processes of the coupled phosphorylation in the preparations studied.

In the literature there are findings testifying to the inhibitory influence of the barbiturates on hexokinase from brain tissue. In a previous paper [2] we were convinced that inhibition of hexokinase activity greatly limits the speed of the processes of oxidative phosphorylation in the "large granules" (from the liver). It was natural that a hypothesis was put forward that in the above-mentioned experiments, the level of phosphorylation in the renal granules of the "amytalized" rabbits was dependent on the inhibition of the hexokinase reaction by the barbiturate. In order to verify this supposition, we compared the P:O value in the large granules isolated

TABLE 1

Coefficient of Oxidative Phosphorylation (P:O) in Suspension of Granules in a Centrifugate (in Extracts) from the Kidneys and Their Sodium Amytal Content

No. of experiment	P:O			Sodium amytal content (μg/mg albumin)	
	Extract	Granules	Centrifugate	Granules	Centrifugate
1*	2.0	—	—	—	—
2*	1.6	—	—	—	—
3*	1.8	—	—	—	—
4	2.2	0	—	—	—
5	1.8	0.4	2.4	—	—
6	—	0.6	2.5	—	—
7	—	0.9	2.4	—	—
8	—	—	—	1.4	0.5
9	—	—	—	3.9	1.3
10	—	—	—	7.0	2.7
11	—	—	—	8.6	4.8
12	—	0.8	2.2	4.6	2.3
13	—	0.8	3.0	6.5	3.5
14	—	0.6	2.7	9.9	4.4
15	—	0.8	2.3	6.3	4.9
16	—	1.1	2.3	5.8	2.5
Mean	1.9	0.7**	2.7	6 0	3.0

* Experiments Nos. 1, 2, and 3 conducted by I. I. Niselovska.

** P:O in renal granules of the control rabbit (i.e., without introduction of sodium amytal) was equal on average to 1.8 (see Table 2).

TABLE 2

Coefficient of Oxidative Phosphorylation (P:O) in Suspension of Granules from Kidneys of the Control Rabbits and Rabbits Subject to Introduction of Sodium Amytal (With and Without Addition of Yeast Hexokinase)

No. of experiment	Control rabbits	No. of experiment	"Amytalized" Rabbits	
			Without addition hexokinase	With addition hexokinase
17	2.1	23	0.9	1.6
18	2.0	24	1.0	1.6
19	1.8	25	1.0	1.8
20	1.5	26	1.3	2.0
21	1.9	27	0.9	1.7
22	1.6	—	—	—
Mean	1.8	—	1.0	1.7

from the rabbits, kidneys, with and without addition of yeast hexokinase to the suspensions. As control we studied P:O in a suspension of granules isolated from the kidneys of rabbits which had not been subject to sodium amytal (killed by decapitation).

The results of the experiments set out in Table 2 show that addition of hexokinase removes the uncoupling effect of sodium amytal, and that consequently this effect may be associated with the inhibition of hexokinase reaction. Specially devised experiments showed that addition of yeast hexokinase to the suspension of the large granules, isolated from a kidney or liver of the control rabbits, had no effect on the level of oxidative phosphorylation. (P:O) in them.

The results of our experiments do not allow us to draw definite conclusions about the effect of the intravenously administered sodium amytal on the processes of oxidative phosphorylation in the tissue of the organism in vivo. The fact that enzyme systems of phosphorylation of the centrifugates do not react to the introduced barbiturate makes one highly cautious in interpreting the significance of the results with large granules. It is possi le that in the cells of the organism where mitochondria are to found in close contact with the remaining cytoplasm, the introduced barbiturate does not have a perceptible effect on the enzyme systems of phosphorylation. This fact was observed in the experiments of I. I. Niselovska (see Table 1) where the composition of the extracts included mitochondria. In other words, it is possible that the uncoupling effect of the barbiturates in the suspension of large granules, both in our experiments and in those conducted by Brody and Bain, were the result of a certain "lack of full value" of the enzyme systems of the granules as a result of their artificial isolation from the cell.

Independent of the considerations set out, the fact which we demonstrated of unequal distribution in the renal cells of sodium amytal introduced into the organism in our opinion deserves attention. Of interest is the study of the distribution of the introduced barbiturates between the cyto-structural formations of other organs and in particular in the cells of the central nervous system.

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