THE EFFECT OF SODIUM AMYTAL ON THE RESPIRATION AND PHOSPHORYLATION OF THE SYMPATHETIC GANGLION OF THE CAT

E. P. Chetverikova

Laboratory of Biochemistry (Head, Active Member AMN SSSR S. E. Severin) of the Institute of Pharmacology and Chemotherapy of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR S. E. Severin)
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It has been shown by experiments in vitro that barbiturates have the property of inhibiting respiration and phosphorylation in the tissue of the brain and other organs [4, 18]. During sleep or barbiturate narcosis, however, instead of a fall in the content of high-energy phosphorus compounds in the tissues (as might have been expected) on the contrary, an increase in the content of ATP [6] and phosphocreatine [1] is observed. We accordingly investigated the effect of a barbiturate on oxidative phosphorylation in nerve tissue, changes in the functional condition of which could be recorded. The tissue selected was the superior cervical sympathetic ganglion of the cat, and the depression of the function of the ganglion after administration of the narcotic (sodium amytal) was recorded mechanographically.

Information on the metabolism and composition of the superior cervical ganglion is scanty, apart from a fairly large volume of research into the mediators of nerve excitation. The respiration of the sympathetic ganglion was investigated by Larrabee [9] by means of a platinum electrode, and it was found that the intensity of oxygen absorption by the ganglion in the presence of glucose was several times greater than the respiration of peripheral nerve, but did not reach the intensity of this process in the cerebral cortex. A comparatively high glycogen content [10, 20] and an intensive process of glycolysis [7, 16, 17] have been found in the superior cervical ganglion. The presence has been demonstrated of phosphorus compounds, including an adenylic system [2, 8], and of certain enzymes of phosphorus metabolism – adenosinetriphosphatase [3] and acid and alkaline phosphatase [11, 20].

In the present paper findings are presented relating to the action of sodium amytal on respiration and phosphorylation of the ganglion tissue, investigated in a Warburg apparatus.

METHOD

Cats were anesthetized with urethane, and the superior cervical ganglia extracted, weighed, sliced longitudinally, and placed in an incubation mixture in the flask of a Warburg apparatus. The usual weight of the ganglion was 10-13 mg. In order to detect the absorption of oxygen by such a small quantity of tissue, special vessels were used with a volume of about 3 ml, which led to a three- to fivefold increase in the sensitivity of the method by comparison with experiments using the vessels of normal capacity. The volume of the incubation mixture in the vessel was 0.3 ml, and in the central portion of the vessel was added 0.1 ml of alkali. Incubation was continued for 30 minutes at 36°. The vessels were saturated with oxygen or nitrogen, depending on the aim of the experiment.

For the estimation of phosphorylation the incubation mixture contained (in mM/ml) α-ketoglutaric acid neutralized with NaOH (20), $MgCl_2$ (10), KCl (13), ATP (1), sodium salt of ethylenediaminotetraacetic acid (1), K_2HPO_4 (15), NaF (20) and glucose (20). The mixture also contained hexokinase (0.1 ml of the enzyme per sample), which was obtained by the method of Berger and co-workers [13], the isolation being carried as far as stage 3a.

The decrease in mineral phosphorus was determined by the difference between its content in the test samples before and after incubation, and in certain experiments two tests were performed before incubation: 1) 0.3 ml of incubation mixture + 0.3 ml 5% trichloroacetic acid, and 2) 0.3 ml of mixture + 0.3 ml 5% trichloroacetic acid + ganglion. The P content in the two samples before incubation was identical to within 5 μg (see Table 2), and only the former was therefore performed in the experiments with sodium amytal. After incubation 0.3 ml 5% trichloroacetic acid was added. The contents of the experimental and control vessels were transferred to 0.6 ml of 2.5% trichloroacetic acid in a mortar, the ganglion was macerated, and the suspension centrifuged. The mineral phosphorus content of the trichloroacetic extract was determined by the method of Berenblum and Chain [12].

TABLE 1. Respiration and Phosphorylation in the Superior Cervical Ganglion of the Cat

Weight of ganglion (in mg)	Absorption of oxygen (in μg-, atoms per ganglion)	Consumption of P (in μg-, atoms per ganglion)	P:O ratio
12	0,63	0,60	1,0
15	0,63	1,24	1,9
14	0,80	1,19	1,5
12	0,57	0,66	1,2
11	0,57	0,44	0,8
10	0,50	0,55	1,1
16	0,51	0,65	1,2
10	0,40	0,47	0,9
13	0,63	0,60	0,9
10	0,53	0,68	1,3
13	0,63	0.80	1,3

TABLE 2. Content of Mineral Phosphorus (in μg) in Samples after Incubation of the Ganglion in Anaerobic Conditions

Before incubation		Mean		Difference
without tissue	with tissue	Before incubation	after incubation	
173,6	178,8	176,4	175,6	−0,8
153,9	158,4	156,2	166,3	+10,6
142,6	150,3	146,5	146,0	+0,5
153,1	157,1	155,0	160,2	+5,2
152,5	157,3	154,9	165,0	+10,1
156,0	156,0	156,0	162,6	+6,6

During extraction with butanol, the test tube contained 0.2 ml trichloroacetic extract, 2.2 ml butanol, 0.4 ml ammonium molybdate, and 1.6 ml water. The colorimetric test with $SnCl_2$ was performed on 0.5 ml of butanol extract. Sodium amytal was injected intravenously in the form of a 1% solution, slowly and in association with artificial respiration. The physiological state of the ganglion was estimated mechanographically by the reaction of the nictitating membrane to stimulation of the preganglionic trunk of the sympathetic nerve with the electric current. The source of current was an electronic stimulator generating impulses with a duration of 0.5 millisecond and a frequency of 25 cps. The stimulation lasted 10 seconds, and the intervals between stimuli were of 3-5 minutes.

RESULTS

The determinations of the endogenous respiration of the ganglion in an incubation mixture containing only Na_2HPO_4 (5mM), $MgCl_2$ (10 mM), and NaCl (148 mM) showed that in these conditions the ganglion tissue is capable of absorbing oxygen at a constant rate for several hours. The mean value of Q_{O_2} for 6 experiments was 4.1. The addition of 20 mM of glucose stimulated respiration, and the mean value obtained in these circumstances (Q_{O_2} = 5.3) was somewhat higher than that found by Larrabee for the superior cervical ganglion of rats by means of the platinum electrode. The addition of other oxidation substrates–pyruvic acid (5 mM), together with malic (2 mM) or ketoglutaric (20 mM)–also stimulated the absorption of oxygen by the ganglion, although in these experimental conditions the absorption of oxygen was not accompanied by absorption of mineral phosphorus from the incubation medium.

To detect the presence of phosphorylation, the tissue was incubated with hexokinase and glucose as a phosphate group acceptor, and with fluoride to depress the phosphatases present in the ganglion. The composition of the incubation mixture was close to that used by Case and McIlwain for the investigation of oxidative phosphorylation in a brain homogenate [15]. As substrate we used ketoglutaric acid. The results obtained are shown in Tables 1 and 2. As may be seen from Table 1, in an atmosphere of oxygen, consumption of mineral phosphorus from the incubation medium took place, to the extent of 0.7 μg-atoms per ganglion, on the average.

In the experiments in which no hexokinase was added, phosphorylation did not always take place. If no fluoride was added to the incubation mixture, an increase in inorganic phosphorus was observed.

It must be pointed out that consumption of mineral phosphorus took place when its content in the incubation mixture was comparatively high-about 5μg-atoms. The initial phosphorus concentration, however, could not be decreased, for a lowered content in the mixture led to a fall in the intensity of phosphorylation. Special experiments of a methodic character showed that against this background changes in the inorganic phosphorus content could readily be detected, starting from 5 μg. Values of consumption of phosphate exceeding this amount could therefore be regarded as significant.

It may be seen from Table 2 that no consumption of phosphorus took place in an atmosphere of nitrogen, but that a slight increase in its content was observed. The absence of phosphorylation in anaerobic conditions showed that the consumption of phosphorus in an atmosphere of oxygen could be attributed to oxidative phosphorylation.

In the next series of experiments, we investigated the effect of sodium amytal on oxidative phosphorylation in the ganglion tissue. Sodium amytal has the strongest ganglion-blocking properties of the narcotics [4,9]. The block due to sodium amytal passed off rapidly (Fig. 1,b), and repeated injections of the bartiturate were required in order to sustain it. For determination of respiration and phosphorylation, ganglia were extracted at different periods after a complete block of transmission had been obtained. Another ganglion, extracted from the same cat before administration of sodium amytal, served as a control. As a first step it was established that the values of respiration in the right and left ganglia were

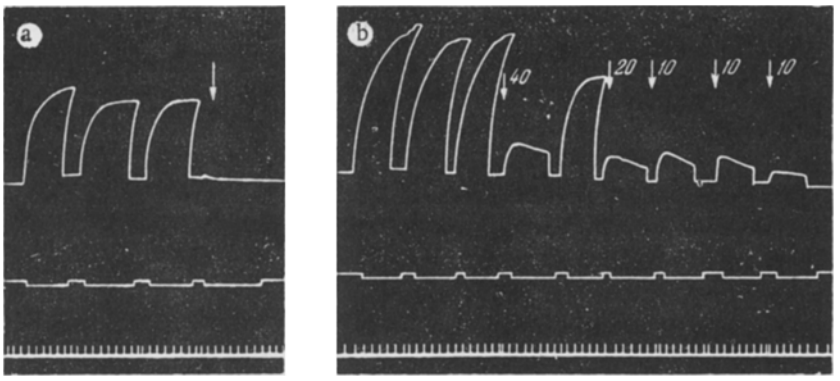

Fig. 1. Effect of sodium amytal on the transmission of impulses through a ganglion. a)A single injection of sodium amytal (30 mg/kg body weight); b) repeated injection of sodium amytal. Significance of the curves (from above down): contraction of the nictitating membrane, stimulation marker, time marker (0.5 second). The arrows denote injection of sodium amytal, and the figures, the dose in mg/kg body weight.

closely similar, and that the stimulation to which the experimental ganglion was usually subjected did not affect respiration and phosphorylation.

A single injection of sodium amytal in a dose of 30 mg/kg caused a transient block of transmission (Fig. 1, a) and considerable depression of respiration for the first 10 minutes of incubation (Fig. 2, a), after which it recovered. A more prolonged depression of transmission was achieved by the repeated administration of sodium amytal (Fig. 1, b). In spite of the large amounts of barbiturate injected, the same results were obtained as after a single injection — depression of respiration at the beginning of incubation and subsequent recovery (Fig. 2, b).

The findings with respect to respiration and phosphorylation in the experimental and control ganglia throughout the period of incubation are given in Table 3. It may be seen from this table that the consumption of mineral phosphorus in the control and experimental ganglia of the same animal was equal, although the level of phosphorylation in the individual animals differed very greatly. The experiments in an atmosphere of nitrogen showed that anaerobic phosphorylation was not present in the ganglion after administration of sodium amytal. The respiration of the experimental ganglion throughout the period of incubation was, on the average, 10% lower than that of the control. Since the margin of error of determination of phosphorus consumption was

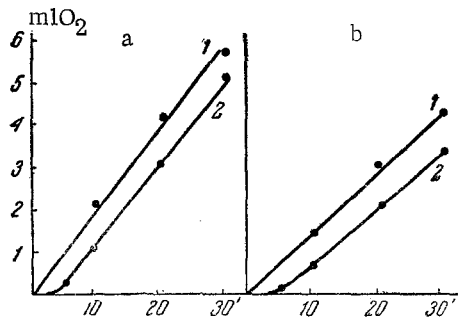

Fig. 2. Effect of sodium amytal on absorption of oxygen by the ganglion in the full incubation mixture. 1) Before injection of sodium amytal; 2) after injection; a) dose of 30 mg/kg injected; b) dose of 70 mg/kg body weight injected.

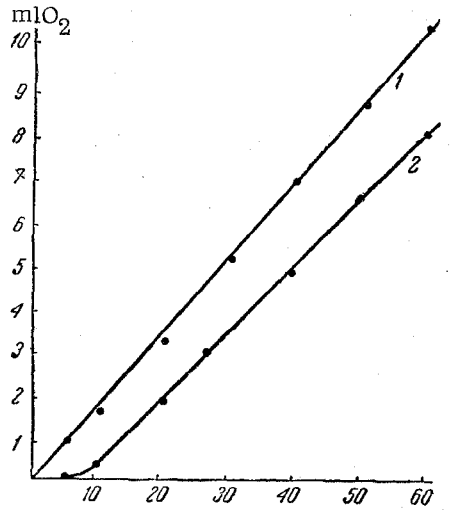

Fig. 3. Effect of sodium amytal (70 mg/kg) on absorption of oxygen by the ganglion in an incubation medium containing NaCl, K_2HPO_4 and ketoglutaric acid. 1)Before injection of sodium amytal; 2) after injection.

TABLE 3. Respiration and Phosphorylation in the Superior Cervical Ganglion of the Cat after Administration of Sodium Amytal (in μg-atoms /10 mg tissue)

Dose of sodium amytal (in mg/kg)	Duration of block (in minutes)	Absorption of O_2		Consumption of P	
		control	experiment	control	experiment
20	No block	0,45	0,45	0,47	0,50
30	5	0,50	0,40	0,40	0,20
30	5	0,47	0,40	0,38	0,30
30	5	0,54	0,56	0,70	0,77
70	15	0,51	0,46	0,53	0,51
90	30	0,54	0,52	0,30	0,30
100	35	0,40	0,30	0,20	0,20
120	35	0,59	0,54	0,26	0,31
150	50	0,60	0,54	0,60	0,60

high, a decrease of respiration by 10% could not be reflected in the magnitude of phosphorylation. It may, however, be concluded from the data on phosphorylation that, in spite of the high doses of sodium amytal and the complete inactivation of the ganglion, sodium amytal had no inhibitory action. It should be mentioned that in the few experiments in which the effect of barbiturates has been studied in vivo, no inhibitory action of the drugs has been found,either [5, 14].

Sodium amytal causes a readily reversible block of transmission, accompanied by a transient fall in respiration. The restoration of respiration after the block may be connected with the presence of substances in the incubation mixture which have a favorable influence on this process and, in particular, on phosphorylation (KCl, ATP, glucose–hexokinase, versene). Experiments were accordingly carried out with a simplified incubation mixture and an increased sodium content (NaCl–148 mM, K_2HPO_4–15 mM, ketoglutaric acid – 20 mM). As may be seen in Fig. 3, the same result was obtained as with the full mixture – an almost complete cessation of oxygen absorption was observed during the first ten minutes, after which the initial rate of respiration was restored.

SUMMARY

Respiration (studied manometrically) and phosphorylation were investigated in the tissue of the superior cervical sympathetic ganglia of cats. Reduction of the mineral phosphorus was determined in the presence of α-ketoglutaric acid and fluoride; hexokinase and glucose served as phosphate group acceptors. Reduction of the mineral phosphorus was observed in an atmosphere of oxygen; no binding of the phosphate occurred in anaerobic conditions.

Sodium amytal blocking of excitation transmission through the ganglion induced a strong short-term inhibition of the oxygen absorption. Since the rate of respiration was later restored, the respiratory values, and especially those of phosphorylation, measured during the whole period of incubation (30 minutes), did not materially differ.

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