AMYTAL RESISTANCE IN ANIMALS ADAPTED TO COLD, AND SOME FEATURES PECULIAR TO THE MECHANISM OF ITS NARCOTIC ACTION

S. P. Maslov and I. N. Ivashkina

From the Department of Vertebrate Zoology (Head, Prof. N. P. Naumov)
of the Bio-Geological Faculty of the M. N. Lomonosov Order of Lenin
and Order of the Red Banner State University
(Presented by Active Member of the Akad. Med. Nauk SSSR S. E. Severin)
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Adaptation of homeothermic animals to a low temperature in the surrounding environment, which is achieved by means of several serial supercoolings of the organism, is accompanied by a disturbance in oxidation and phosphorylation (including "free" oxidation) in the skeletal muscles, ensuring survival of the animal under these conditions [2]. With a more prolonged adaptation to low temperature, one also observes a lowering of the P/O coefficient in the mitochondria of the liver of experimental animals [11,12,15,19-22].

The disturbance in oxidation and phosphorylation, observed in these models, is always coupled with a clear change in the structure of the mitochondria, a result of their swelling. In the works of a number of authors, carried out in vitro, it was shown that in this type of disturbance the respiration associated with phosphorylation is blocked by amytal at the level of diphosphopyridine nucleotide [8-10, 13,14,16,17]. The connection between the sensitivity of respiration to amytal and the structure of the mitochondria is caused by the fact that when they swell there is a decrease in the strength of the link between the diphosphopyridine nucleotide and the membrane of the mitochondria. As a result, it begins to escape from the latter, and its oxidation becomes amytal-resistant.* Simultaneously, the interlinking between respiration and phosphorylation decreases.

These data justify postulating that amytal resistance in the respiration of animals under the physiological conditions of the experiment can be used to control a disturbance in oxidation and phosphorylation in vivo. The possibility of this control was tested by us on white mice adapted to low temperature (adult females, weighing approximately 26 grams). Decreasing the interlinking of oxidation and phosphorylation in this model was described earlier.

EXPERIMENTAL METHOD

Adaptation to the low temperature was carried out according to the method described previously [8], at a temperature of -15° for a period of less than 10 hours, an interval at which the mice retained their viability at this temperature. In individual cases, where the indicated level of adaptation was not attained after 10 trainings, the animals were used in an experiment with a lower level of adaptation. In the experiment, the animals were picked on the day after the last chilling. Sodium amytal was injected subcutaneously, in the form of a 0.6% aqueous solution, in a dosage of 115 mg per kg of weight. Prior to this, the total level of gas exchange was determined in the mice, using the method of Kalabukhov [1], at a chamber temperature of 25°. At 40 minutes after the amytal injection the animals were again placed in the gas exchange chamber, and the level of gas exchange was again determined.

*Thus, non-phosphorylating respiration is blocked by amytal in those cases where the disturbance is not related to swelling of the mitochondria, for example in association with 2,4-dinitrophenol, and in a number of other cases [6].

EXPERIMENTAL RESULTS

Table 1 shows that the total level of gas exchange in the animals adapted to cold, upon injection of amytal, was loweredone third as much (15% against 45%) as in the controls (the difference is statistically significant). Also statistically significant was the difference in the level of respiration of the control and experimental animals after amytal inection. The bounds of individual variation in the sensitivity of total gas exchange to amytal were also altered correspondingly in the controls and the animals adapted to cold (Table 2). Thus, the observed differences in inhibition of total gas exchange by amytal, in the control and experimental animals, confirms the data obtained earlier on the disturbance of oxidation and phosphorylation in animals adapted to cold. This makes it possible to use amytal for controlling a disturbance in oxidation and phosphorylation in vivo.

Special attention is merited the great individual variation in sensitivity of the total level of gas exchange to amytal in the control animals (see Table 2). It is known that the degree of interlinking of respiration and phosphorylation in the mitochondria (in vitro) varies considerably, even within the bounds of one series of experiments with uniform conditions for extraction of the mitochondria. The possibility is not excluded that the scattering of the data, observed in this case, is largely the result of individual differences in the degree of interlinking between oxidation and phosphorylation in the individual animals, and is not due to minimal differences in the conditions of extraction. This explanation would conform nicely with the marked individual variability in the sensitivity of the animals to amytal which we observed.

TABLE 1. The Action of Amytal on the Gas Exchange of Control Animals and Animals Adapted to Cold, and the Link Between the Narcotic Action of Amytal and Its Capacity to Suppress Gas Exchange

Animals	No. of animals	Oxygen consumption (in ml per 100 grams of weight of the animal per hour)		Level of respiration after injection of amytal (in percents of the original)*
		before injection of amytal	after injection of amytal	
Control	25	418 ± 13	232 ± 28	55.0 ± 6.5
Adapted to cold	25	410 ± 17	341 ± 22	85.2 ± 5.4
After injection of amytal:				
in the state of deep sleep . .	42	409 ± 12	192 ± 16	47.5 ± 3.9
in the state of light sleep . .	16	427 ± 17	356 ± 19	83.9 ± 3.6
lively animals	8	420 ± 27	446 ± 36	107.1 ± 2.7

*The data in this table was calculated separately for each animal and then totaled. This method was selected because it makes it possible to appraise errors in the obtained result. Using this calculation, there is always a minimal deviation from the percent relationship obtained from the mean values.

We noted that the increase in the resistance of total gas exchange to amytal in the animals adapted to cold correlated with the decrease in narcotic action of the amytal in these animals. We recorded the depth of slumber according to external signs, allowing the animals to be divided into 3 groups: 1) those sleeping at the moment that the gas exchange was determined, and having slept uninterruptedly for a very long period of time (the majority, for over 2–3 hours)–deep sleep; 2) those sleeping at the moment that the gas exchange was determined, but awakening even during the course of the gas exchange determination–light sleep; 3) those awake. As can be seen from Table 3, the percent of animals in which amytal showed a narcotic action was much lower in the group of mice adapted to cold than in the control. This agress with the data indicating that the narcotic action of barbiturates is proportional to their capacity to inhibit tissue respiration [18].

For a more graphic demonstration of this fact, we combined the results obtained on the control mice and the mice adapted to cold, relevant to the intensity of narcotic action of the amytal on the animal (the numbers in this project were somewhat increased, due to the additon of the control animals from a parallel series of experiments). From the data presented, it is apparent that the narcotic action of amytal is proportional to its capacity to inhibit the gas exchange of the animal (see Tables 1 and 2). The difference between the results obtained on the first group of animals (deep sleep) and on the other two is statistically significant.

TABLE 2. Individual Differences in the Reaction of Respiration to the Injection of Amytal in the Control Animals and Animals Adapted to Cold, and the Link Between These Differences and the Depth of the Narcotic Action of Amytal

Animals	Number of animals	Level of respiration in the individual animals after injection of amytal (in percents of the original)							
		0—20	20—40	40—60	60—80	80—100	100—120	120—140	140—160
Control	25	1	11	3	5	3	1		1
		4	44	12	20	12	4		4
Adapted to cold	25		2	2	5	11	3	2	
			8	8	20	44	12	8	
After injection of amytal: in a state of deep sleep	42	1	20	8	9	3			1
		2,4	47,6	19,1	21,4	7,1			2,4
in a state of light sleep	16			2	3	9	2		
				12,5	18,25	56,25	12,5		
lively animals	8					5	1	2	
						62,5	12,5	25	

Note. In the numerator—the number of animals with the given level of gas exchange after injection of amytal; in the denominator—the percent of the total number of animals in the group.

At the same time, our data contradict the McIlwain interpretation of the mechanism of barbiturate's narcotic action [18]. In the opinion of this author, the basic route of action of barbiturates is a depression in the use of adenosine-triphosphate and creatine phosphate, which leads to a secondary inhibition in respiration after filling up the system of acceptors for macroergic phosphate. Thus, this hypothesis connects the mechanism of narcotic action of the barbiturates exclusively with their action on phosphorylating respiration. However, from this point of view it is difficult to explain why there is an increase in the resistance of animals adapted to cold against the narcotic action of amytal, since in this case the high level of oxidation is ensured by non-phosphorylating respiration.

From this point of view, it is also difficult to explain the data of E. P. Chetverikova [7], which shows that an increase in the level of respiration through oxidation of succinate injected into the blood causes awakening of animals that were narcotized with barbiturates. Succinate is oxidized directly by a flavin enzyme, avoiding diphosphopyrine nucleotide and the stage sensitive to amytal. Apparently, it must be postulated that the level of respiration is connected with the narcotic action of amytal also by some sort of more direct means than was postulated in the indicated hypothesis. Obviously, the level of respiration itself, without its connections to the level of oxidative phosphorylation, may be essential for preserving the functional activity of the central nervous system.

TABLE 3. Difference in the Intensity of the Narcotic Activity of Amytal in the Control Animals and the Animals Adapted to Cold

State of the animals after the amytal injection	Control animals		Animals adapted to cold	
	abs. no.	%	abs. no.	%
Deep sleep	20	80	8	32
Light sleep	5	20	9	36
Awake	0	0	0	32

SUMMARY.

As demonstrated, amytal resistance of respiration in the cold-adapted albino mice, in which respiration and phosphorylation disturbances were noted formerly, was much higher than in the control animals. This fact confirms the uncoupling of respiration and phosphorylation following cold adaptation and permits the use of amytal for control of this phenomenon when the uncoupling mechanism is connected with the increased amytal resistance. It was also shown that amytal resistance of respiration in the cold-adapted animals correlates with their increased resistance to the narcotic action of amytal. This result confirms the data of other authors on the connection between the hypnotic effect of barbiturates and their ability to inhibit the respiration, but is against the Mc Ilwain interpretation of their hypnotic effect mechanism. Since, of the given model, a high respiration level is maintained at the expense of non-phosphorylation reactions, it is supposed that in definite physiological conditions there may exist a more direct relationship between the respiration level and the functional cerebral activity.

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