THE INFLUENCE OF PHARMACOLOGIC SLEEP UPON THE CONTENT OF CERTAIN LABILE PHOSPHORUS COMPOUNDS IN VARIOUS ORGANS AND TISSUES

A. M. Alekseeva and L. G. Magazanik

From the Department of Biochemistry (Chairman: Yu. M. Gefter) 1st Leningrad Medical Institute

(Received March 7, 1956. Presented by Acting Member Acad. Med. Sci. USSR S. E. Severin).

In the present communication we present results of investigations into the influence of pharmacologic sleep upon the creatine phosphate (CP) in the brain and internal organs. Part of our material has already been published [2].

The experiments were done on white rats. Sleep was produced by the subcutaneous introduction of a 2.5% solution of sodium amytal in the dose of 0.1 g. of the dry preparation to 1 kg wt. Such a dose produces a sleep lasting about 3 hours. Pain reflexes are preserved.

The following tissues were studied: brain, skeletal muscle, heart, liver, kidneys, spleen and gonads.

As is known, CP has a very labile bond. Therefore the brain samples have to be taken with definite precautions.

We used in our work a technique that we had worked with previously, [3]. The rats under light ether narcosis were immersed by the back portion of their head into a vessel with liquid oxygen. It is interesting to observe that when this is done the rat continues to breath almost up to the completion of freezing. . The blood stays bright red, being saturated with oxygen. Besides this, it should also be noted that the use of this method of freezing the brain does not produce convulsions, which is very important in studying muscles. We attempted also other methods of obtaining brain tissue, in part, by freezing the entire rat without narcosis. But with this method we always obtained lower levels of CP in the brain.

Therefore we recommend our method of freezing only the head, (especially under narcosis) when labile bonds are being analyzed in brain tissue, the freezing beginning from the posterior portion. By means of this method we were able not only to obtain higher levels of labile phosphorus bonds but also a smaller variation in the brain as well as in the muscle.

In Table I, are given the data on the content of CP in the normal brain, as well as during the period of sleep produced by sodium amytal.

As can be seen from Table I, the CP content of the brain during sleep is increased. In the period of awakening, when the rats are beginning to make feeble movements, the CP content diminishes, although it does not reach the levels established in control animals. The maximum elevation of CP in the brain occurs about one hour after the beginning of sleep.

What, then, is the mechanism of the heightening and subsequent lowering of the CP level in the brain during the period of sleep? The rapidity with which the heightening is attained, as well as the return lowering almost to base level, compels the supposition that the process of redistribution takes place among the various fractions of acid-soluble phosphorus.

We attempted to prolong the sleep in animals by additional introduction of the hypnotic (2 hours after the beginning of sleep), supposing that there might be prolongation of the sleep and an increase in the CP. We were unsuccessful in this as the animals withstood the additional hypnotic very poorly, convulsions being frequently observed; the level of the CP in these animals dropped.

TABLE 1

CP Content in the Brain of White Rats During Normal and Sodium Amytal Induced Sleep

Control animals	Pharmacologic sleep			
	5 minutes	1 hour	2 hours	2½-3 hours (moment of awakening)
	Creatinine from CP (in mg %)			
35.3	40.5	53.4	49.4	42.4
37.7	44.4	54.9	46.0	48.7
31.0	40.1	58.3	45.8	48.3
32.3	42.1	55.6	46.4	35.6
31.7	22.4	51.8	44.0	43.9
38.6	45.3	52.0	49.2	38.6
35.8	42.5	51.5	44.5	50.2
30.1	47.8	54.7	48.2	37.1
30.3	41.3	53.3	47.3	—
36.1	40.3	52.9	47.4	—
38.4	41.6	50.1	46.8	—
35.1	—	49.8	—	—
34.4	—	—	—	—
Average 34.3	42.5	53.2	46.8	43.1

TABLE 2

The Phosphorus Content in Skeletal Muscles in White Rats During Normal and Sodium Amytal Induced Sleep

Control Animals				After 1 hour and 30 min. of sleep			
Inorganic phosphorus in (mg %)	Phosphorus (from ATP in mg %)	Creatinine from CP (in mg %)	Acid soluble phosphorus in (mg %)	Inorganic phosphorus (in mg %)	Phosphorus from ATP (in mg %)	Creatinine from CP (in mg %)	Acid soluble phosphorus (in mg %)
32.0	27.7	174	132	32.0	26.4	206	127
29.1	26.0	157	128	27.3	32.0	219	137
34.3	32.0	206	136	30.5	40.5	260	156
30.0	31.2	207	142	23.0	36.0	239	144
37.0	30.7	211	141	22.2	30.3	201	120
25.8	24.4	181	122	18.7	32.5	222	121
21.1	39.0	193	139	24.1	36.4	227	136
30.0	42.0	208	143	27.3	34.9	235	142
20.0	31.0	185	124	19.0	35.0	232	135
28.5	28.2	189	140	22.0	29.8	215	129
Average 28.8	31.0	191	133	Average 24.5	33.4	226	135

According to these findings it is difficult to think that sleep serves the period of restoration of the high-energy phosphorus bonds which have been used up in the period of wakefulness, as is the opinion of some investigators [5]. All that the rat has to do is to begin feeble movements at the end of the sleep period to have the CP content begin dropping almost to normal.

The changes in the CP levels during the period of sleep in rats seem to point, rather, to the conclusion that there is really a weakening in some other chemical processes during both sleep and wakefulness. It is possible that the indicated changes in the CP level during sleep are connected with a diminution in the processes of oxidation and glycolysis. Owing to this the consumption of high-energy phosphorus bonds is reduced and in accord with the lowered metabolic rate, their equilibrium with other phosphorus compounds is restored, i.e., there must occur, apparently, changes comparable to those observed in the muscles when they change from a condition of rest to intensive work and vice versa.

TABLE 3

The CP Content in the Organs of White Rats Normally and in the Period of Sleep Caused by Sodium Amytal

Organ	Creatinine from CP (in mg %)		Organ	Creatinine from CD (in mg %)	
	Normal	Sleep 1 hr. 30 min.		Normal	Sleep 1 hour 30 min.
Heart	10.4 15.4 19.2 25.8 19.8 14.2 —	22.0 15.0 18.1 14.2 16.3 13.8 22.0	Gonad	2.2 0.0 0.0 0.7 0.0 0.0 —	0.0 1.2 0.1 0.0 0.0 0.5 0.4
Average	17.5	18.2	Average	0.5	0.3
Liver	3.8 3.8 2.2 3.7 3.5 0.0 —	4.7 3.9 3.8 2.5 4.5 3.2 2.8	Spleen	2.3 2.0 1.1 2.5 1.6 2.7 —	2.8 4.9 3.1 3.7 2.2 3.2 3.0
Average	2.8	3.6	Average	2.0	3.2
Kidneys	1.1 1.5 2.6 3.1 0.2 1.9	3.3 2.1 1.8 3.0 5.4 1.5 2.3			
Average	1.7	2.8			

It appears to us that the supposition concerning the redistribution of the acid-soluble phosphorus fraction, is confirmed by the findings of our experiments in which we determined separate fractions of the acid-soluble phosphorus in the muscles of rats both in the waking state and in the period of sodium amytal induced sleep, [Table 2].

The creatine phosphate in these experiments was determined, as in the brain, by creatinine and adenosinetriphosphate (ATP) determined by the phosphorus which separated after boiling it for ten minutes in 0.1 N HCl after preliminary precipitation with barium acetate. The inorganic phosphorus was determined by the difference between the phosphorus determined by the method of Fiske-Subbarow in the fraction inorganic phosphorus + CP phosphorus, and the phosphorus calculated for creatinine from CP.

As seen from the data in Table 2, in the muscles of rats during the period of sleep, for 1 hour and 30 minutes there continue certain changes in the phosphorus content of certain fractions of the acid soluble phosphorus. Especially noticeable is the elevation in the CP level (on the average about 18%) and some lowering of the inorganic phosphorus level (around 15%).

We could not determine any marked increase in the ATP level during the period of sleep, as was determined in the work of S.F. Epshtein [5], although it should be noted, that this author produced sleep in rats by a somewhat different method which was somewhat more prolonged. It is a cause for surprise that S. F. Epshtein found low levels of CP phosphorus in normal rats (on the average, 18.8 mg). Even if we determined CP by the old method (by its phosphorus), we found levels averaging 40 mg %. The values of the inorganic phosphorus in the muscles of normal rats appear, contrariwise, quite high (61.3 mg %). It is to be regretted that the author does not state how he obtained his material and how he made the phosphorus determinations.

In Table 3 are shown the results of experiments tracing the influence of pharmacologic sleep upon the CP content in other organs of white rats.

The tabulated results shown in Table 3 appear to prove that the heights of the CP levels of the various organs in the animals (in liver, kidneys, spleen), with the exception of the seminal vescicles in the period of sleep are somewhat higher than in the waking state. But it must be said that these and other quantities are so small that we can only speak of traces of CP. It is difficult to talk of an accumulation of this substance in the period of inhibition. The increase is so insignificant that it is within the limits of the errors of the method (e.g., the data on the CP content in the heart). The absence of CP in the gonads deserves further examination. It cannot be found here either in the waking state or in the period of sleep. In spite of the high level of creatine in this organ, apparently synthesis of CP for some reason does not occur here.

Our experiments permit the following conclusions.

In the period of sleep induced by sodium amytal, there occurs a definite elevation of CP in the brains of rats. At awakening the level falls, remaining, however, somewhat above base level.

During the period of sleep there also is a noticeable CP elevation in the muscles of rats as a result of a redistribution of the fraction of acid-soluble phosphorus, at which time there is a definite decrease in the quantity of inorganic phosphorus. The ATP level is elevated very slightly.

In the liver, kidneys, and the spleen, during the period of sleep as well as during the period of wakefulness there are only traces of CP. In the heart its level during the period of sleep is practically unchanged. In the seminal vescicles, CP is not found either during sleep or wakefulness.

LITERATURE CITED

[1] Alekseeva, A. M. Acid Catalytic Reaction Converting Creatine into Creatinine and the Formation of Creatinine in the Organism,* Dissertation (Leningrad, 1952).

[2] Alekseeva, A. M. Biokhimiya 16, 2, 97-103 (1951).

[3] Epshtein, S.F. Biochemistry of the Nervous System* (Kiev, 1954).

[4] Shapot, V. S. Uspekhi Sovremennoi Biol. 34, 2, 234-257 (1952).

[5] Epshtein, S. F. Biochemistry of the Nervous System* (Kiev, 1954), pp. 211-219.

* In Russian.