CHANGES IN CERTAIN ASPECTS OF LIPID METABOLISM IN RATS WITH EXPERIMENTAL INSOMNIA

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Adaptation of the organism to external environmental conditions is based on processes of biochemical adaptation associated with changes in the activity of enzyme systems [5, 6]. Because of the special importance of prolonged neuropsychic stress in the etiology of hypertension and atherosclerosis [1, 3], the study of the effect of prolonged deprivation of sleep on certain aspects of lipid metabolism is of the utmost importance.

In the present investigation the model for reproduction of stress was experimental insomia, by means of which a state of stress could be produced and the nervous processes ultimately and completely exhausted. The object of the investigation was to study the character of possible changes in lipid metabolism in rats.

Prolonged deprivation of sleep in animals can be regarded as a powerful stressor, to the action of which the organism responds by the nonspecific, generalized mobilization of its internal reserves. In this sense the effect of sleep deprivation should be similar to the effect of other stressors which, as many investigators have shown, give rise to characteristic changes of lipid metabolism, starting with the intensification of lipolysis and the mobilization of nonesterified fatty acids (NEFA) [2, 9, 11, 14, 15]. However, in each individual case, depending on the properties of the stressor, the response of the organism, including the generalized reaction expressed, in particular, by mobilization of the lipids, must present specific features.

No reports could be found of investigations to determine the effect of sleep deprivation on lipid metabolism. On a priori grounds it may evidently be assumed that prolonged sleep deprivation, on the one hand, must produce generalized changes in lipid metabolism characteristic of the state of stress, and on the other hand it must lead to specific changes characteristic for each concrete case.

EXPERIMENTAL METHOD

The experimental animals were male Wistar rats weighing 150-270 g. The animals were kept in a special slowly revolving drum (constructed by A. A. Pokrovskii and I. E. Malakhov), in which they could not sleep because they were compelled to move continuously in order to maintain the horizontal position.

The model of insomnia which was used is not a pure model. There is no doubt that definite importance during the action of a general pressor on animals is attached to the continuous and enforced movement, which must evidently cause fatigue, not so much of the muscles working in these circumstances (the distance covered by the rats in the course of the 24 h was relatively small), as of the nerve centers which received a continuous stream of afferent impulses from the proprioceptors. In this investigation, the combined action of at least two factors was therefore studied: true insomnia which, in the authors' opinion, was still the chief factor, and intensified proprioceptive impulsation. It was not possible to differentiate these two factors in the present investigation.

For convenience of description, the state of the experimental rats will subsequently be called experimental insomnia.

The animals received a standard diet and water ad lib. Preliminary experiments showed that in these conditions, the animals died mainly on the 5th or 6th day depending on their age. The rats were sacrificed 1 and 4 days after being placed in the drum. Control animals kept in ordinary conditions and receiving the same diet were sacrificed along with the experimental rats. The concentration of NEFA [12],

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TABLE 1. Effect of Sleep Deprivation on Content of Lipids and Their Fractions in Blood Plasma and Liver of Rats

Index	Control		After 1 day		P	After 4 days		P
	No. of rats	M±σ	No. of rats	M±σ		No. of rats	M±σ	
Blood plasma Total lipids (in mg%)	19	198±16	18	255±14	<0,001	32	304±44	<0,001
β-lipoproteins (in mg%)	18	220±14	18	300±13	<0,001	18	353±33	<0,001
Cholesterol (in mg%)	23	38±4,2	18	49±3,1	<0,001	34	57±5,2	<0,001
NEFA (in meq/liter)	20	0,302±0,021	18	0,595±0,052	<0,001	17	0,423±0,164	<0,01
Liver (in mg%)	21	2670±368	18	3790±162	<0,001	37	4000±578	<0,001
Cholesterol (in mg%)	18	223±22	18	336±17	<0,001	27	432±38	<0,001

total lipids [13], β-lipoproteins [8] and cholesterol [16] in the blood plasma were determined. The content of total lipids [7] and cholesterol [16] and the activity of tributyrinase and triacetinase [4] in the liver were determined. The activity of the enzyme was expressed in units (in micromoles of butyric or acetic acid obtained during hydrolysis of tributyrin or triacetin by 1 g liver tissue per minute).

EXPERIMENTAL RESULTS

When the rats were deprived of sleep they lost approximately 20% of their body weight in 4 days; the weight of the liver, on the other hand, increased in this period on the average by 10-15%.

Results showing the changes in the investigated lipid fractions in the blood plasma and liver of the rats after deprivation of sleep for 1 and 4 days are given in Table 1.

Clear changes in lipid metabolism were found 24 h after the beginning of the experiment. At this time increases were observed in the concentration of total lipids (by 29%), β-lipoproteins (by 36%), cholesterol (by 29%) and, in particular, NEFA (by 97%) in the blood plasma, and increases were also found in the contents of total lipids (by 42%) and cholesterol (by 50%) in the liver. After 4 days the concentrations of total lipids, β-lipoproteins, and cholesterol in the blood plasma showed a further increase although the NEFA concentration in the plasma has begun to fall, but still remaining much above its initial level. In the liver the content of total lipids and cholesterol at this time likewise showed a further increase.

In connection with these changes in lipid metabolism of the experimental animals, shown by the accumulation of lipids and of their fractions in the liver and blood plasma, it was interesting to consider possible changes in the activity of the lipid metabolism enzymes in the liver, notably tributyrinase and triacetinase, hydrolzying glycerol esters with fatty acids of low molecular weight and, thereby, responsible for the further oxidation of these acids. The results obtained are given in Table 2.

Twenty-four hours after the beginning of enforced sleep deprivation no significant changes in the tributyrinase and triacetinase activity in the liver tissue of the animals was found. Marked changes in the activity of these enzymes were observed after 4 days, when the tributyrinase activity was reduced by approximately 60% and the triacetinase activity by 36%.

Hence, in rats deprived of sleep lipids accumulated in the liver and blood plasma, and in the later periods, this was accompanied by marked depression of tributyrinase and triacetinase activity in the liver tissue. To examine the detailed mechanisms of the observed changes further investigations are necessary, but certain hypotheses may be put forward even on the basis of the existing material. Attention is first drawn to the fact of the marked increase in NEFA concentration in the blood plasma of the animals 24 h after the beginning of the experiment, undoubtedly a response of the organism to the agent used. In this respect the effect of experimental insomnia was to some extent similar to the action of other stressors, although a distinguishing feature of the model used was the long duration of its action. The NEFA mobilized in large quantities in the blood stream from the adipose tissue are partially used by working organs

TABLE 2. Effect of Sleep Deprivation on Enzyme Activity in Liver Tissue of Rats

Enzyme	Control		After 1 day		P	After 4 days		P
	No. of rats	M±σ	No. of rats	M±σ		No. of rats	M±σ	
Tributyrinase (in units)	26	110±13	12	113±9	>0,05	16	50±5	<0,001
Triacetinase (in units)	26	18,6±0,6	12	19,0±1,3	>0,05	16	12,8±0,6	<0,001

(heart, muscles), but since the work performed by the animal was small, the NEFA evidently passed mainly into the liver, where the synthesis of triglycerides and cholesterol from them with the subsequent formation of β-lipoproteins and their passage into the blood were sharply intensified.

Since the utilization of β-lipoproteins, like that of NEFA, by the working organs was not significantly increased, the intensification of the processes described above, starting from mobilization of NEFA, led to the accumulation of lipids in the liver and blood. In the early stages, despite important changes in the content of lipids in the liver, the tributyrinase and triacetinase activity in the liver remained unchanged. Later, with an increase in the duration of enforced sleep deprivation of the animals, which may probably be regarded as a disturbance of the adaptive reactions of the organism, the content of lipids in the liver tissue and blood increased still further. Four days after the beginning of the experiment, i.e., 1-2 days before death of the animals, the concentration of NEFA in the blood plasma fell slightly below that found in the initial period, although it still remained above the original level. This was possibly the result of the developing exhaustion of the neurohumoral mechanisms maintaining the organism in a state of prolonged stress in response to the continuous stressor action. At this time, the tributyrinase and triacetinase activity in the liver tissue was significantly lowered.

Hence, the reaction of adaptation during experimental insomnia, as expressed by an increase in the content of lipids and cholesterol in the blood and liver tissue and attaining a considerable magnitude, at the same time has a harmful influence on the organisms, for this reaction may be regarded as facilitating atherosclerosis, which is associated with an increase in the content of lipids and cholesterol in the liver and blood.

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