ADAPTATION TO PERIODIC HYPOXIA REDUCES ETHANOL CONSUMPTION AND ABSTINENCE-RELATED VISCERAL DAMAGE DURING ALCOHOL WITHDRAWAL IN CHRONIC ALCOHOLIC ANIMALS

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Adaptation to periodic hypoxia is known to involve activation of central stress-limiting systems, and in particular, those like the serotoninergic [1] and opioidergic [2] systems. This adaptation, which is essentially a course of sessions of hypoxia, each of which is followed by reoxygenation, leads to a regular increase in activity of the antioxidant enzymes in the liver, heart, and brain [3, 4, 5]. During long-term alcohol administration several mutually opposite changes develop: activity of the stress-limiting systems is reduced [6, 7, 8], lipid peroxidation (LPO) is activated, LPO in the liver being realized through the cytochrome P-450 system [8]. Withdrawal of ethanol regularly induces a stress reaction [9] which, because of the reduced efficacy of the stress-limiting systems, becomes excessive and may play an important role in the development of the abstinence syndrome. The negative role of the stressor induced action of LPO, developing in the liver under these circumstances, may with a high degree of probability be aggravated by the fact that during chronic ethanol consumption it is the substrate of action of the H_2O_2 generated by cytochrome P-450 [8], but in the absence of ethanol, cell membrane lipids may act as this substrate during the period of abstinence, and abstinence-related damage may take place. These considerations led us to suggest that adaptation to periodic hypoxia may limit alcohol consumption due to activation of stress-limiting systems, and also abstinence-related damage due to an increase in power of the antioxidant systems in the liver and other organs.

The aim of this investigation was to assess the effect of adaptation to periodic hypoxia on alcohol consumption and on the severity of the syndrome of abstinence-related damage to the liver and heart in chronic alcoholic animals with established ethanol dependence.

EXPERIMENTAL METHOD

Altogether four series of experiments were carried out on male Wistar rats of the same age, kept simultaneously in the same animal house. Series I consisted of control animals kept in the animal house for 11.5 months; series II consisted of animals kept in the animal house for the same period of time but subjected to alcoholization from the beginning of their stay through the provision of free choice between water and 15% ethanol solution. Animals of series III were kept in the animal house for the same period of time and after 8.5 months of alcoholization they underwent adaptation to hypoxia, while still continuing to receive alcohol. Series IV consisted of control

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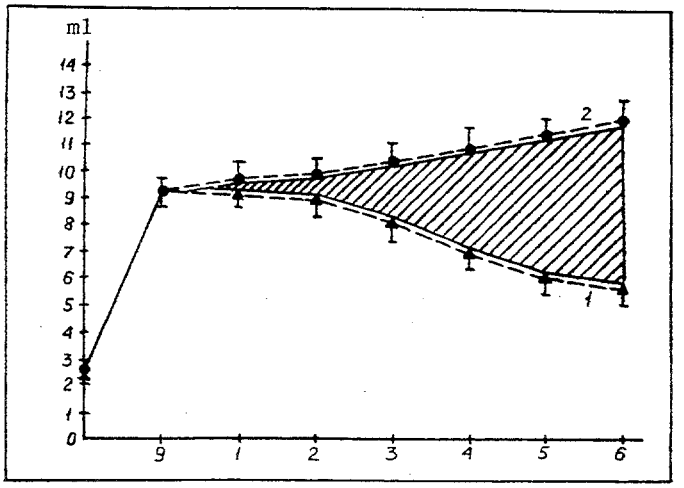

Fig. 1. Effect of adaptation to periodic hypoxia on consumption of 15% ethanol solution by alcoholic unadapted animals (top curve) and alcoholic animals adapted to periodic hypoxia (bottom curve). Shaded zone characterizes quantitatively reduction of ethanol consumption evoked by adaptation. Ordinate, quantity of ethanol solution consumed (in ml). Abscissa, duration of adaptation (in weeks). 1) Adapted animals, 2) unadapted.

animals, kept in the animal house for the same period of time, and after 8.5 months they were adapted to periodic hypoxia for 40 days. Adaptation to hypoxia was carried in a pressure chamber, in which the animals were kept for 5 h daily During the 1st week the animals were gradually lifted up to an altitude of 1000 to 5000 m in the course of five sessions, and subsequent sessions of hypoxia were carried out at that altitude. Throughout the experiment the daily consumption of ethanol solution was recorded. A withdrawal syndrome was induced by preventing the animals from reaching the drinking bowl containing ethanol solution for 48 h. The severity of the withdrawal syndrome was assessed on the basis of the degree of abstinence-related analgesia and the quantity of ethanol consumed after resumption of access to the drinking bowl [7]. The intensity of abstinence analgesia was determined as the length of time the animal stayed on a metal plate heated to 51°C [10]. Liver damage in the abstinence syndrome was assessed by measuring the concentrations of LPO products in the liver tissue. Conjugated dienes were determined as in [11] and TBA-positive products as in [13]. Serum levels of fructose-monophosphate aldolase (FPA, as in [12]) and gamma-glutamyl transpeptidase (GGT, as in [14]) were determined simultaneously. An increase in the serum concentration of these enzymes is traditionally used as a test of liver damage. To assess disturbances of cardiac contractility, the pressure in the left ventricle was recorded electromanometrically and on the basis of the resulting curve, the developed pressure was calculated. The electrical stability of the heart was assessed by determining the threshold of ventricular fibrillation. The minimal strength of current of a single premature pulse, triggered by the R wave by means of an electrical stimulator, leading to the development of fibrillation, was recorded. In a parallel study, conjugated dienes and TBA-positive products were determined in the ventricular myocardium of animals of the same series by the same methods as in the liver.

EXPERIMENTAL RESULTS

The curves in Fig. 1 demonstrate the effect of adaptation to daily alcohol consumption by chronic alcoholic rats. They show that 8.5 months after the beginning of alcohol intake, ethanol consumption in the animals of the

TABLE 1. Effect of Adaptation to Hypoxia on Intensity of Physical Ethanol Dependence

Series of experiments	Ethanol consumption, ml		Time staying on hotplate (sec) after withdrawal of ethanol for 48 h
	before withdrawal of ethanol	After withdrawal of ethanol for 48 h	
Control (n = 12)	—	—	65,4±13,3
Adaptation (n = 12)	—	—	68,1±12,6
Alcoholization (n = 15)	12,3±2,7	26,8±3,5 $p<0,01$	146,4±11,3
Alcoholization + adaptation (n = 15)	5,8±0,7	6,2±0,9 $p>0,1$	79,0±6,7
Significance of differences:			
p_{1-3}	—	—	<0,001
p_{1-4}	—	—	>0,1
p_{3-4}	<0,01	<0,001	<0,01

Legend. Here and in Tables 2 and 3, n denotes number of animals.

TABLE 2. Hepatospecific Enzymes and LPO Products in Blood Serum and Liver during Adaptation of Chronic Alcoholic Animals to Hypoxia

Series of experiments	Gamma-glutamyl transpeptidase (/l)	Fructose-1-phosphate aldolase(/l)	Conjugated dienes, optical density units/mg protein	Malonic dialdehyde, nmoles/mg protein
Intact (n = 12)	28,4±3,1	2,5±0,12	0,39±0,05	1,77±0,36
Adaptation (n = 12)	32,1±2,9	2,3±0,15	0,37±0,04	1,51±0,41
Alcoholization (n = 18)	61,6±7,9	5,2±0,35	1,18±0,09	6,4±0,59
Alcoholization + withdrawal of ethanol (n = 12)	89,8±11,2	7,7±0,81	1,64±0,19	8,7±0,72
Alcoholization + adaptation (n = 18)	36,5±4,2	4,0±0,36	0,68±0,081	3,18±0,34
Alcoholization + adaptation + withdrawal of ethanol (n = 12)	36,3±5,1	4,2±0,29	0,73±0,069	3,43±0,37

Legend. Significance of differences shown in text.

two groups was the same at about 9.0 ml. Later the alcohol consumption of the control animals was increased, and after 6 weeks it exceeded 12.0 ml. In the adapted animals, on the other hand, a decrease in alcohol uptake was observed, and took place quite quickly, starting from the 3rd week of adaptation; by the end of the 6th week the animals consumed 5.8 ml of alcohol, i.e., only half as much as in the control. Thus adaptation of the chronic alcoholic animals significantly reduces their ethanol consumption.

The data in Table 1 show that 48 h after withdrawal of alcohol the animals remained on the hotplate twice as long as in the control: marked abstinence-related analgesia was observed in them. In the chronic alcoholic animals subjected to adaptation to hypoxia these manifestations were absent. Similarly the alcoholic animals, after access to ethanol was resumed, consumed twice as much ethanol solution immediately after the enforced interruption of its supply than before withdrawal. Alcoholic adapted animals, in the same situation, consumed as much ethanol as before withdrawal. Thus adaptation also depresses important components of the abstinence syndrome such as abstinence-related analgesia and the increased ethanol consumption after its enforced withdrawal.

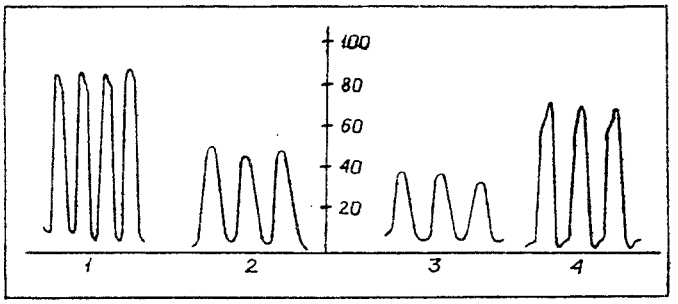

Fig. 2. Effect of adaptation to periodic hypoxia on abstinence-related weakening of cardiac contractions in chronic alcoholic animals 2 days after ethanol withdrawal. Electrometric curve of pressure in left ventricle of rats: 1) control, 2) chronic alcoholization + gradual adaptation to period hypoxia + ethanol withdrawal. Explanation in text.

There are at least three points worthy of note in Table 2. First, alcoholization itself causes liver damage, which is expressed as a roughly twofold increase ($p < 0.05$) in the blood levels of two hepatospecific enzymes, namely GGT and FPA. These high blood enzyme levels are created against the background of activation of LPO in the liver, manifested as a three- to fourfold increase ($p < 0.001$) in the concentrations of conjugated dienes (CD) and TBA-positive products in the liver tissue. During adaptation these manifestations of liver damage caused by alcoholization were still present, but were significantly weaker.

Second, abstinence leads to a sharp increase both in the blood levels of hepatospecific enzymes and activation of LPO in the liver. Parameters characterizing these two phenomena were found to be increased by 3.5-5 times compared with the control ($p < 0.001$).

Finally, the most important conclusion arising from the data in Table 2: adaptation of alcoholized animals to hypoxia sharply reduces the abstinence-related rise of the blood enzyme levels, which ceases to be significant. The concentration of LPO products under the influence of adaptation was reduced by more than half compared with the control animals, but nevertheless, the increase in the concentration of these products compared with intact animals still remained. On the whole it is evident that adaptation to hypoxia, by reducing alcohol consumption, limits liver damage during chronic alcoholization, and protects the liver against abstinence-related damage by an even greater degree.

In the next stage of the experiments we assessed the effect of adaptation to periodic hypoxia on the electrical stability and contractile function of the heart during chronic alcoholization and subsequent withdrawal of ethanol. The curves in Fig. 2 reflect the results of typical experiments and show that alcoholization by itself induced marked weakening of contraction, but subsequent withdrawal led to catastrophic depression of the contractile function of the heart. Adaptation to periodic hypoxia largely abolished this depression. Quantitatively these results and also those of determination of LPO products are shown in Table 3. Clearly under the influence of chronic alcoholization the threshold of electrical stability of the heart was lowered by about 40% ($p < 0.01$) compared with the control, but during abstinence it fell by 2.5 times, a direct indication of increased probability of ventricular fibrillation. The developed pressure also fell during alcoholization by more than 30% ($p < 0.01$) compared with the control, but during abstinence it fell critically. Corresponding to these severe disturbances of the contractile function of the heart marked activation of LPO processes took place, as shown by a twofold increase in the concentrations of CD and TBA-positive products during alcoholization and a threefold increase in their values during the abstinence syndrome ($p < 0.01$).

TABLE 3. Effect of Adaptation to Hypoxia on Disturbances of Cardiac Contractility and Activation of LPO during Chronic Alcoholization and Withdrawal of Ethanol

	Series of experiments	Heart rate, beats/min	Developed pressure, mm Hg	Intensity of functioning (double product)	Threshold of fibrillation, mA	Conjugated dienes	TBA-positive products
I.	Control (n = 19)	480±13	100±9,3	48 000±121	8,6±1,0	0,14±0,02	0,35±0,04
II.	Adaptation (n = 13)	470±16	105±8,1	49 350±130	8,3±1,3	0,14±0,03	0,39±0,07
III.	Alcoholization (n = 12)	342±9,0	68±4,3	23 256±39	4,8±0,6	0,28±0,04	0,81±0,09
IV.	Alcoholization + withdrawal of ethanol (n = 12)	315±16	42±7,2	13 230±115	2,9±0,4	0,41±0,06	1,12±0,13
V.	Alcoholization + adaptation (n = 9)	352±18	88±6,9	30 976±124	6,8±0,07	0,21±0,03	0,52±0,06
VI.	Alcoholization + adaptation + withdrawal of ethanol (n = 9)	348±17	92±7,8**	32 016± ±133**	6,9±1,2**	0,22±0,05*	0,61±0,09**

Legend. Significance P_{IV-VI}: *p < 0.05, **p < 0.01.

Thus adaptation abolishes disturbances of electrical stability of the heart and of the force of cardiac contraction observed in the abstinence syndrome and limits the activation of lipid peroxidation, which may play a role in these disturbances.

On the basis of these results it is useful to examine at least 2 aspects: first, the damaging action of abstinence, and second, the mechanism by which adaptation to periodic hypoxia abolishes the abstinence syndrome.

Alcohol withdrawal in people and animals habituated to it leads, as we know, to a marked stress reaction, manifested as increased synthesis and destruction of catecholamines in the brain [15], an increase in the concentration of catecholamines in the cerebrospinal fluid and of their metabolites in the blood [19], and also an increase in the glucocorticoid concentration in the blood, which is proportional to the duration and severity of abstinence [19]. This nonspecific stress syndrome evidently activates a dominant (which is quite specific) of alcoholics that determines their craving for ethanol. Like any stress, this situation is accompanied by manifestations of analgesia [17] and, at the same time, it enables maximal realization of the dominant addiction, namely an increase in alcohol consumption after termination of its withdrawal (Table 1).

Under abstinence conditions stress may be particularly strong because the preceding alcoholization, as was pointed out above, reduces the efficiency of function of the stress-limiting systems [6, 7, 8]. Strong stress of this kind can cause activation of LPO in the heart and can also disturb its electrical stability (Table 3).

Another mechanisms which plays a role in the formation of the abstinence syndrome is realized mainly in the liver. During chronic alcoholization the cytochrome P-450 system generates an increased quantity of hydrogen peroxide, which plays a definite role in ethanol oxidation [8]. After withdrawal of ethanol, the target of action of the excess of peroxide moves to the cell membrane lipids, and activation of LPO in the liver and the release of enzymes into the blood, take place, as was shown above (Table 2). This liver damage logically ought to disturb the detoxicating function of the organ, which in turn leads to increased concentrations of ammonia and also of biogenic amines, absorbed from the intestine, in the body. This factor may increase the severity of the abstinence syndrome and, in particular, may cause profound disturbances of the strength of the cardiac contractions and the electrical stability of the heart (Table 3). This is in agreement with data on arrhythmias and the comparatively high mortality among people with an abstinence syndrome [18, 19].

Taking these facts into consideration, we can examine some possible mechanisms of adaptive defense against the abstinence syndrome. First, the main discovery from this investigation, reduction of the craving for alcohol and its consumption taking place under the influence of adaptation (Fig. 1) must itself inevitably reduce the intensity of the abstinence syndrome, for a smaller alcohol consumption must lead to a less marked alcohol withdrawal syndrome. The mechanism of this important phenomenon is not yet clear, but the possibility cannot be ruled out that marked and, in some cases, total activation of the stress-limiting systems, leading to a tenfold increase in release of opioid

peptides from the adrenals in stress situations in particular [2], a twofold increase in the serotonin concentration in the brain [1], and a similar increase in prostaglandin levels in the blood and tissues [20], collectively inhibit the alcohol-motivated behavioral dominant and weaken the craving for alcohol and withdrawal stress.

These investigations are of both practical and theoretical importance.

The practical aspect is connected with the fact that adaptation to periodic hypoxia in recent years has been successfully used for the treatment of neuroses allergic diseases [6], and psychoses [22]. This, together with the results of our own experiments, enable us to recommend the study of the possible use of adaptation of this kind in the treatment of forms of alcoholism in which the abstinence syndrome plays a key role.

The theoretical aspect of the problem is that the mechanism of the reduction of alcohol consumption and of abolition of the abstinence syndrome under the influence of adaptation to hypoxia, discovered previously [23] and now confirmed, deserves further study by the combined use of methods of modern neurochemistry and ethology.

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