REDUCTION OF ALCOHOL CONSUMPTION OF RATS BY CENTRAL α-ADRENOBLOCKERS: ROLE OF LIVER ALDEHYDE DEHYDROGENASE ISOZYMES

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KEY WORDS: alcohol intoxication; α-adrenoblockers; aldehyde dehydrogenase isozymes; rat liver.

The noradrenergic neurotransmitter system has been shown to play an important role in the central mechanisms controlling alcohol consumption, and substances depressing activity of the noradrenergic system of the brain can inhibit the craving for alcohol [1, 3]. Hence it follows that the search for substances reducing the craving for alcohol can best be undertaken among central adrenoblockers. There is evidence that substances acting on brain α-adrenoreceptors reduce the craving for alcohol most effectively [3]. However, the mechanism of the inhibitory action of α-adrenoblockers on alcohol consumption has not been studied. The writers showed previously that in the early stages of experimental alcoholism (contact of rats with ethanol for 10 days) the central α-adrenoblocker IÉM-611 reduced voluntary consumption of ethanol solution by the animals [5]. Later, when the action of IÉM-611 on enzymes of ethanol metabolism in the liver was studied, it was found to have no significant effect on alcohol dehydrogenase (AlDH) activity, but considerably reduced aldehyde dehydrogenase (AdDH) activity. It seems probable that the inhibitory action of IÉM-611 on ethanol consumption is mediated by changes in liver AlDH activity. It is logical to suppose that the effect of IÉM-611 on AlDH activity in this situation may be realized through central mechanisms or direct inhibition of the enzyme. To test this hypothesis, effects of IÉM-611 and of another central α-adrenoblockers, phenoxybenzamine, on activity of individual AlDH isozymes were compared in vivo and in vitro. The effect of both α-adrenoblockers on ethanol consumption by rats at different stages of experimental alcoholism also was investigated.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-350 g were used. As a first step, under conditions of free choice between water and ethanol solution, animals consuming 15-30 ml of 15% ethanol solution per kilogram body weight daily were selected. The method of screening was described in [5]. The rats were then divided into control and experimental groups so that the original level of ethanol consumption was as close as possible in the two groups.

The preparations IÉM-611 in doses of 30 and 15 mg/kg and phenoxybenzamine in a dose of 10 mg/kg were injected subcutaneously into the rats, daily for 6-14 days. Animals of the control group received physiological saline subcutaneously. The action of these substances was studied in three series of experiments (the number of animals in each group in series I and II was not below 12, and the number of experiments in the 3rd series is indicated in Table 1.

In series I the ability of the preparations to affect the formation of a craving for alcohol was estimated from the time course of ethanol consumption by rats. Administration of the preparations began immediately after 10-days screening. The animals were given freedom of choice between water and 15% ethanol solution. The volume of water and ethanol consumed was recorded throughout the period of injection of the preparations.

In series II the ability of the preparations to reduce the craving for ethanol in rats exposed beforehand to alcohol for 6 months also was evaluated from the time course of ethanol consumption throughout the period of administration of the preparations and the first week after their discontinuation.

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TABLE 1. Effect of Central α-Adrenoblockers IÉM-611 and Phenoxybenzamine on Rat Liver AlDH Activity (M ± m)

Substance, dose	Period of injection, days	Number of experiments	AlDH activity, nmoles NADH/min/mg protein		
			AlDH (total activity)	AlDH I	AlDH II
Physiological saline	6	18	21,7±2,5	8,1±1,2	12,7±1,3
	12	17	22,9±1,5	8,1±1,2	14,3±2,0
IÉM-611, 30 mg/liter	6	6	13,6±1,8*	5,8±0,8*	7,7±0,9*
	12	3	12,1±2,2**	4,7±0,8*	7,4±1,5*
IÉM-611, 15 mg/liter	6	6	7,9±1,3**	4,2±0,7*	3,4±0,9**
	12	6	8,8±1,4**	3,8±0,7*	5,0±0,9**
Phenoxybenzamine, 10 mg/kg	6	6	15,1±1,9*	4,9±0,4*	10,2±1,8
	12	6	20,3±3,1	4,7±1,0*	15,3±2,5

Legend. *p < 0.05, **p < 0.01.

In series III the effect of the preparations on AlDH isozyme activity was investigated in the liver mitochondria of rats [11] receiving water alone to drink for 2 months after screening, without contact with ethanol. Protein in the liver was determined by Lowry's method [8].

In experiments in vitro IÉM-611 and phenoxybenzamine were added to the incubation medium for measurement of AlDH activity in concentrations of between 10^{-9} and 10^{-2} M.

The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

During the formation of craving for alcohol (10 days of contact with ethanol, i.e., the first stage of experimental alcoholism as defined by Burov [2]) the central α-adrenoblockers IÉM-611 and phenoxybenzamine reduced consumption of ethanol solution (Fig. 1). A marked effect of the preparations developed actually during the first week of administration: after 6 days the reduction of ethanol consumption in response to IÉM-611 was 29% compared with the control and 40% compared with initial consumption; phenoxybenzamine reduced consumption by 37 and 48%, respectively. During administration of the preparations for 12 days, the effect achieved was maintained but was not intensified. After administration of IÉM-611 in different doses (15 and 30 mg/kg) the decrease in ethanol consumption was virtually identical, i.e., increasing the dose up to 30 mg/kg caused no marked potentiation of the effect.

After rats consuming ethanol chronically for 6 months (2nd stage of experimental alcoholism according to Burov) had an established craving for alcohol, only IÉM-611 had a marked inhibitory action of their ethanol consumption (Fig. 2). The reduction of ethanol consumption 1 week after the beginning of injection of the preparation was 45% compared with the control and 54% compared with initial consumption. This effect was maintained during 2 weeks of administration, and ethanol consumption was reduced in 90% of the animals studied. Ethanol consumption 1 week after the withholding of IÉM-611 was reduced in 100% of the animals tested (a reduction of 65% compared with the control and 72% compared with the original consumption), evidence of the lasting therapeutic effect of the preparation. Phenoxybenzamine significantly reduced alcohol consumption 1 week after the beginning of its administration (by 37% compared with initial consumption), and in the 2nd week and after discontinuation of the preparation, its effect was no longer exhibited.

Thus phenoxybenzamine was effective only in stage 1 of experimental alcoholism, whereas IÉM-611 inhibited ethanol consumption by animals in stages 1 and 2.

The writers showed previously that IÉM-611 increases the ratio between activity of ethanol-oxidizing enzymes in the liver: cytoplasmic ADH and mitochondrial AlDH. An increase in the ADH/AlDH ratio was due to the inhibitory action of IÉM-611 on AlDH [4, 5]. AlDH is known to be represented in the liver as two different molecular forms: AlDH I (with low K_m for acetaldehyde) and AlDH II (with high K_m for acetaldehyde) AlDH I is active in the presence of low concentrations of acetaldehyde (50-100 μM), but AlDH II is active also at high concentrations (1 mM or over) [9]. In chronic alcohol poisoning the acetaldehyde concentration in the liver and blood is increased and, as a result, the contribution of AlDH II to acetaldehyde oxidation increases [7, 10].

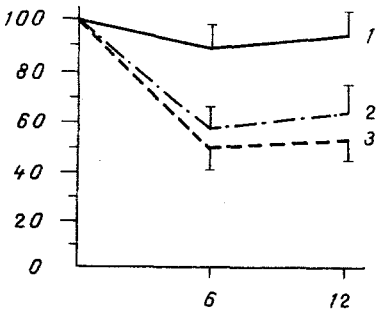

Fig. 1

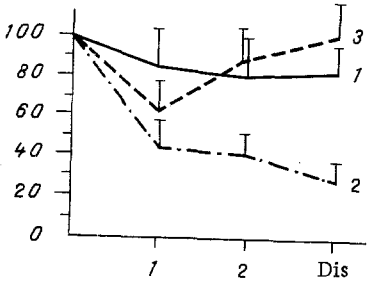

Fig. 2

Fig. 1. Effect of phenoxybenzamine (3) and IÉM-611 (2) on ethanol consumption by rats during period of formation of alcohol motivation. 1) Control. Abscissa, period of administration of preparations (in days); ordinate, consumption of 15% ethanol solution (in %).

Fig. 2. Effect of phenoxybenzamine (3) and IÉM-611 (2) on ethanol consumption by the rats after exposure to alcohol for 6 months. 1) Control. Abscissa, period of injection for preparations (in weeks); Dis) discontinuation of preparation, 1st week; ordinate, consumption of 15% ethanol solution (in %).

This investigation showed that in the course of 6 and 12 days after injection of IÉM-611 (15 mg/kg) the specific AlDH activity fell approximately threefold compared with the control; the compound significantly inhibited both AlDH I and AlDH II (Table 1). With an increase in the dose of the compound to 30 mg/kg its inhibitory ability was weakened a little. Phenoxybenzamine (10 mg/kg, administered for 6 and 12 days, inhibited the specific activity of AlDH I almost by half compared with the control but had no significant effect on AlDH II (Table 1). When ethanol enters the body, ADH catalyzes its conversion into acetaldehyde, and AlDH catalyzes oxidation of acetaldehyde to acetic acid. Inhibition of AlDH activity therefore leads to the accumulation of highly toxic acetaldehyde in the body, and this tends to reduce the animals' alcohol consumption [6]. Probably IÉM-611 and phenoxybenzamine affects voluntary ethanol consumption through their regulating effect on the blood acetaldehyde level. In stage 1 of experimental alcoholism, when the acetaldehyde concentration in the body is still low, and when mainly AlDH I is functioning, inhibition of the enzyme by phenoxybenzamine for IÉM-611 leads to accumulation of the toxic metabolite. After long-term alcoholization of the animals the acetaldehyde concentration in the body is significantly raised [6], and both AlDH I and AlDH II are evidently "working." Further accumulation of acetaldehyde in the liver and blood, and its aversive effect can therefore arise only in response to administration of IÉM-611, which differs for phenoxybenzamine by inhibiting both AlDH isozymes.

In experiments in vitro phenoxybenzamine and IÉM-611, in concentrations of 10^{-9} to 10^{-4} M, had no effect of AlDH activity. The molecular weight of phenoxybenzamine and of IÉM-611 is 303 and 465, respectively. The blood plasma volume of the rats is about 4% of its body weight. It is easy to calculate from these figures that, with the doses of the substances used (phenoxybenzamine 10, IÉM-611 15 mg/kg, subcutaneously), their blood level did not exceed 10^{-4} M, i.e., in vitro the preparations could not directly inhibit AlDH. Reduction of AlDH activity in response to injection of IÉM-611 and phenoxybenzamine was evidently due to their central action. Probably α-adrenoreceptors participate in the central regulation of liver AlDH activity.

Reduction of voluntary ethanol consumption in rats by administration of IÉM-611 and phenoxybenzamine is thus due to a certain extent to their central action. However, it is an interesting fact that IÉM-611 was found to be most effective at the same stages of experimental alcoholism. The difference between the action of these two preparations on the consumption of ethanol by animals with an established craving for it is evidently connected with the absence of an inhibitory effect of phenoxybenzamine against AlDH II, and with the ability of IÉM-611 in vivo to inhibit both AlDH isozymes.

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DIFFERENCES IN EXCITATORY ACTION OF ACETYLCHOLINE AND METHACHOLINE TO HIGH-THRESHOLD C-FIBER MECHANOSENSITIVE CUTANEOUS SENSORY UNITS IN CATS

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Acetylcholine (ACh) and acetyl-β-methylcholine (methacholine, MCh), introduced through the blood stream into the intercellular space of the cat small intestine, excite the interoceptor of that organ, and, in concentrations as low as 1-10 ng/ml, induce relatively small and slowing rising pressor reflexes [3, 11]. An increase in the MCh concentration, even by 10^5-10^6 times, does not change the character of the reflexes. By contrast, if the ACh concentration is increased up to 10 μg/ml, it begins to act as a nocigenic stimulus: the pressor reflexes acquire the features characterizing the circulatory components of the nocifensive (defensive) response [2, 4, 11]. Beginning with a concentration of 5-50 μg/ml ACh behaves as an algogenic substance: if applied to the human skin denuded of the epidermis it elicits pain [10], MCh does not have this property even in a concentration of 1 mg/ml [10]. The information given above prompted us to compare responses of the same cutaneous sensory units (SU) when excited by ACh and MCh. We considered that such a comparison would reveal the differences between the character of responses of SU induced by ACh within the concentration range in which it exhibits its action of a nocigenic and algogenic (for man) stimulus. We shall consider responses only of those SU whose excitation threshold by a mechanical stimulus is high, and which therefore may be accepted as nociceptors [5, 12].

EXPERIMENTAL METHOD

Two segments of a subcutaneous nerve were isolated in nine cats, anesthetized with chloralose (40 mg/kg) and urethane (600 mg/kg): at the level of the knee joint, where the nerve was placed on a bipolar stimulating electrode, and in the proximal third of the thigh, where microbundles of fibers were isolated from the nerve. The nerve was tightly ligated proximally. Impulses of single C-fibers were derived by a monopolar technique by a platinum

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