ASSESSMENT OF RAPID DEVELOPMENT OF STABLE ALCOHOL MOTIVATION IN RATS FOR TESTING POTENTIAL ANTIALCOHOLIC DRUGS

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Preclinical assessment of the activity of potential antialcoholic drugs is undertaken on animals after an 8-month period of voluntary alcohol consumption, with assessment of the development of pathological craving for alcohol on the basis purely of its average daily consumption [2]. This model needs to be improved because it takes far too long to select animals which have developed addiction for alcohol, because of the specific effect of the additional aging factor, which is not taken into account, the stress factor due to isolation of the animals, and also the inadequate final outcome for the animals, namely "chronic alcoholics."

The aim of this investigation was to try to shorten as much as possible the period of voluntary alcohol consumption by modifying the taste of the alcohol solution, and also to introduce an additional parameter for assessing pathological craving for alcohol by using periodic overindulgence as a procedure causing exacerbation of existing craving. At the same time, the factor of isolation stress was abolished by keeping the animals alternately in isolation and under standard animal house conditions. To confirm the appropriateness of the proposed model for preclinical evaluation of the specific activity of potential antialcoholic drugs, we used a known antidepressant with a stimulating component of its action, namely pirlindol (pyrazidol), which was chosen because of indications that these pharmacological properties are combined in the pharmacotherapy of alcoholism [1]. The known nootropic agent piracetam, widely recommended in chronic alcoholism [3] but, according to some data, having no effect on the formation of alcohol motivation [4], was used as the reference substance.

EXPERIMENTAL METHOD

Experiments were carried out on 74 noninbred male rats weighing 180-200 g at the beginning of the investigation. The animals were divided initially into experimental (46 rats — group 1) and control (28 rats — group 2) groups and tested in individual cages for preference for: a 0.1% solution of saccharine and water, a 0.1% solution of saccharine in 15% ethanol and water, and a 15% solution of ethanol and water under conditions of free choice of each fluid. The order of presentation of the solutions was randomized. The level of consumption of each solution was estimated for one week, after which, in order to minimize the factor of isolation stress, the rats were kept for three days in common cages, and received only water as their source of drinking fluid, thus giving rise to a situation of deprivation of each test solution. Immediately after this the animals were returned to individual cages, and provided with access to water and to one of the solutions hitherto disallowed. After 90 min the level of consumption of each fluid was estimated. The rats of group 1 were then returned to common cages for two months, and were offered the choice between water and a 0.1% solution of saccharine in 15% ethanol; the animals of group 2 drank water and an aqueous solution of saccharine at the same times under similar conditions. After two months of alcoholization, rats of both groups were tested by the scheme described above. After animals with a stable and high level of alcohol consumption, whether tested for a week or for 1.5 h, had been selected, in order to prove the adequacy of the chosen model the possible antialcoholic activity of pirlindol (10 mg/kg) and piracetam (400 mg/kg), injected intraperitoneally daily at the same time

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TABLE 1. Triangular Matrix of Correlations of Features Studied

No. of feature							
2	—0,06						
3	0,14	0,14					
4	—0,23	0,34*	—0,06				
5	0,19	0,06	0,42*	0,02			
6	—0,02	0,05	—0,16	0,12	0,03		
7	0,37*	—0,17	0,27	—0,15	0,46*	0,30*	No. of feature
	1	2	3	4	5	6	

Legend. No. 1) consumption of 0.1% saccharine solution before alcoholization (ml/kg/day); No. 2) consumption of 15% ethanol solution before 2 months of alcoholization (ml/kg/day); No. 3) consumption of sweet ethanol before alcoholization (ml/kg/day); No. 4) consumption of 15% ethanol after 2 months of alcoholization (ml/kg/day); No. 5) consumption of sweet ethanol after alcoholization procedure (ml/kg/day); No. 6) consumption of 15% ethanol after alcoholization, after ethanol deprivation (ml/kg/90 min); No. 7) consumption of sweet ethanol after procedure of 2 months of alcoholization after alcohol deprivation (ml/kg/90 min).

of day was studied, by testing their effect on the mean daily consumption of sweet alcohol and the possibility of their stopping alcohol consumption after its deprivation. For this purpose an additional group of alcoholized and selected animals was used. Control rats were given physiological saline in equivalent volumes. The results were subjected to statistical analysis by comparison of mean values by the T test, and also by factor analysis using the Varimax procedure [5].

EXPERIMENTAL RESULTS

Factor analysis of the parameters of consumption of the different solutions revealed a group of significant correlations ($p < 0.05$), which were combined into four factors (Tables 1 and 2). Factor I had significant loadings on values of consumption of sweet water and sweet alcohol before alcoholization and as a result of deprivation, and it can be interpreted as preference for sweet ethanol. Factor II has strong loadings on values of consumption of unsweetened 15% alcohol before and after alcoholization and can be defined as a tendency toward its consumption. Factor III has significant loadings only on values of consumption of the two alcohol solutions, under conditions of alcohol thirst (after its deprivation), and it can be interpreted as a craving for alcohol irrespective of its taste. By analogy with the known clinical manifestations of a craving for alcohol, it is this factor which is particularly interesting, especially when expressed as consumption of a sweet solution of 15% ethanol after deprivation for 3 days. No significant correlations that could serve as the basis for distinguishing the factors described above could be found by factor analysis. This indicates that the reason why the factor interpreted as a craving for ethanol itself is present in the experimental group is the procedure of 2-month alcoholization. In rats of the experimental group, after 2-month alcoholization, the average daily consumption of sweet ethanol was 32.9 ± 4.3 ml/kg, and the one-time consumption after alcohol deprivation was 3.2 ± 0.8 ml/kg. To assess the time course of ethanol consumption during alcoholization, we continued administering alcohol to these animals for a further 6 months, and at the end of that time the above parameters had values of 36.8 ± 5.2 and 4.0 ± 1.0 ml/kg, respectively, which does not differ significantly from the level of alcohol consumption recorded after two months of alcoholization. Incidentally, the animals refused to take the test alcohol solutions if alcoholization was preceded by three days of deprivation, which confirmed once again that craving for ethanol is formed during the two months of alcoholization of the rats when kept under standard conditions.

Thus alcoholization with a 15% solution of ethanol with added saccharine for 2 months is sufficient to select animals with a marked craving for alcohol. The more attractive taste of the solution evidently leads to an increase in the doses of ethanol consumed. Elements of the specific effect of saccharine on the development of narcotic dependence likewise cannot be ruled out, because we know that keeping animals for 1-2 months on saccharine solution modifies tolerance to morphine formed subsequently [6].

TABLE 2. Matrix of Factor Loadings for Features Studied after Rotation of Axes

No. of feature	No. of factor		
	I	II	III
1	0,47	—0,42	0,08
2	0,14	0,77	—0,08
3	0,76	0,13	—0,34
4	—0,08	0,78	0,18
5	0,80	0,09	0,09
6	—0,03	0,12	0,91
7	0,66	—0,30	0,52

Legend. Conventional numbers of features the same as in Table 1.

TABLE 3. Effect of Pirlandol and Piracetam on Consumption of Sweet Ethanol Solution by Alcoholized Rats (mean values for groups and confidence interval at $p < 0.05$; n, number of animals in group)

Preparation	Period of injections of prepn.		Period after end of injection of drug	
	mean daily consumption of ethanol (ml/kg/day)	one-time consumption after alcohol deprivation (ml/kg/90 min)	mean daily consumption of ethanol (ml/kg/day)	one-time consumption after alcohol deprivation (ml/kg/90 min)
Control (n = 10)	39,3±12,4	4,1±1,5	55,4±12,2	3,6±2,1
Pirlindol, 10 mg/kg (n = 11)	36,7±9,9	1,2±1,6*	42,8±16,6	1,6±1,1*
Control (n = 11)	34,1±11,1	3,3±1,8	33,9±9,0	3,0±2,0
Piracetam, 400 mg/kg (n = 10)	35,7±10,9	2,9±1,7	32,3±10,2	4,6±2,6

Legend. *$p = 0.02$, **$p = 0.14$ — difference from control.

In additional experiments carried out on 42 animals which had undergone the alcoholization procedure described above, the effect of pirlindol and piracetam on the character of ethanol consumption in doses sufficient to exhibit their specific activity, was estimated. The experiments showed that pirlindol induces a marked decrease in ethanol consumption under alcohol thirst conditions (after alcohol deprivation), and this can be interpreted as weakening of alcohol motivation. This tendency partly remains even after injections of the drug were discontinued (Table 3). Piracetam had no significant effect on the parameters tested. The results are in agreement with the known data on indications for substances with an antidepressive type of action to produce abstinence from alcohol [1, 3], and the fact that piracetam does not possess this specific activity [4].

It can thus be recommended, on the basis of these results, that a procedure of two-month alcoholization followed by subsequent testing of the animals, used in the investigation described above, can be recommended for experiments to discover and study drugs with an antialcoholic type of action.

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