DIFFERENCES BETWEEN DOSE-EFFECT CURVES FOR ETHANOL IN MICE OF DIFFERENT LINES FOR LOCOMOTION AND STANDING

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The dose-effect curve of ethanol (1-5 g/kg, perorally) based on the study of locomotion in four male mice of four different lines (SHR, BALB/c, C3HA, C57BL/6) is characterized by a gradual rise to a peak (at 3 g/kg for all lines except C3HA, for which it is 5 g/kg) and a subsequent fall. The curve based on the study of standing erect is flat and falls steadily to zero. The excitatory effect of ethanol (1.5-3 g/kg) can therefore be measured as an increase in locomotion. The influence of different preparations (or other factors) on this effect must be assessed simultaneously with respect to locomotion and standing, for because of the change in direction of the curve a decrease in locomotion could indicate either weakening or strengthening of the excitatory action of ethanol. The parallel decrease in the frequency of standing indicates potentiation of the action of ethanol, whereas an increase in the frequency of standing indicates weakening of its action.

KEY WORDS: ethanol; dose-effect curves; locomotion; standing; mice of various lines.

Ethanol, in different doses, is known to cause different changes in individual components of motor activity in laboratory animals [1, 3], and the same doses of ethanol [2] and of other psychotropic drugs [4] evoked different responses in mice of different genetic lines.

The object of the present investigation was accordingly to plot dose-effect curves for ethanol, using mice of different lines, and to look for a component of motor activity that would be reliable for evaluating the action of ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on male mice of about the same age (3 months) and equal weight (22-23 g) belonging to four different lines: albino SHR (150 animals), albino BALB/c (80 mice), C57BL/6 (80 mice), and C3HA (100 mice). Experiments were carried out twice a week, and each individual mouse was used not more often than once a week. Ethanol was given perorally through a tube in doses of 1, 2, 3, 4, and 5 g/kg as aqueous solutions of 10, 20, 30, 40, and 50% (v/v), respectively, in a dose of 0.01 ml/g body weight. In some experiments, during determination of the threshold excitatory and narcotic doses, ethanol was given in doses of 0.5 and 6 g/kg respectively. Control animals received equal volumes of distilled water. The action of ethanol on locomotion (crossing the arms of a cross drawn on the floor of a chamber measuring 20×16×11 cm and the number of times the animal stood up on its hind limbs 15 min after its administration) was measured over a period of 2 min by means of a key-actuated counter. The experiments were carried out during the afternoon (noon to 3 p.m.) with natural illumination and at a temperature of 20-21°C in the winter and spring months. The animals were deprived of food and water during the experiments. The mice were kept in the animal house in cages measuring 60×30×20 cm in groups of 40-50 animals. The mice were weighed before the beginning of each experiment and at once transferred either to separate cages (6×6×9 cm), in which they could not see each other, or to metal boxes measuring 20×16×11 cm in groups of three animals.

In the experiments of series I, mice isolated after weighing were given ethanol and put back in the cages. The mice also were placed singly in the counting chamber. In the experiments of series II, after administration of ethanol to all three individuals in the group, the mice were returned to the boxes, also three at a time. All three mice were placed in a

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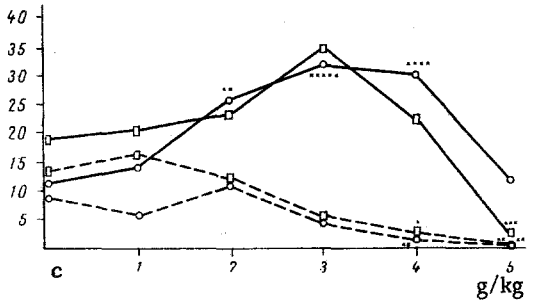

Fig. 1

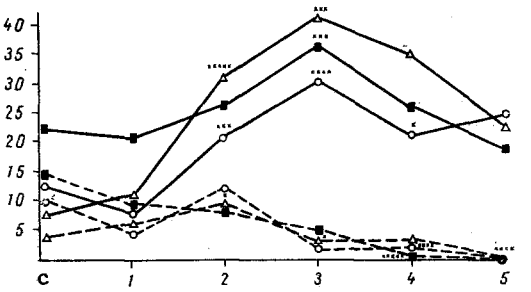

Fig. 2

Fig. 1. Dose-effect curves for ethanol based on locomotion and standing by isolated and grouped SHR mice. Abscissa, dose of ethanol (in g/kg); ordinate, absolute readings of counter at intervals of 2 min. Circles denote mice in isolation, squares mice in group. Continuous line represents locomotion, broken line standing. Each point is mean of eight isolated mice or eight groups, each of three mice. Significance of differences from control (administration of distilled water): * $P<0.05$; ** $P<0.02$; *** $P<0.01$; **** $P<0.002$; ***** $P<0.001$.

Fig. 2. Dose-effect curves for ethanol based on locomotion and standing by isolated BALB/c, C3HA, and C57BL/6 mice. Triangles) BALB/c, circles) C3HA, squares) C57BL/6. Remainder of legend as in Fig. 1.

counting chamber 15 min after administration of ethanol and the motor activity of one of them was measured (any one, without special choice, usually the first by its identification number in the records). The means of their standard errors were compared by Student's t-test.

EXPERIMENTAL RESULTS

With an increase in the dose of ethanol from 1 to 5 g/kg the curves of locomotion and the curves of standing for all lines of mice began to diverge (Figs. 1 and 2). Whereas the locomotion curves rose to reach a peak at a dose of 3 g/kg, the number of standings almost immediately began to fall steadily (after a dose of 2 g/kg), and in every case was close to zero at a dose of 5 g/kg. In a dose of 2 g/kg ethanol significantly increased the locomotion of SHR, BALB/c, and C3HA mice, but not of C57BL/6 mice (single animals). It was this dose that was used repeatedly on SHR mice when studying the effect of different preparations on the excitatory effect of ethanol. Its reproducibility was relatively high: Of the 25 control groups, with eight mice in each group, the increase in locomotion was significant (on average by 94% of its initial level) in 16 groups and not significant (on average by 53% of the initial level) in nine groups. In a dose of 3 g/kg ethanol significantly increased locomotion in all cases (the highest point), but in a dose of 4 g/kg it did so only in C3HA mice. A dose of 5 μ/kg caused a decrease in locomotion in mice of all groups except C3HA. A decrease in locomotion in the C3HA mice was observed when a dose of 6 g/kg was given (not shown in Fig. 2). The curves of locomotion and standing for mice in a group did not differ greatly from the curves of motor activity of mice in isolation (Fig. 1).

The change in direction of the locomotion curve is of decisive importance when the results of experiments to study the effect of preparations (or any other factors) on the excitatory effect of ethanol are interpreted, for the decrease in the intensity of motor excitation evoked by ethanol could indicate both weakening of the action of ethanol (a shift to the left toward smaller doses) or its potentiation (a shift to the right, toward larger doses). As Figs. 1 and 2 show, a correct interpretation of interaction between a drug and ethanol is possible only on the basis of simultaneously obtained data for changes in the frequency of standing: if locomotion was reduced and standing was more frequent (or showed little change), the action of ethanol was weakened, but if locomotion was reduced and standing became less frequent, the action of ethanol was potentiated. Therefore, whereas the excitatory action of ethanol itself on the muscles can be reliably measured by the use of a single index: an increase in the intensity of locomotion, to assess the effect of various drugs on the excitatory action of ethanol, the frequency of standing must be measured at the same time as locomotion.

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EFFECT OF PHENAZEPAM ON ETHANOL INTAKE IN RATS

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The new Soviet tranquilizer phenazepam, if given by daily intraperitoneal injection to rats in a dose of 1 mg/kg for 3 weeks, can depress the craving for ethanol developed beforehand by administration of a 5% solution of alcohol for 2 months as the only source of fluid. The mechanism of this effect is probably connected with changes in the activity of the hypothalamic neurosecretory centers observed under these conditions. The property thus revealed evidently also explains the efficacy of phenazepam in the treatment of patients with chronic alcoholism.

KEY WORDS: tranquilizers; treatment of alcoholism; hypothalamic neurosecretion.

Phenazepam (7-bromo-5-o-chlorophenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one) is the first Soviet tranquilizer of the benzodiazepine series, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, and the Physicochemical Institute, Academy of Sciences of the Ukrainian SSR. Clinical trials have demonstrated its great efficacy in the treatment of patients with chronic alcoholism [2]. However, the mechanism of its antialcoholic action has not yet been established, and the investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing initially 140-160 g, in which alcohol dependence was formed by the method described previously [1]. For 3 weeks one group of these animals received phenazepam in a near-therapeutic dose (1 mg/kg). The drug was given by intraperitoneal injection, as a suspension with Tween-80, 30 min before the animal was placed in an individual cage. The rats of the other group served as the control and received injections of water (with Tween-80) under similar experimental conditions. Observations on these animals continued for 3 weeks, during which they were allowed free choice of fluid for drinking. The effects of phenazepam on the water intake of previously intact rats was studied at the same time. The experimental conditions, the time of the last observations, and the control corresponded to those for the animals receiving ethanol. To study the mechanism of action of phenazepam under conditions of alcohol dependence, parallel with observations on the ethanol and water intake of the animals of the above groups, the state of the hypothalamic-hypophyseal neurosecretory system (HHNS), which plays the leading role in the formation of adaptive reactions [4], was studied. For this purpose, the experimental, control, and intact animals were decapitated at times of the experiments characterized by the greatest changes in the intake of the various fluids, i.e., 24 h before beginning or 1 and 21 days after the end of administration of the drug or water. The brain and pituitary were fixed in 96% ethanol or Bouin's fluid and embedded in paraffin wax. Sections of the hypothalamus and pituitary were stained with toluidine blude by Nissl's method and with paraldehyde-fuchsin by the Gomori—Gabe method, with counterstaining with Halmi's mixture. The morphological and

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