PHARMACOLOGICAL CORRECTION OF EXPERIMENTAL ALCOHOL EMBRYOPATHY

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Parental alcoholism has an adverse effect on child development in the antenatal and postnatal periods. The combination of disturbances arising in this situation has been called alcohol embryopathy or the fetal alcohol syndrome [1]. This disease is based on damage to the nervous system, which is manifested as delayed psychomotor development, memory disturbances, and intellectual impairment [1, 10]. These facts have stimulated the development of various experimental models with which to study the mechanisms of the teratogenic action of alcohol [2].

A particularly urgent task is the search for rational treatment to correct the disturbances arising in the progeny as a result of the pathogenic action of alcohol on the fetus. For this purpose, we have used for the first time a synthetic enkephalin analog, the hexapeptide dalargin (synthesized by M. I. Titov, All-Union Cardiologic Scientific Center).

EXPERIMENTAL METHOD

Experiments were carried out on 210 noninbred rats. The mothers of the experimental animals had been given 15% ethanol solution throughout pregnancy as the only source of fluid, whereas mothers of the control animals had received water. This experimental model, it was assumed, can provide phenocopies of genetic changes which may arise in the progeny of parents with chronic alcoholism. Dalargin was injected subcutaneously in 5-day courses, either into the mother rat from the 16th through the 20th days of pregnancy in a dose of 15 μg/kg in 0.5 ml physiological saline (series I) daily, or into young rats from the 7th through the 11th days after birth in a dose of 10 μg/kg in 0.02 ml physiological saline daily (series II). Some of the experimental and control animals received physiological saline at the same time and in the same volumes; in series II, half of the young rats in each litter received dalargin, the other half received physiological saline. A group of intact control animals (no treatment of any kind) also was distinguished.

When the progeny attained sexual maturity, a defensive conditioned reflex (CR) of two-way active avoidance was formed in 80 rats in a shuttle box. Flashes with a frequency of 1 Hz for 5 sec were used as the conditioned stimulus: the greatest number of presentations was 60 per experiment and the interval between them lasted 1 min. The criterion of reflex formation was 5 consecutive correct runs.

Succinate dehydrogenase (SDH) activity was determined in tissues of the sensomotor and visual areas of the cortex in 30 animals aged 2 months. Nartsissov's quantitative histochemical method [7], by which SDH activity can be determined in conventional units (μmoles formazan/mg tissue), was used for this purpose. The localization of the enzyme in the tissues was studied in sections by the method in [9]. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test [3].

EXPERIMENTAL RESULTS

The offspring of the mother rats receiving alcohol were retarded in their physical development compared with the control. The average weight of the newborn rats subjected to antenatal alcohol poisoning was lower ($p < 0.01$) than in the control (5.7 and 6.4 g, respec-

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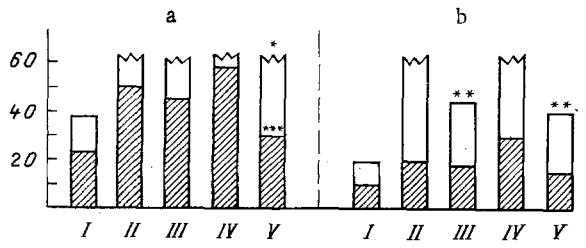

Fig. 1

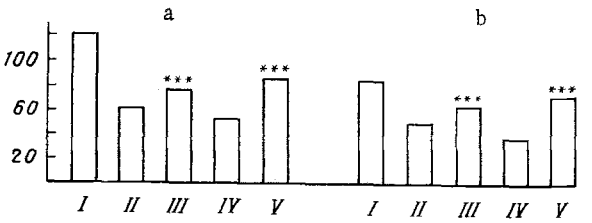

Fig. 2

Fig. 1. Formation of defensive two-way avoidance CR. Ordinate, number of combinations of conditoned and uncondictioned stimuli in experiment required for appearance of CR (shaded part of columns) and for consolidation of CR (total height of columns); zigzags indicate that more than 60 combinations were required for consolidation. a) 1st day; b) 2nd day of experiment. I-V: Group of animals: I) control; II, III) experimental group of series I (II — no treatment, III — dalargin at end of embryogenesis); IV, V) experimental groups of series II (IV — without treatment; V — dalargin in early ontogeny). Asterisks indicate significance of differences between groups III and II, and V and IV: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Fig. 2. SDH activity in sensomotor (a) and visual (b) cortex (conventional units). Remainder of legend as to Fig. 1.

tively). Stillborn rats (8.3% of the total number of newborn) were found in half of the experimental litters, whereas in the control litters there were no stillborn rats. As a result of antenatal alcohol poisoning, the early postnatal mortality also was increased: in the experimental groups 39% of the young rats died (including stillborn) during the first two weeks of life, compared with 16.3% in the controls ($p < 0.01$). Dalargin did not affect physical development.

Control animals of the two series receiving dalargin and physiological saline did not differ significantly from each other or from intact animals with respect to any of the parameters of conditioned-reflex activity and tissue respiration studied. For convenience of subsequent description, we therefore pooled the results for all control animals into a single common group (Fig. 1, group I).

Alcohol poisoning during the intrauterine period of development significantly delayed 2-way avoidance conditioning in the adult rats. The experimental animals (Fig. 1, group II, IV) required significantly more combinations than the control rats for the appearance of a conditioned response. To consolidate the reflex, these animals required on average more than 120 combinations, and for that reason most rats of groups II and IV did not achieve the criterion of consolidation on either the 1st or the 2nd day of the experiment.

Injection of dalargin considerably improved the parameters of conditioned-reflex activity in the antenatally alcoholized animals. Rats receiving dalargin (Fig. 1, groups III, V) required significantly fewer combinations than those not receiving this compound for CR to appear and to be consolidated, although they needed rather more than the control animals.

It will be clear from Table 1 that despite some delay in the rate of reflex formation compared with the control, the majority of antenatally alcoholized rats receiving dalargin reached the criterion of reinforcement on the second day of the experiment (group III 85.7, group V 66.7%). They did not differ significantly with respect to this parameter from the control group.

The results of the histochemical tests are given in Fig. 2. The level of SDH activity in the sensomotor cortex in the control group was higher than in the visual cortex. This may be associated with differences in the structural and functional organization of these two brain structures.

A significant ($p < 0.001$) fall in SDH activity in the cerebral cortex (Fig. 2, groups II, IV) was found in rats subjected to alcohol poisoning in the intrauterine period of development. Administration of dalargin (groups III, V) partially compensated the disturbances of tissue respiration caused by intoxidation. The SDH level in animals receiving dalargin was higher ($p < 0.001$) than in antenatally alcoholized rats not receiving it.

TABLE 1. Formation (in percent) of Defensive Two-Way Avoidance CR

Group of animals	1st day of expt.		2nd day of expt.	
	appearance of CR	consolidation of CR	appearance of CR	consolidation of CR
I (n=40)	90	82,5	97,4	94,7
II (n=9)	44,4	11,1	66,7	22,2
III (n=7)	85,7	28,6	100	85,7
IV (n=13)	38,5	0	66,7	16,7
V (n=12)	83,3	33,3	91,7	66,7

Legend. Explanation of groups given in Fig. 1.

A layer by layer study of SDH localization showed an uneven distribution of the enzyme in animals subjected antenatally to alcohol intoxication: it appeared in certain parts of the cortex of these areas with sharply reduced or with normal enzyme activity. This pattern of distribution of SDH was observed in the sensomotor cortex in layers II and III and in the visual cortex in layers II, III, and V. Injection of dalargin not only compensated the quantitative lowering of the SDH level caused by intoxication, but also led to a more uniform distribution of the enzyme in the tissue, with ultimate normalization of oxidative processes in the mitochondria.

Dalargin thus had a corrective action when used in the treatment of disturbances caused by alcohol intoxication in the intrauterine period of development. No difference could be found in the effect of dalargin in different models of treatment. When used for the treatment both of the mother and of the offspring, in early ontogeny, dalargin considerably improved the parameters of conditioned-reflex activity in antenatally alcoholized animals and led to normalization of their tissue respiration.

The experimental data obtained on the pathogenesis of alcohol embryopathy lead to the conclusion that the disturbances of higher nervous activity and behavior were caused by organic changes in the brain, such as dystrophic changes in the neurons and their processes, and ultrastructural disturbances of the mitochondria [8]. According to Popova [8], the morphological changes observed are largely determined by cerebral anoxia. The lowering of the DSH level which we found is also evidence of anoxic phenomena in the brain of antenatally alcoholized animals. It is thus very probable that the therapeutic effects of dalargin are based, on the one hand, on its antianoxic properties [6], and on the other hand, on the nerve-growth properties of the preparation and its ability, as an opioid peptide, to activate the repair capacity of the organism, inducing intensive growth of tissue where it is damaged or underdeveloped [4, 5]. The effectiveness of treatment is also evidently determined by the fact that dalargin was injected during a period of rapid development of the nervous system, namely at the end of embryogenesis and in early postnatal development, i.e., before maturation of the brain is complete, and a substance of dalargin type can stimulate the body's own compensatory powers.

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