Effects of Individual Fractions of Cerebrospinal Fluid from Drug Addicts Treated by Liquor Adsorption on Behavior of Recipient Rats

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Effects of individual fractions of the liquor preparation from drug addicts, eluted from filters after liquor adsorption session, on rat behavior in an elevated plus-maze was analyzed. Fraction I activated and fraction IV inhibited orientation and exploratory activity and increased anxiety of experimental animals. Effects of fraction IV coincided with the effect of total preparation on rat behavior in an elevated plus-maze, which indicates that this fraction contains substances actively accumulating in the liquor of drug addicts and partially responsible for functional changes observed during abstinence.

Key Words: *drug addiction; liquor adsorption; rats, behavior*

Liquor adsorption is now often used for detoxication and treatment of abstinent syndrome in drug addicts. This method purifies the cerebrospinal fluid from toxic endogenous compounds accumulated during drug addiction and responsible for well known symptoms of abstinence [2]. Toxic products eluted from the adsorbent induce various, primarily depressive changes in adaptive behavior in animals [1,4].

In the present study we fractionated the material obtained during liquor adsorption in order to analyze the reactive components of the cerebrospinal fluid adsorbed on the filter.

MATERIALS AND METHODS

Liquor adsorption sessions were carried out in alert male drug addicts aged 17-28 years starting from the early period of abstinence. Filters obtained after carbofiltration were washed with tap water from the outside and with distilled water from inside (2×150 ml for 30 min) followed by 3-min centrifugation at 1500 rpm The substances precipitated on filters were extracted with 96% ethanol containing 0.05 M ammonium. As a dehydratant, ethanol prevents the formation of hydration shells and promotes protein transition into a soluble state. This mixture extracts also some lipid components. The extracts were evaporated and fractionated by thin layer chromatography on Silufol plates (150×150 mm, Kavalier) in 2:1 chloroform: methanol mixture. After chromatography the plates were divided into 2 parts; 2-cm strips were detached from left and right edges and developed in iodine vapor. Color spots were encircled and Rf was calculated. Developed strips were applied onto the plate and silica gel from the sites corresponding to developed spots and intervals between them was scraped and extracted with the solvent for 30 min. After centrifugation the extract from each tube was evaporated and used in physiological experiments. The following fractions were analyzed: I) between the start and the first spot; II) first spot (Rf=0.28±0.017); III) between the first and second spots; IV) second spot (Rf=0.79±0.015).

Effects of individual fractions on rat behavior were evaluated in an elevated plus-maze (EPM). Apart from anxiety parameters (duration of stay in closed and open arms), number of runs and rearings, duration of grooming and freezing were recorded. The rats were

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tested for 5 min. Forty male Wistar rats (200-250 g) were used. Test fractions (5 μl) were administered intranasally with a micropipette. The fractions were dissolved to a concentration ensuring the optimal physiological effect selected in preliminary experiments for the total preparation [4]. To controls normal saline in the same volume was administered. The results were statistically processed using Student's *t* test.

RESULTS

Individual fractions exerted different effects on animal behavior in EPM. The most pronounced effect was observed after challenge with fraction IV, which significantly increased rat anxiety, *i. e.* reduced the time spent in the open arms and prolonged the time spent in closed arms (Fig. 1). It also significantly inhibited motor activity (Fig. 2, *a*) and prolonged grooming and freezing in experimental rats (Fig. 2, *b*) in comparison with the control.

Fraction I caused opposite behavioral changes. The animals spent more time in open arms and less in closed arms (Fig. 1), which indicated their lower anxiety. Moreover, this fraction increased horizontal motor activity (Fig. 2, *a*). Fraction III significantly decreased the time of grooming (Fig. 2, *b*), but had no effect on other behavioral parameters; the same was observed after challenge with fraction II (Figs. 1, 2).

Hence, the effects of different fractions on rat behavior in EPM were different. Fractions IV and I exerted opposite effects on rat anxiety and locomotor activity. Only fraction IV significantly prolonged freezing, while other fractions did not affect this parameter. In addition, this fraction prolonged the duration of grooming.

Administration of the total preparation decreased motor activity in EPM, increased anxiety, and prolonged grooming and freezing [4]. All these effects were observed after challenge with fraction IV, hence this fraction contains all substances actively accumulated in the cerebrospinal fluid of drug addicts and at least partially responsible for functional disorders observed during the abstinence.

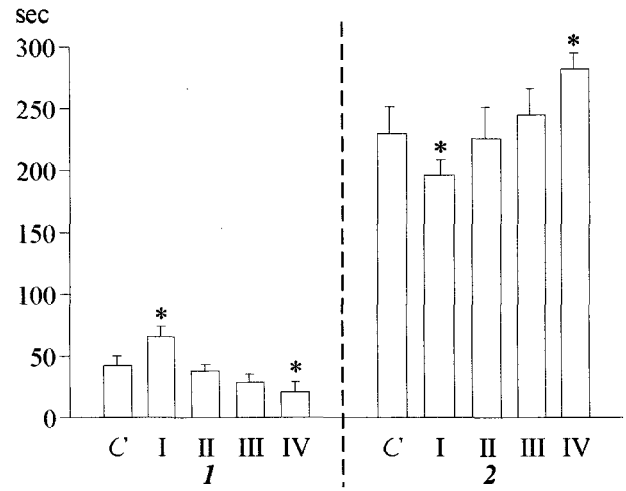

Fig. 1. Rat anxiety in the control (*C*) and after intranasal instillation of individual liquor fractions from drug addicts (I-IV). Duration of stay in open (*1*) and closed (*2*) arms. Here and in Fig. 2: *$p<0.05$ *vs.* the control.

The conditions of liquor fractionation suggest the presence of protein or peptide substances. The fact that pronase treatment of the total liquor preparation from drug addicts decreased, but not completely abolished its inhibitory effect on orientation and exploratory activity of rats [1] indicates that peptides are not the only active components of the liquor. Iodine-stained fraction IV substances can contain unsaturated fatty acids, including arachidonic acid, whose inhibitory effects at the cell and systemic levels are well known [3,5-7]. All this does not rule out the presence of ac-

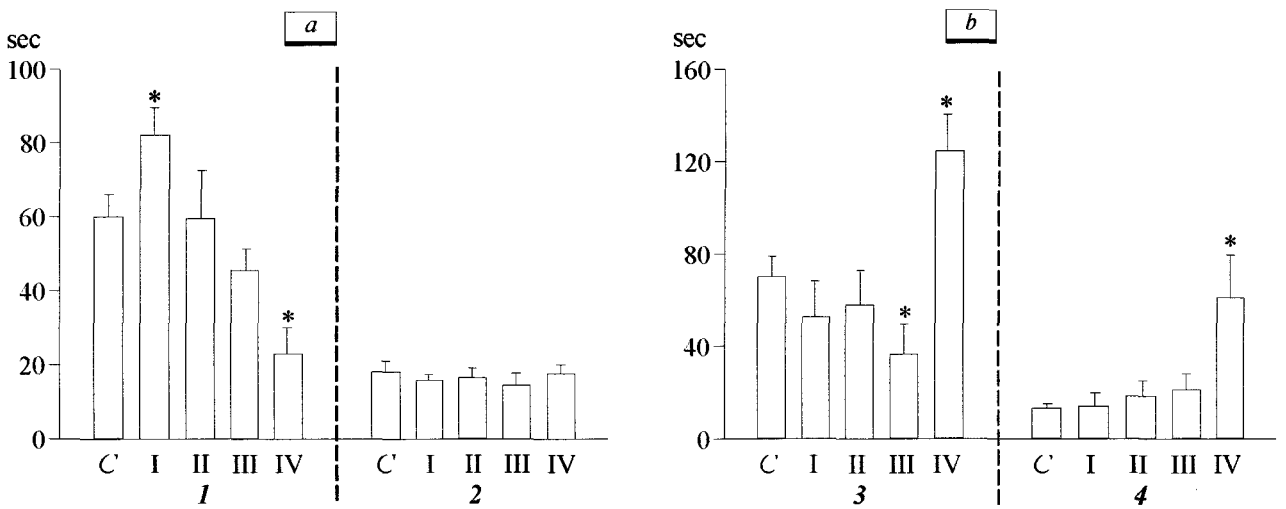

Fig. 2. Rat behavior in an elevated plus-maze in the control (*C*) and after instillation of liquor fractions from drug addicts (I-IV). *a*) Orientation and exploratory activity: number of crossed squares (*1*) and rearings (*2*); *b*) duration of grooming (*3*) and freezing (*4*).

tive compounds of other chemical nature in the liquor of drug addicts. This hypothesis needs special investigations.

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