of interconversion of GABA-rc conformers, differing in the conductance of the chloride ionophore. It follows from the scheme that $ED_{50,m}\ ED_{50,k}^{-1} = k_3k_2^{-1} > 1$ and is stationary in the BD group investigated; ≈1 for their antagonists (stabilizers of the [B]-form [14]), and <1 for their counteragonists (stabilizers of the [C]-form of BD receptor [13]). Potentiation of the effects observed in the presence of the combined anticonvulsant action of different types of GABA-rc modulators (BD and BB) assumes the existence of more than two ([A], and [B] and [C]) conformational states of GABA-rc, which under physiological conditions definitely determine the recorded parameters of the biological system.

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EFFECTIVENESS OF ADRENERGIC TRANSMISSION AND MECHANISMS CONTROLLING IT IN RATS EXPOSED PRENATALLY TO ETHANOL

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KEY WORDS: noradrenalin secretion; ethanol; prenatal development; rat brain.

Prenatal exposure to ethanol leads to disturbances of conditioned-reflex activity [7, 10, 11]. It was shown previously that conditioned reflex formation and preservation correlate with specific changes in the effectiveness of adrenergic transmission: with changes in the intensity and stability of noradrenalin (NA) secretion [3]. The intensity of NA secretion is controlled by α- and β-autoadrenoreceptors, whose physiological role is to stabilize NA secretion in a series of consecutive depolarizations and to realize the process of frequency potentiation [4-6].

This paper describes the study of the effects of prenatal exposure to ethanol on the intensity and stability of NA secretion in a series of consecutive depolarizations and on activity of the autoreceptors regulating these processes.

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TABLE 1. Basic Parameters of Intensity of ^{3}H-NA Secretion in Control and Experimental Animals (M ± m)

Experimental conditions	S_1	SV_{S_1}	S_m	CV_{S_m}
Control (n = 5)	2,23±0,21	12,3±4,4	2,48±0,26	15,5±1,78
Experiment (n = 6)	1,96±0,22	18,9±6,9	2,35±0,22	24,4±3,4*

Legend. *p < 0.05 compared with control, n) number of animals.

EXPERIMENTAL METHOD

Noninbred female albino rats received 2-2.5 ml of 40% ethanol solution, in a dose of 4-5 g/kg body weight, by gastric tube 5 times a week from the 5th through the 21st days of pregnancy. Experimental and control rats were kept under identical animal house conditions. Males from the progeny of these animals were investigated when reaching the age of 7 months (weight 300-350 g). The intensity of ^{3}H-NA secretion was investigated by the section transfer method [1, 2]. From each animal four tangential sections were cut through the parietal cortex 0.3 mm thick (altogether 8 sections from control and experimental animals) and they were incubated simultaneously in Krebs–Ringer–buffer medium (1 mM Na-phosphate and 15 mM bicarbonate buffer, pH 7.25, saturated with a mixture of 95% O_2 and 5% CO_2) for 10-15 min at 37°C. Next 20 μCi of DL-^{3}H-NA (specific activity 20 Ci/mmole; "Amersham," England) was added to the incubation medium with a final concentration of 2.5×10^{-10} M NA, and incubated for 10-15 min. The sections were washed simultaneously but individually for 30 min by parallel transfer into pure incubation media. After rinsing, spontaneous and evoked ^{3}H-NA secretion was determined from each section. Spontaneous secretion was determined by measuring radioactivity accumulated in 1 ml of medium during incubation of the section in it for 2 min. Evoked secretion was determined after incubation of the section for 2 min in 1 ml of medium containing 20 mM KCl. The intensity of secretion (S_1) for each section was determined as the ratio between radioactivity during evoked secretion and radioactivity during spontaneous secretion. After depolarization the sections were washed for 30 min with incubation medium and the intensity of secretion was again determined (S_2). The intensity of secretion was determined 4 times in each section (S_1-S_4). Half of the sections were washed 15 min before determination of S_2 with incubation medium containing 10^{-9} M L-NA ("Serva," West Germany). The sections were washed 15 min before determination of S_4 with incubation medium containing 10^{-8} M NA. Spontaneous and evoked secretion of radioactivity in these cases is determined in the presence of given concentrations of exogenous NA. The coefficient of regulation of NA secretion by autoadrenoreceptors (C) at 10^{-9} M NA was calculated as the ratio of the value of S_2/S_1 in the presence of NA to the same value measured in parallel sections, tested without NA, at 10^{-8} M NA — the ratio S_4/S_3. The intensity of secretion S_1 and S_m was calculated individually for each animal by averaging four values of S_1 for each section and averaging all 10 values of S without the addition of NA for all sections from each animal. By averaging in this way it was possible to calculate coefficients of variation (CV) of the test parameters individually for each animal.

EXPERIMENTAL RESULTS

Prenatal exposure to ethanol did not affect the intensity of NA secretion S_1 and S_m (Table 1). By contrast, values of autoreceptor activity differed strongly from each other. The coefficient of regulation of secretion in the control animal (Fig. 1a) showed a characteristic trend depending on the exogenous NA concentration [4]: low NA concentrations increased secretion of ^{3}H-NA (at 10^{-9} M NA — 1.11 ± 0.06) whereas higher concentrations reduced it (at 10^{-8} M NA — 0.87 ± 0.05; $p < 0.05$). Autoreceptors form a "level of stable secretion" [4] (the point of intersection of the descending straight line with the abscissa), namely 4×10^{-9} M NA. In the experimental animals no characteristic relationship was observed between the coefficient of regulation of secretion and the exogenous NA concentration (Fig. 1b). These data show that a "level of stable secretion" was not formed in the experimental animals. With the NA concentrations used, the autoreceptors were virtually not activated. Individual analysis showed that characteristic dependence of autoreceptor activity on the exogenous NA concentration was observed in all the control animals (Fig. 2a), but individual differences in the

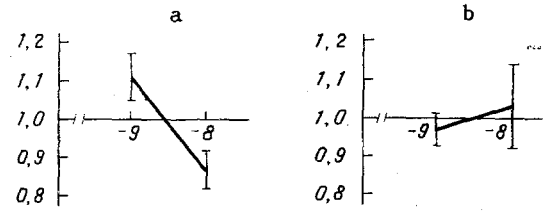

Fig. 1. Dependence of coefficient of regulation of ^{3}H-NA secretion on logarithm of endogenous NA concentration in control animals (a) and in animals exposed prenatally to ethanol (b). Abscissa, NA concentration (in M); ordinate, value of C.

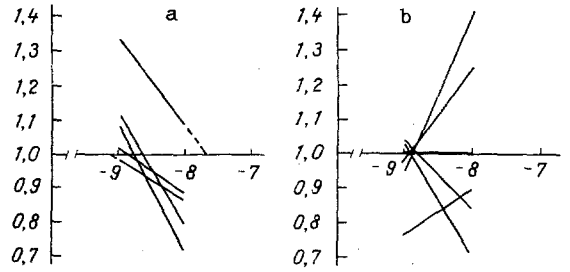

Fig. 2. Individual graphs of dependence of coefficient of regulation of ^{3}H-NA secretion on logarithm of exogenous NA concentration in control animals (a) and animals exposed prenatally to ethanol (b). Legend as to Fig. 1.

"level of stable secretion" were very great — from 9×10^{-10} to 4.3×10^{-8} M NA (Fig. 2a, broken lines), and CV = 167.0% with quite small fluctuations of the intensity of secretion (Table 1). Only in two of the experimental rats was characteristic autoreceptor activity found (Fig. 2b) with "levels of stable secretion" of 2.5×10^{-9} and 3.1×10^{-9} M NA respectively. The coefficient of regulation of secretion in one animal at 10^{-9} and 10^{-8} M NA was 1. In three animals the reaction proceeded in the opposite direction: with an increase in NA concentration the value of C rose.

Changes in the intensity of ^{3}H-NA secretion during consecutive depolarization compared with the first corresponded to autoreceptor activity. If the characteristic dependence of autoreceptor activity on exogenous NA concentration was present, leading to the formation of a "level of stable secretion" (Fig. 1a), self-intensification and stabilization of secretion were observed during subsequent depolarizations (S_n) (Fig. 3a). The value of S_n/S_1 was significant for the 2nd, 3rd, and 4th depolarizations. The difference between S_3/S_1 and S_4/S_1 was 3%. In the experimental animals self-intensification of secretion was observed, but not its stabilization (Fig. 3b). In that case the value of (S_n/S_1) also was significant at the 2nd, 3rd, and 4th depolarizations. Moreover S_3/S_1 differed significantly from S_2/S_1 by 24%, and from S_4/S_1 by 14% ($p = 0.05$). Thus absence of the characteristic dependence of the value of C on the exogenous NA concentration in animals exposed prenatally to ethanol led to destabilization of ^{3}H-NA secretion. Destabilization of secretion also was reflected in the value of the coefficients of variation of the intensity of ^{3}H-NA secretion. The value of CV_{S1} for the experimental animals was higher than for the controls, by 54% (Table 1). Despite this considerable difference, it was not significant because of the high values of the error of the arithmetic mean. The reason for this is that the value of CV_{S1} is an individual feature for each animal (calculation from 4 values of S_1). In the case of CV_{Sm}, when this value was calculated from ten values of S individually for each animal, a significant increase in the coefficient of variation by 57% was found for animals exposed prenatally to ethanol (Table 1).

It was shown previously [4] that the presence of a "level of stable secretion" in graphs of autoadrenoreceptor activity is an essential condition for normal functioning of adrenergic terminals. Under these circumstances processes of depression or facilitation of NA secretion followed by its stabilization can take place in a series of consecutive presynaptic depolarizations. The "level of stable secretion" determines the intensity of NA secretion as it depends on the degree of depolarization or the frequency of stimulation [4, 5]. Consequently,

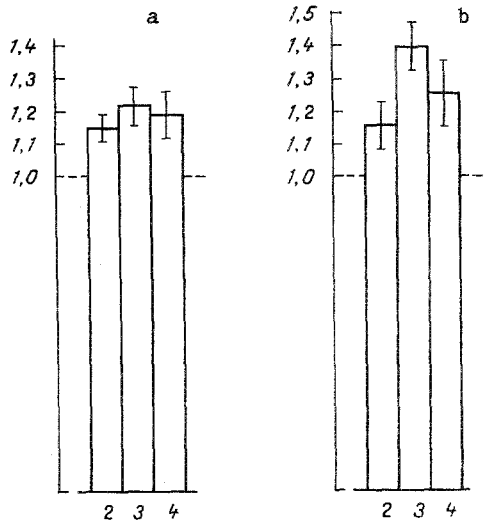

Fig. 3. Changes in intensity of ^{3}H-NA secretion during repeated (at intervals of 30 min) depolarizations of cerebral cortical sections from control animals (a) and animals exposed prenatally to ethanol (b). Abscissa, serial no. of depolarization; ordinate, S_n/S_1 (ratio of subsequent depolarizations to first depolarization, taken as 1 and indicated by broken line).

autoreceptors provide for mechanisms of synaptic plasticity such as synaptic depression, presynaptic facilitation, and frequency potentiation. Disturbances of autoreceptor activity lead to a disturbance of the processes of synaptic plasticity described above and to the destabilization of secretion. Results of this kind have been obtained only in animals exposed prenatally to ethanol, but they were also observed in control animals during the spring period [4, 6]. Characteristically, in the animals of both groups with disturbance of autoreceptor activity, disturbances of higher nervous activity, expressed as worsening of ability to form a conditioned reflex, also may appear [6, 7]. Evidently worsening of conditioning in these animals is connected with disturbances of the mechanisms of plasticity in adrenergic transmission, which are controlled by autoadrenoreceptors. We defined these disturbances as destabilization of secretion.

Naturally disturbances of higher nervous activity are determined not only by the adrenergic system of the brain. In this particular case the adrenergic system is regarded as an indicator of the general level of function of the brain, when disturbed by prenatal exposure to ethanol. We know that exposure to this same pathological factor has a powerful effect on the protein metabolism of the animal brain [7, 9], it lowers the cyclic nucleotide concentrations [8] and disturbs protein phosphorylation processes [7]. Corresponding correlation is observed here between data in the literature and our own results for besides other mechanisms, the cyclic nucleotide and protein phosphorylation systems also participate in realization of their functions by autoadrenoreceptors. Autoreceptors are integral membrane proteins; consequently, disturbance of their activity may also be linked with disturbances of protein metabolism caused by prenatal exposure to ethanol, and this may be manifested very early — during embryonic development of the rats, before the nervous system is fully developed [12]. Although protein metabolism had returned to normal in animals exposed prenatally to ethanol, at the time they were investigated in the present experiments, this still does not guarantee that the function of integral proteins was normalized.

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EFFECT OF ANTHRACYCLINE ANTIBIOTICS ON IONIC CURRENTS AND ON Na^+/Ca^{++}-EXCHANGE-RELATED CONTRACTION OF FIBERS OF THE FROG ATRIUM

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The use of anthracycline antibiotics as antitumor agents has been delayed by their cardiotoxicity and, in particular, by the development of cardiomyopathy, heart failure, and fatal arrhythmias [1, 8, 15]. The toxicity of drugs of this class is linked with the formation of semiquinone radicals of anthracyclines and activation of a chain of intracellular free-radical reactions, leading to oxidative damage of the cell structures [10, 14]. Previous investigations, aimed at studying the effects of the cumulative cardiotoxicity of anthracycline derivatives, showed that their action leads to inhibition of contractivity of isolated papillary muscles, to a change in the parameters of action potentials (AP), and to a steady decline of Na-conductance, confirming that anthracyclines act on the cell systems involved in coupling of electrical and mechanical activity [1, 3].

In the investigation described below, a method of simultaneous recording of AP, ionic currents, and contractions [6] was used to study the effect of rubomycin and its less toxic nitroxyl analog, emoxyl (ruboxyl) [7] on the pathways of entry of Ca^{++} ions into the heart cells and the possible role of these changes in the mechanism of the cardiotoxic action of anthracyclines.

EXPERIMENTAL METHOD

Experiments were carried out on atrial trabeculae of *Rana ridibunda*. The heart fibers used in the experiments had a diameter of 0.1-0.15 mm and a length of 3.5-5 mm. The fibers were perfused with standard Ringer's solution (in mM): NaCl — 110, KCl — 2.5, $CaCl_2$ — 1.8, $MgCl_2$ — 1.0, $NaHCO_3$ — 2.4, glucose — 5.5 (pH 7.4-7.5). Rubomycin* was obtained from "Khimfarmreaktiv" and emoxyl was synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR; both were used in a concentration of 100 mg/liter.

Ionic currents were investigated under voltage clamp conditions by the double sucrose gap method, using a four-electrode circuit for current clamping [11, 12]. Contraction of the trabeculae were measured at the same time, using an optical recording method [6] (the amplitude of contractions was normalized relative to the maximum). Ionic currents were measured by the use of standard circuits. The Ca current (I_{si}) was measured by testing voltage steps 70 msec in duration and 40-130 mV in amplitude immediately after preliminary membrane depolarization by 35-40 mV for 150 msec, causing inactivation of the fast Na-current. The Ca-current was

*Alternative name daunorubicin — translator.

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