L-PYROGLUTAMYL-D-ALANINE AMIDE NORMALIZES FUNCTIONS OF THE DEVELOPING RAT BRAIN DISTURBED BY ANTENATAL ALCOHOLIZATION

R. U. Ostrovskaya, S. S. Trofimov, T. A. Gudasheva, N. M. Smol'nikova, E. P. Nemova, S. V. Krapivin, N. M. Savchenko, M. L. Tsirenina, A. N. Ushakov, E. I. Mel'nik, and A. B. Kampov-Polevoi

UDC 616.831-008.1-053.2-02:616.89-008.441.13-055.52]-085.214.3.547.745]-092.9:

KEY WORDS: pyroglutamyl-dipeptides, peptide analogs of piracetam, nootropics, alcohol-induced embryonic encephalopathy

Disturbance of development of the nervous system of the offspring is a serious consequence of parental alcoholism [1, 5]. Alcohol-induced embryonic encephalopathy is manifested as various degrees of disturbance ranging from dysmorphogenesis of the brain to disturbances of its functional characteristics. Elucidation of the pathogenetic mechanisms of the damaging action of ethanol on the developing brain has attracted attention to the class of nootropics as potential correctors of these disturbances. Nootropics increase the resistance of the brain to various damaging influences [12]. The principal representative of this class, namely piracetam, increases the rate of incorporation of precursors into nucleic acids and proteins, accelerates adenine nucleotide turnover, and activates adenylate kinase [13]. Ethanol delays protein synthesis [6], inhibits Na,K-dependent ATPase, and depresses the cAMP level [14]. The question whether these opposite biochemical effects are realized at the physiological level in the intact organism is still largely unexplained. It has been shown that when learning ability is impaired as a result of chronic alcoholization, piracetam does not bring about reliable normalization. When chronic alcoholics are treated with piracetam, even in high doses, a therapeutic effect was obtained only in some of the patients [10]. A group of nootropic compounds, with activity 2-3 orders of magnitude greater than that of piracetam, has been developed in the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. Their creation was based on ideas of the receptor mechanism of action of piracetam and on the role of oligopeptides based on pyroglutamate as possible ligands of these receptors [4]. High nootropic activity has been found [8] in one of the stable peptide analogs of piracetam, namely L-pyroglutamyl-D-alanine amide (PGA). The aim of the present investigation was to study the effects of this compound on a model of disturbances of higher integrative functions of the brain in the offspring of rats subjected to antenatal alcoholization, using a combination of behavioral, biochemical, and electrophysiological methods of investigation.

EXPERIMENTAL METHOD

Alcohol was administered to 20 mature noninbred female albino rats throughout pregnancy. Ethanol in a dose of 5 g/kg, in the form of a 25% solution, or the corresponding volume of water (control group) was injected through a gastric tube. Young rats, offspring of females not receiving alcohol, were given subcutaneous injections of 0.9% NaCl solution from the 8th though the 19th day after birth (group 1), whereas offspring of alcoholized animals were given 0.9% NaCl solution (group 2) or PGA in a dose of 1 mg/kg (group 3) at the same time. Experiments were carried out only on young male rats. The time course of body weight, the time of morphological maturation (growth of the hair, opening of the eyes, descent of the testes), and the formation of motor activity were evaluated. On the 20th day eight young rats were taken from each group for the biochemical experiments.

Laboratories of Psychopharmacology and Drug Toxicology, Institute of Pharmacology, Academy of Medical Sciences of the USSR. Research Center for Molecular Diagnoses, Ministry of Health of the RSFSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 12, pp. 613-616, December, 1990. Original article submitted October 10, 1989.

 0007-4888/90/0012-1664$12.50

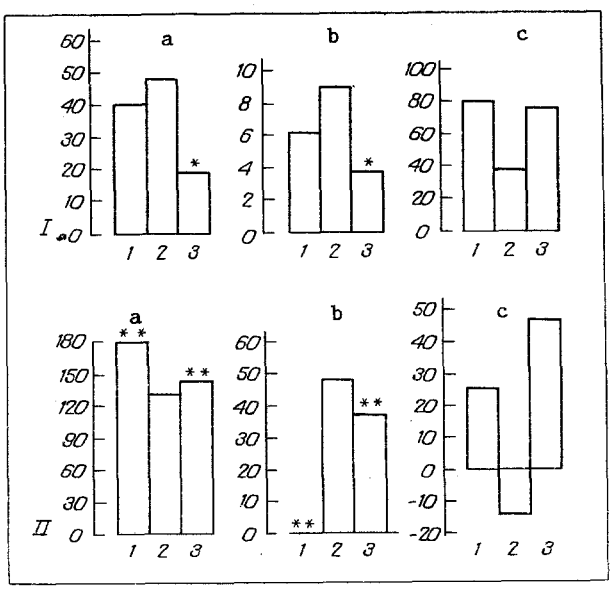

Fig. 1. Disturbance of higher integrative functions: avoidance of a stress situation (I) and ability to learn in the CPAR test (II) by ethanol and their correction by PGA. 1, 2, 3) Groups 1, 2, and 3 respectively. 1: a) latent period of diving (in sec); b) latent period of emerging on to gauze (in sec); c) number of rats completing the task (in %); II, a) latent period of visiting dark compartment (in sec); b) duration of stay in darkness (in sec); c) change in number of rats not visiting dark compartment 2 weeks after learning, compared with number of animals not visiting dark compartment 1 day after formation of CPAR, taken as 100%. $*p < 0.05$, $**p < 0.01$ compared with group 2 by Wilcoxon—Mann—Whitney U test.

The methods used to determine levels of serotonin, noradrenalin, and dopamine in brain tissue and blood plasma, and of phosphatidylserine, phosphoinositides, and cholesterol, were described previously [7]. Behavioral experiments were carried out in the following order: evaluation of emotional reactivity on a three-point scale at the age of 1 month, study of the ability of the young rats to avoid a stress situation, simulated [2] at the age of 1.5 months. Learning ability, in a model of conditioned passive avoidance reflex (CPAR) was investigated by the method in [11] at the age of 2 months. Brain electrical activity was studied after 1 month in the sensomotor cortex and dorsal hippocampus. The method of recording potentials, of Fourier analysis of the power spectrum, and calculation of the coefficient of interhemispheric asynunetry was described previously [3].

EXPERIMENTAL RESULTS

Under the conditions of the alcoholization model used, no animals were born with visible deformities: no significant changes could be found in the parameters of physical development, except slight (by 2 days) delay in the time of opening the eyes; PGA corrected the delay relating to this parameter, The dynamics of formation of the simplest parameters of the animal's state (movement coordination, acrophobia, turning over reflex) under the conditions of the relatively mild model of alcoholization which we used was unchanged. Meanwhile, in this offspring we observed disturbances of functions with a more complex organization, and later in their formation. They were found to have emotional hyperreactivity (the mean value of the response to handling was 1 in group 1 and 1.8 in group 2, and this was weakened by PGA (1.3 in group 3). In young rats with alcoholic mothers, the extrapolation avoidance reaction was disturbed. Early postnatal treatment with PGA increased the number of animals capable of diving beneath the lower edge of a cylinder almost to the control level, while the latent period of this reaction was actually reduced to lower values in the control (Fig. 1a). This facilitation of the avoidance reaction, as we showed previously in experiments on mature rats, is characteristic of the action of nootropics [9]. Formation of a CPAR, based on the inborn tendency of rats to prefer a dark, confined space (the burrowing reflex) was carried out in a two-compartment chamber. In the dark compartment, the rat was subjected to a single episode of unavoidable painful electrical stimulation through the

TABLE 1. Correction by PGA of Biochemical Parameters of the Brain and Blood in Offspring of Alcoholized Rats

Experimental conditions	Brain						Blood			
	serotonin	DA	NA	PI	PS	C/P	serotonin	DA	NA	EE
Prenatal injection of water + 0.9% NaCl solution postnatally	22,5±0,8	7,8±0,5	2,3±0,2	2,8±0,3	23,0±1,0	1,2±0,05	378±73	6,1±2,9	9,2±2,9	6,4±0,4
Prenatal alcoholization + 0.9% NaCl postnatally	19,6±1,0	9,3±0,9	2,3±0,2	4.8±0,5	12,0±0,7	0,8±0,05	624±134	0,2±0,1	6,8±1,7	0,5±0,5
Prenatal alcoholization + PGA postnatally	24,8±1,1	8,6±0,6	1,9±0,1	1,2±0,2	14,1±0,7	0,9±0,02	961±130	0,2±0,05	9,4±1,5	4,7±0,5

Legend. DA) Dopamine, NA) noradrenalin (in ng/10 mg brain tissue or in g/ml blood plasma), PI) phosphatidylinositides, PS) phosphatidylserine (in % of total lipids), C/P) molar ratio cholesterol/phospholipids, EE) endogenous ethanol (in μg/ml blood).

electrified floor. Preservation of the skill was tested 2 weeks after training, and compared with the data obtained 1 day after training. The preservation of passive avoidance skill was found to be disturbed in the offspring of alcoholized mothers (Fig. 1b). The earlier postnatal administration of PGA partially corrected this increased forgetfulness, increasing the latent period of visiting the dark compartment, reducing the duration of stay in it, and increasing the number of animals not visiting the dangerous compartment. According to the last parameter, the effect of this peptide analog of piracetam was particularly demonstrative. In the control group this parameter was increased, evidence, it would seem, of a prolonged process of consolidation as a result of reminding when the test was carried out 1 day after training. In the offspring of the alcoholics it was reduced, but in rats also subjected to antenatal alcoholization, treated in the early postnatal period with the dipeptide, it was actually higher than in the control group.

Sensomotor cortical electrical activity of the offspring of alcoholized rats was characterized by increased relative power of the high-amplitude slow δ-waves (1 and 6% in groups 1 and 2 respectively) and weakening of the power of the high-frequency β-band (16 and 9% in groups 1 and 2 respectively) compared with the offspring of intact animals. There was also an increase in interhemispheric differences with respect to amplitude of the power spectrum and its dominant frequency (the values in groups 1 and 2 were 1 and 1.13 respectively), indicting a decrease in the degree of coordination of their activity under the conditions of this particular model of pathology. Activity of the dorsal hippocampus was not significantly changed compared with the control animals. In the progeny of alcoholized rats, treated with PGA, a tendency was noted for the parameters of cortical electrical activity to return to normal (reduction of the relative power of the δ-band to 3% and an increase in the level of β-activity to 13%), as well as reduction of the degree of interhemispheric asymmetry. The parameter of asymmetry was actually lower (0.67), than in the control animals, and this can be regarded as a manifestation of facilitation of interhemispheric connections, characteristic of nootropics [3].

Antenatal alcoholization led to changes in all the biochemical parameters studied in the offspring. There was a sharp fall in the endogenous ethanol level, which was abolished by early postnatal administration of PGA. The neurotransmitter balance was disturbed in the offsping: the serotonin concentration in brain tissues was depressed (probably due to increased release into the blood) and the dopamine level was raised. At the same time we found a marked increase in the phosphatidylinositide concentration and a decrease in phosphatidylserine. In the offspring of alcoholized animals exposed to early postnatal PGA therapy these changes were considerably weakened or completely absent (Table 1). Phosphatidylinositides and phosphatidylserine are ascribed an important role in the regulation of release of acetylcholine and biogenic amines. It has been shown that injection of phosphatidylserine abolishes the amnesic effect of scopolamine [15]. It can be tentatively suggested that the change which we found in these phospholipids is one cause of the worsening of learning ability and of other disturbances of higher integrative functions in the offspring of alcoholized females. Considering ideas on the triggering role of membrane phospholipids in maintenance of the neurotransmitter balance, it may be postulated that the normalizing effect of PGA on neurotransmitter processes is mediated through normalization of the lipid components of the membrane. In turn, these mechanisms may be triggered by the primary response of the nootropic with the receptor, the presence of which was postulated on the basis of much neuropharmacological evidence described previously [4]. Although the mechanisms of the observed effect require further study,

the fact of the normalizing influence of the compound on a combination of electrophysiological, behavioral, and biochemical parameters may provide the basis for further analysis of the role of the new type of highly active nootropics, which are peptide analogs of piracetam, as correctors of the functional disturbances in alcohol-dependent embryonic encephalopathies.

LITERATURE CITED

1. I. P. Anokhina, I. V. Man'kovskaya, K. V. Mashilov, and E. V. Kudinov, Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), pp. 42-53.
2. N. A. Bondarenko, A. V. Val'dman, and V. A. Kamysheva, Byull. Éksp. Biol. Med., No. 7, 35 (1981).
3. T. A. Voronina, S. V. Krapivin, and N. N. Bogdanov, Vestn. Akad. Med. Nauk SSSR, No. 2, 17 (1987).
4. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, et al., Khim.-Farm. Zh., No. 11, 1322 (1985).
5. R. I. Kruglikov and M. Ya. Maizelis, Alcoholism and Offspring [in Russian], Moscow (1987).
6. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Neirokhimiya, **2**, No. 3, 288 (1983).
7. E. I. Mel'nik, M. L. Tsirenina, A. N. Ushakov, et al., Vopr. Med. Khim., No. 3, 33 (1989).
8. R. U. Ostrovskaya, T. A. Gudasheva, S. S. Trofimov, et al., Byull. Éksp. Biol. Med., No. 11, 576 (1987).
9. N. M. Savchenko, R. U. Ostrovskaya, and Yu. V. Burov, Byull. Éksp. Biol. Med., No. 8, 170 (1988).
10. S. Binder and P. Doddabela, Med. Klin., **71**, 711 (1976).
11. J. Bureš and O. Burešova, J. Comp. Physiol. Psychol., **56**, No. 2, 268 (1963).
12. C. Giurgea, Actual. Pharmacol., **25**, 115 (1972).
13. W. J. Nicholson and O. L. Wolthuis, Biochem. Pharmacol., **25**, No. 2, 2241 (1976).
14. L. Volicer, R. Mirin, and B. I. Gold, J. Stud. Alcohol., **38**, No. 1, 11 (1977).
15. A. Zanotti, L. Valzelli, and G. Toffano, Psychopharmacology, **90**, 274 (1986).