EFFECT OF BRAIN ACID EXTRACT PRODUCTS ON ^{3}H-DIAZEPAM RECEPTION IN INBRED MICE

Yu. A. Blednov, Yu. L. Fedoseev,
N. N. Bogdanov, M. L. Gordei,
and S. B. Seredenin

UDC 615.214.22.015.4:612.82.015.3/.076.9

KEY WORDS: benzodiazepine receptor; neuropeptides.

Chromatographic fractionation of acid brain extracts of C57BL/6 (B6) and BALB/c (C) mice, with opposite types of behavior in the open field test during exposure to emotional stress and administration of benzodiazepine tranquilizers [2], revealed marked interlinear differences between chromatogram profiles and content of ACTH-immunoreactive peptides (ACTH-IP) in the individual fractions.

To study whether these products may participate in the mechanisms of formation of inherited features of emotional-stress reactions, their effect on specific binding of ^{3}H-diazepam by brain synaptosomal membranes of animals of these same lines was investigated.

EXPERIMENTAL METHODS

Experiments were carried out on B6 and C mice obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The animals were kept 10 to a cage on a standard diet with alternation of 12 h daylight and 12 h darkness.

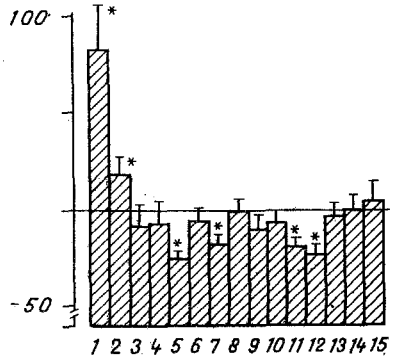

Fig. 1

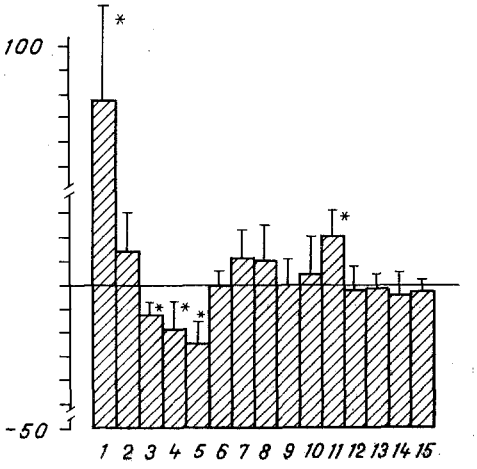

Fig. 2

Fig. 1. Effect of contents of brain fractions from B6 mice on ^{3}H-diazepam reception by synaptosomal membranes. Here and in Fig. 2: abscissa, Nos. of fractions; ordinate, ^{3}H-diazepam binding (in per cent of control – horizontal line); *$P < 0.01$ compared with control.

Fig. 2. Effects of contents of brain fractions from C mice on ^{3}H-diazepam reception by synaptosomal membranes.

Research Laboratory of Pharmacokinetics, N. I. Pirogov Second Moscow Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 294-296, September, 1986. Original article submitted October 14, 1985.

 0007-4888/86/0009-1206$12.50

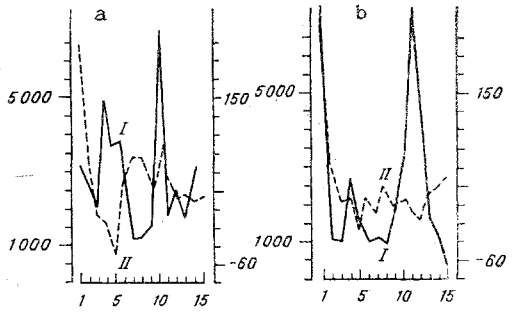

Fig. 3. Content of ACTH-IP (I) in brain fractions of C (a) and B6 (b) mice and effect (II) of contents of these fractions on ^{3}H-diazepam binding with synaptosomal membranes. Abscissa, Nos. of fractions; ordinate, on left – content of ACTH-IP (in pg ACTH/g tissue), on right – binding of ^{3}H-diazepam (in percent of control).

Acid brain extracts were fractionated by high-efficiency liquid chromatography. Acetonitrile was removed from the product by evaporation on a rotary evaporator and the residue was frozen at −40°C and lyophilized. Before the beginning of the experiment the lyophilized material of each fraction was dissolved in 4 ml of Tris-citrate buffer, pH 7.4, and used in the ^{3}H-diazepam binding experiments.

To study the membrane fraction, after removal of the cerebellum and brain stem the remainder of the brain was homogenized in 25 ml of 50 M Tris-citrate buffer, pH 7.4. The homogenate was centrifuged at 45,000 g for 25 min in a 42 L5-50 rotor (Beckman, Austria). The residue was resuspended in the initial volume of buffer and centrifuged under the same conditions.

The membrane material was washed 3 consecutive times. The residue was resuspended in 75 ml of Tris-citrate buffer and used in subsequent experiments. All procedures concerned with purification and isolation of the membrane preparations were carried out at 0-4°C. The suspension, 1 ml in volume, containing from 150 to 250 µl protein, was incubated with N-methyl-^{3}H-diazepam (specific radioactivity 71 Ci/mmole, from Amersham Corporation, England) in a final concentration of 1 nM. Incubation was carried out in an ice bath for 30 min. A reaction was stopped by rapid filtration through GF/B glass fiber filters (Whatman, England), and then washed twice with cold buffer. Radioactivity was counted in an LS-100C scintillation counter (Beckman).

Nonspecific binding was determined in the presence of unlabeled diazepam in a final concentration of 10 µM; it did not exceed in magnitude 20% of the total. The final ethanol concentration in the incubated sample did not exceed 0.5%.

EXPERIMENTAL RESULTS

Receptor interaction is regarded as the principal stage in realization of the effects of endogenous regulator hormones, of neurotransmitters, and also of many drugs. Fundamental research has demonstrated the role of receptors in the genesis of several hereditary nervous and mental diseases [4].

After the discovery of specific binding sites of the benzodiazepine tranquilizers in 1977 it was shown that these membrane formations, functioning in combinations with the GABA receptor and Cl^--ionophore [3], are not only the key stage in the mechanism of action of benzodiazepines [7], but they also participate actively in responses to emotional stressors [8, 9]. Changes in the receptor complex induced by endogenous compounds, which can be described on the basis of differences in its ability to bind labeled benzodiazepines, must therefore be considered as important evidence that the substances studied can take part in the regulation of emotional behavior.

The results of experiments with chromatographically fractionated acid brain extracts from B6 mice showed that the contents of fraction 1 considerably increased specific ^{3}H-diazepam binding by synaptosomal membranes isolated from the brain of mice of the same line.

A similar but weaker effect was given by products of fraction 2. Reduced binding of the radioligand was observed if fractions 5, 7, 11, and 12 were added to the incubation medium (Fig. 1).

When material obtained from C mice was used a significant increase in binding of the labeled product was observed under the influence of fractions 1 and 11, whereas fractions 3, 4, and 5 had an inhibitory action (Fig. 2).

It will thus be evident that acid brain extracts from B6 and C mice contains substances capable of modifying the functional state of the benzodiazepine receptor complex. Qualitative and quantitative interlinear differences were found in the effect of the test products on radioligand reception.

Comparison of the effectiveness of action of the test fractions on the benzodiazepine receptor with their ACTH-IP content revealed the following facts. First, the appearance of activity modulating ^{3}H-diazepam reception, both stimulating and inhibiting, correlated with elevation of the ACTH-IP level in that fraction, which was particularly marked in C mice (Fig. 3a). Second, the interlinear differences were very distinct. For instance, whereas in fractions 1 and 2 an increase in the ACTH-IP concentration in mice of both lines corresponded with the presence of stimulating activity, and with the presence of inhibitory activity in fractions 4 and 5, in the case of fractions 11 and 12 elevation of the ACTH-IP level in C mice was accompanied by strengthening of reception, but by its depression in B6 mice (Fig. 3b). Taken as a whole these data, evidence of definite binding between ACTH-IP and the benzodiazepine receptor, agree with information in the literature on the role of ACTH and related peptides in the regulation of emotional behavior [1], the ability of ACTH to modify the characteristics of GABA-receptors in the rat brain [5], and the effect of ACTH and of benzodiazepine tranquilizers on microviscosity of synaptosomal membranes [6, 10]. It can therefore be tentatively suggested that the specific differences observed in the composition of ACTH-IP in the brain (and also, perhaps, of other products of its acid extract) are one stage in the mechanism of formation of hereditary characteristics of emotional behavior. The results of the present investigation provide a basis for future research to test this hypothesis.

LITERATURE CITED

1. A. V. Val'dman and V. P. Poshivalov, Pharmacologic Regulation of Intraspecific Behavior [in Russian], Leningrad (1984), pp. 170-174.
2. S. B. Seredenin and A. A. Vedernikov, Byull. Éksp. Biol. Med., No. 7, 38 (1979).
3. E. Costa and A. Guidotti, Ann. Rev. Pharmacol. Toxicol., 19, 531 (1979).
4. G. Kato and T. Ban, Prog. Neuro-Psychopharmacol. Biol. Psychiat., 6, 207 (1982).
5. D. A. Kendall, B. S. McEwen, and S. J. Enna, Brain Res., 236, 365 (1982).
6. T. Mennini, A. Ceci, S. Caccia, et al., FEBS Lett., 173, 255 (1984).
7. R. W. Olsen, Ann. Rev. Pharmacol. Toxicol., 22, 245 (1982).
8. V. V. Petkov and S. Yanev, Pharm. Res. Commun., 14, 739 (1982).
9. P. Soubrie, M. H. Thiebot, A. Jobert, et al., Brain Res., 189, 505 (1980).
10. C. J. Van Dongen, M. Hershkowitz, H. H. Zwiers, et al., in: Aging of the Brain, W. H. Gispen and J. Traber, eds., Amsterdam (1983), pp. 101-114.