8. G. V. Kovalev, A. A. Spasov, and N. A. Bogachev, New Data on Eleuterococcus and Other Antistress Agents [in Russian], Vladivostock (1981), pp. 51-56.
9. F. Z. Meerson, S. I. Krasikov, V. M. Boev, and V. E. Kagan, Byull. Éksp. Biol. Med., No. 7, 17 (1982).
10. F. Z. Meerson, V. E. Kagan, Z. V. Beresneva, et al., Teor. Prakt. Fiz. Kul't., No. 8, 14 (1983).
11. F. Z. Meerson and M. G. Pshennikova, Adaptation to Stress Situations and Physical Exertion [in Russian], Moscow (1983).
12. B. Z. Simkhovich, D. V. Meirena, Zh. V. Shutenko, et al., Calcium Metabolism in the Physiology and Pathology of the Cardiovascular System [in Russian], Part 2, Tomsk (1988), pp. 179-180.
13. A. A. Spasov, O. V. Ostrovskii, A. V. Tsibanev, et al., Pharmacology and Scientific–Technical Progress [in Russian], Tashkent ( ), pp. 352-353.
14. P. Hochachka, and J. Somero, Biochemical Adaptation [Russian translation], Moscow (1988), pp. 98-229.

EFFECT OF DIAZEPAM AND OF N^6-CYCLOHEXYLADENOSINE ON LEVEL OF DIAZEPAM-BINDING INHIBITOR IN THE HIPPOCAMPUS DURING IMMOBILIZATION STRESS

G. M. Poltavchenko

UDC 615.214.22.015.4:612.82.15.1].07

KEY WORDS: diazepam-binding inhibitor, adenosine derivatives, diazepam, immobilization stress

Adenosine and its stable derivatives possess antistressor and anticonvulsant activity [4, 5]. The physiological effects of this group of preparations have been shown to take place through a system of specific adenosine receptors of the A_1- and A_2-types, located on presynaptic membranes of neurons [8]. Meanwhile, some investigators have shown that purine derivatives can affect the functional activity of the GABA-benzodiazepine receptor-complex (GABA-BD-RC) and, in particular, by displacing diazepam from its specific binding sites [6]. The search for an endogenous modulator of GABA-BD-RC has led to the isolation and identification in brain neurons of a specific protein, diazepam-binding inhibitor (DBI), which, by its action on benzodiazepine receptors, reduces the ability of GABA to bind with specific receptors [1, 3].

In the investigation described below changes in the DBI level were studied in animals with immobilization stress and during its correction by diazepam and by N^6-cyclohexyladenosine, an agonist of A_1-adenosine receptors.

EXPERIMENTAL METHOD

Experiments were carried out on 45 noninbred male albino rats weighing 200-220 g. Immobilization stress was induced in the animals by fixing them for 6 h. The following substances were used in the experiments. N^6-cyclohexyladenosine, an agonist of A_1-adenosine receptors (from the All-Union Technologic Research Institute of Antibiotics and Enzymes of Medical Importance, Leningrad) in a dose of 0.1 mg/kg, and diazepam (from "Polfa," Poland) in a dose of 0.5 mg/kg. The substances were injected intraperitoneally 30 min before the beginning of the experiment and thereafter every 2 h during its course. Animals of the control group received physiological saline. The animals were decapitated. The brain was quickly removed (within 30-45 sec) and homogenized in 10 volumes of hot (80°C) 1 M acetic acid. The homogenate was centrifuged at 48,000*g* for 20 min (at 2-4°C). The supernatant was adjusted to pH 6 and again centrifuged, after which the supernatant was lyophilized. The DBI level

All-Union Technologic Research Institute for Antibiotics and Enzymes of Medical Importance, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 166-167, August, 1990. Original article submitted October 26, 1989.

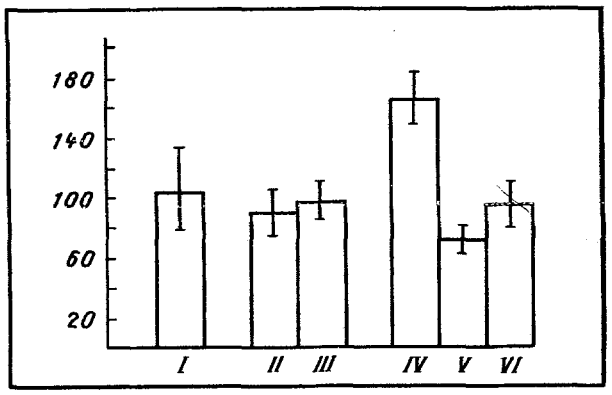

Fig. 1. DBI level in hippocampus during immobilization stress and its correction by N^6-cyclohexyladenosine and diazepam. Abscissa: I) control, II) diazepam (0.5 mg/kg), III) N^6-cyclohexyladenosine (0.1 mg/kg), IV) immobilization stress, V) stress + diazepam, VI) stress + N^6-cyclohexyladenosine. Ordinate, DBI level (in pg/500 μl sample).

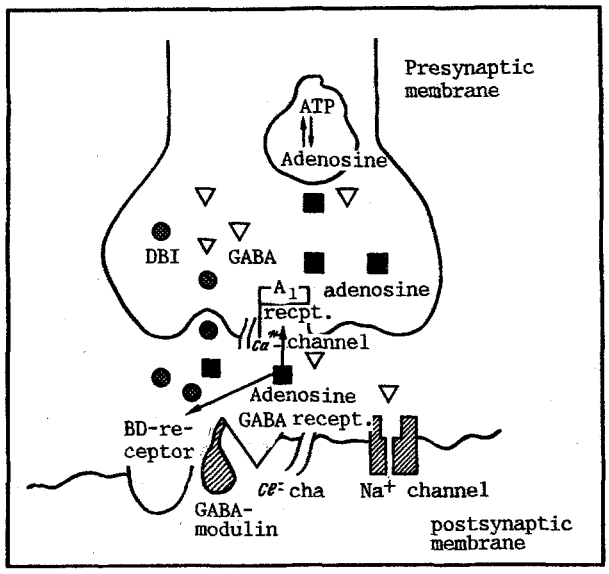

Fig. 2. Hypothetical model of interaction of type A_1 adenosine receptors and GABA-BD-RC. Neurotransmitter GABA, co-transmitter DBI, and adenosine were isolated from presynaptic membrane. Arrows indicate possible pathways of neuromodulating action of adenosine on GABA-BD-RC activity.

was determined by radioimmunoassay, using a test kit from DRG Int. (USA). The experimental data were subjected to statistical analysis by Student's t test on an "Iskra-2264" computer.

EXPERIMENTAL RESULTS

The experiments showed that under the conditions of the chosen model of immobilization stress there was a considerable increase in the DBI level in the rat hippocampus. For instance, the DBI level under these conditions rose by more than 1.5 times (167.3 ± 14.7 pg/sample compared with animals of the control group (104 ± 28.1). Administration of diazepam to intact animals led to a very small decrease, not statistically significant, in the DBI content in hippocampal tissues (90.0 ± 8.3 pg). Injections of N^6-cyclohexyladenosine into animals not exposed to stress likewise did not change the neuropeptide level in the brain tissue.

The use of diazepam during stress led to a significant fall of the DBI level to 72 ± 8.3 pg. The type A_1 adenosine receptor agonist N^6-cyclohexyladenosine had a similar effect: administration of the compound lowered the level of the neuropeptide to 97 ± 8.3 pg, i.e., virtually to its level in the control group (Fig. 1).

Thus, the DBI level rose in the hippocampal structures against the background of immobilization stress. In view of data [2] relating to the neuromodulating role of DBI in GABA-ergic neurotransmission it can be tentatively suggested that the change in the functional state of GABA-DB-RC is an important factor in the mechanisms of development of the stress reaction. The rise of the DBI level during development of the stress reaction evidently leads to a decrease in binding of endogenous diazepam with the receptor sites, followed by a decrease in the ability of GABA to bind with its own receptors and, as a result, by the appearance of behavioral reactions of fear and anxiety in the animals.

Data indicating that the agonist of A_1-adenosine receptors acts similarly to diazepam are particularly interesting. In view of reports in the literature that adenosine derivatives have anxiolytic, anticonvulsant, and antistressor activity, and can also bind with benzodiazepine receptors, the existence of close receptor—receptor bonds can be postulated between adenosine receptors and GABA-BD-RC. However, many aspects of these relationships are not yet clear. At the same time, the following hypothetical model of relationships of this type can be proposed (Fig. 2). During the development of a stress reaction the primary neurotransmitter GABA, the co-transmitter of peptide nature DBI, and also adenosine are released from the presynaptic membrane. We know that adenosine is a presynaptic neuromodulator [7] which, by its action through type A_1 presynaptic receptors, can perhaps reduce the release and accumulation of DBI at GABA-BD-RC, and this may be one of the mechanisms of the antistressor action of adenosine and its derivatives.

LITERATURE CITED

1. E. Costa and A. Guidotti, Ann. Rev. Pharmacol., **19**, 531 (1979).
2. E. Costa, H. Alho, M. F. Santi, P. Ferrero, and A. Guidotti, Prog. Brain Res., **68**, 343 (1986).
3. A. Guidotti, C. M. Forechetti, M. G. Corda, et al., Proc. Nat. Acad. Sci. USA, **80**, 3531 (1983).
4. P. H. Franklin, E. D. Tripp, Ge Zang, et al., Eur. J. Pharmacol., **150**, 202 (1988).
5. P. J. Marangos, S. M. Paul, F. K. Goodwin, and P. Skolnick, Life Sci., **25**, 1093 (1979).
6. S. M. Paul, P. Marangos, and P. Skolnick, Psychopharmacology and Biochemistry of Neurotransmitter Receptors, New York (1980), pp. 666-676.
7. J. W. Phillis and B. H. Wu, Prog. Neurobiol., **16**, 187 (1981).
8. M. Williams, Ann. Rev. Pharmacol., **27**, 315 (1987).