Effect of Antiserotonin Antibodies on Bioelectrical Activity of Sensorimotor Cortex

S. I. Igon'kina, M. L. Kukushkin, L. A. Basharova, L. A. Vetrile, O. I. Mikovskaya, and V. A. Evseev

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The effect of antiserotonin antibodies on basal electrocorticogram was studied in electrophysiological experiments on rats. Intracortical injection of 10 μg antiserotonin antibodies into the sensorimotor cortex induced epileptiform activity in this area. It is assumed that antiserotonin antibodies modulate activity of cortical neurons due to both binding serotonin molecules and interaction with serotonin receptors.

Key Words: *serotonin; antiserotonin antibodies; sensorimotor cortex; electrocorticogram*

Antibodies against neurotransmitters play an important role in the mechanisms of neuroimmunomodulation. The effects of antineurotransmitter antibodies on various physiological functions were described [1,2]. Antiserotonin and antidopamine antibodies were detected in various pathologies of the central nervous system (CNS): drug addiction [4], neurogenic pain syndrome [5,6], alcoholism [7], Parkinson's disease [8,9], and schizophrenia [13]. Active immunization with protein-conjugated serotonin changed the content of serotonin in CNS structures [2,3]. However, the mechanisms underlying the effects of antineurotransmitter antibodies on CNS are little studied. Here we examined the effect of antiserotonin antibodies on electrical activity of the brain cortex.

MATERIALS AND METHODS

The experiments were performed on 17 male Wistar rats weighing 270-320 g. Antiserotonin antibodies were obtained from rabbits immunized with serotonin-protein conjugate according to a routine scheme [2]. For examination of the neurotropic effects, the antibodies were purified by affinity chromatography. Chromotographic column packed with BrCN-activated Sepharose 4B (4 g) in distilled water was washed with 0.001 M HCl buffered physiological saline (0.15 M NaCl, 0.15 M KH_2PO_4, 0.15 M Na_2HPO_4), 0.1 M $NaHCO_3$, and balanced with 0.3 M $NaHCO_3$. Conjugated serotonin-protein antigen (20 mg) in 5 ml 0.3 M $NaHCO_3$ was applied to the column. The obtained immunosorbent was washed with 0.3 M $NaHCO_3$, 0.1 M $NaHCO_3$, and buffered physiological saline to zero eluate extinction at 280 nm. Antiserotonin antibodies (γ-globulin fraction, 40 mg protein) were dissolved in 5 ml buffered physiological saline and applied to the column. On the next day the anti-BSA antibodies were eluted with buffered physiological saline, the column was washed with 0.1 M CH_3COOH, and optical density of each fraction was measured at 280 nm. Purified antiserotonin antibodies were lyophilized and stored at 4°C.

For evaluation of the effect of antiserotonin antibodies on bioelectrical activity of the sensorimotor cortex the antibodies were injected intracortically. Experimental animals (n=9) were injected with 10 μg antiserotonin antibodies, control animals received physiological saline (n=3) or 10 μg γ-globulin from intact rabbits (n=5). The preparations (in 2 μl saline) were injected intracortically at a depth of 200 μ for 2 min

Laboratory of Neuroimmunopathology, Laboratory of Pain Pathophysiology, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Science, Moscow. ***Address for correspondence:*** 4909.g23@g23.relcom.ru. Igon'kina S. I.

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using a glass microcannula connected to a stereotaxic microfeed. Electrocorticogram (EcoG) was recorded before and after injection of antiserotonin antibodies for 4-6 h. To this end, the rats narcotized with urethan (1400 mg/kg intraperitoneally) were fixed in a stereotaxis apparatus (the sites of fixation were treated with 0.5% novocain), the scull was trepanized, and the sensorimotor cortex was exposed. The rats were immobilized with myorelaxin (50 mg/kg, intramuscularly) and maintained on jet ventilation. Body temperature was maintained at 37.5°C. ECoG was recorded monopolarly, using surface silver electrode located close to the microcannula; indifferent electrode was fixed on the frontal bone. The signals were input to a biophysical amplifier (0.1-500 Hz frequency band) of a VC-9 oscillograph (Nihon Kohden) and taped for subsequent processing.

RESULTS

Basal electrical activity of the sensorimotor cortex was characterized by combination of fast (12-16 Hz) and slow (4-6 Hz) low-amplitude oscillations (Fig. 1, *1*, and Fig. 2, *1*). Intracortical injection of antiserotonin antibodies induced a desynchronization reaction within the first 2-3 min associated with predominance of low-amplitude (up to 40 μV) 16-20 Hz rhythm. Starting from 5-10 min postinjection all experimental animals showed short single or grouped high-amplitude discharges (300-500 μV) following at a frequency of 2-3 per sec (Fig. 1, *2*). Twenty minutes after injection of antiserotonin antibodies the periods of paroxysmal activity increased and presented as a dominant high-amplitude 4-6 Hz rhythm (Fig. 1, *3-5*). In animals receiving antiserotonin antibodies this epileptiform activity persisted throughout the whole observation period.

In the control groups the changes in ECoG were different. In rats receiving physiological saline a decrease in oscillation amplitude and an increase in frequency were observed within the first few minutes after microinjection, but then the basal rhythm was restored. In control animals injected with γ-globulin the desynchronization reaction observed within the first few minutes postinjection was followed by a short period of single high-amplitude discharges and recovery of the basal rhythm (Fig. 2, *4-5*). The observed transient changes in ECoG after injection of γ-globulin can be explained by nonspecific binding of some neurotransmitters with immunoglobulins [10].

Thus, intracortical injection of antiserotonin antibodies significantly changed electrical activity of the brain. Intracortical microinjection of these antibodies into the sensorimotor cortex induced persistent epileptiform activity. We assumed that paroxysmal dischar-

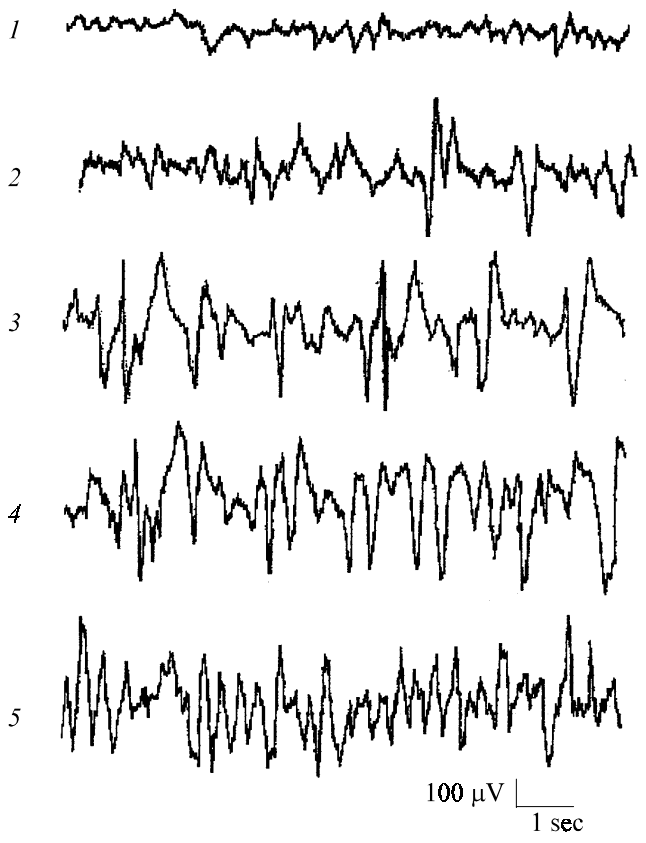

Fig. 1. Bioelectrical activity of sensorimotor cortex after intracortical microinjection of 10 μg antiserotonin antibodies in rat No. 6. *1*) before antiserotonin antibody injection, *2, 3, 4, 5*) 10, 20, 60, and 90 min after antibody injection, respectively.

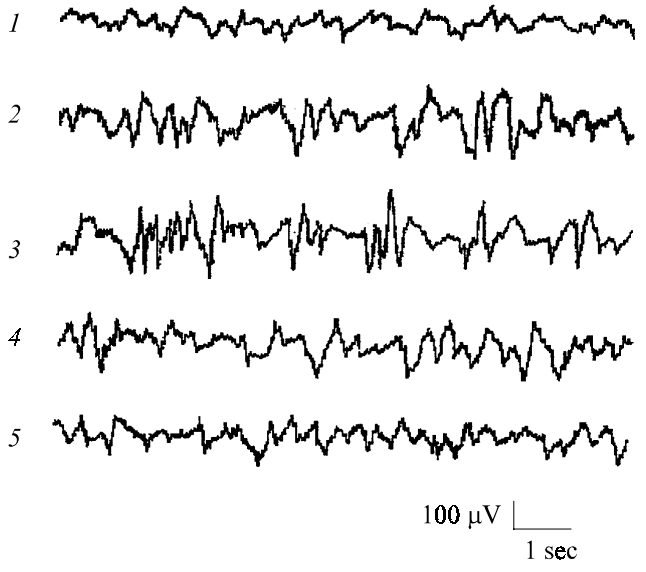

Fig. 2. Bioelectrical activity of sensorimotor cortex after intracortical microinjection of 10 μg γ-globulin in rat No. 6. *1*) before γ-globulin injection, *2*, *3*, *4*, *5*) 10, 20, 60, and 90 min after γ-globulin injection, respectively.

ges in ECoG induced by intracortical injection of antiserotonin antibodies result from decreased inhibitory influences of serotonin on cortical neurons. Neurotransmitter serotonin plays an important role in the modulation of neuronal activity of the brain cortex. Intravenous injection of serotonin reduce ECoG amplitude in the frontal and entorhinal cortex in rats [15], while ionophoretic application of serotonin [11], or electrostimulation of the raphe nuclei, a source of serotonin-containing terminals in the cortex [12] induce inhibitory reactions in sensorimotor cortical neurons.

The mechanisms underlying the effect of antineurotransmitter antibodies on neuronal activity are little studied. We previously showed that active immunization with serotonin-protein conjugate decreased the content of serotonin in the cortex [2,3]. Moreover, the inhibitory effect of antiserotonin antibodies on neuronal receptors was reported. It was shown that anti-idiotypic antiserotonin antibodies blocked the binding of labeled serotonin and agonists of 5-HT_{1B}, 5-HT_{1C}, and 5-HT_2 receptor subtypes to cell membranes isolated from the cortex and striatum [14].

Our findings suggest that the effect of antiserotonin antibodies on the activity of cortical neurons is mediated by serotonin binding to antibodies and their interaction with serotonin receptors.

REFERENCES

1. I. P. Ashmarin and I. S. Freidlin, *Zhurn. Evolyuts. Biokhim.*, **25**, No. 2, 76-81 (1989).
2. L. A. Basharova, L. A. Vetrile, V. A. Evseev, *et al., Fiziol. Zhurn. SSSR*, No. 10, 1367-1372 (1988).
3. N. V. Bobkova, L. A. Plakkhinas, L. A. Basharova, *et al., Pat. Fiziol.*, No. 3, 31-36 (1989).
4. V. A. Evseev, L. A. Basharova, L. A. Vetrile, *et al., Vopr. Narkol.*, No. 1, 33-39 (1995).
5. S. I. Igon'kina, G. N. Kryzhanovskii, V. A. Zinkevich, *et al., Pat. Fiziol.*, No. 2, 6-8 (1997).
6. S. I. Igon'kina, G. N. Kryzhanovskii, M. L. Kukushkin, *et al., Zhurn. Vyssh. Nervn. Deyat.*, **45**, No. 6, 999-1006 (2000).
7. G. N. Kryzhanovskii and V. A. Evseev, *Vestn. AMN SSSR*, No. 3, 10-14 (1988).
8. G. N. Kryzhanovskii, M. A. Atadzhanov, S. V. Magaeva, *et al., Byull. Eksp. Biol. Med.*, **107**, No. 1, 13-16 (1989).
9. G. N. Kryzhanovskii, V. A. Evseev, S. V. Magaeva, *et al., Ibid.*, **112**, No. 11, 470-472 (1991).
10. V. A. Strigin and A. P. Ternovoi, *Nonspecific Properties of Immunoglobulin Preparations*, Moscow (1979) p. 96
11. M. H. Bassant, K. Ennouri, and Y. Lamour, *Neuroscience*, **39**, No. 2, 431-439 (1990).
12. A. J. Beitz, *Serotonin and Pain*, Amsterdam (1990) p. 95-103
13. K. Schott, A. Batra, R. Klein, *et al., Eur. Psychiatry*, **7**, 209-215 (1992).
14. H. Tamir, K. P. Liu, S. C. Hsiung, *et al., J. Neurochem.*, **57**, No. 3, 930-942 (1991).
15. T. Winkler, H. S. Sharma, E. Stilberg, *et al., Neuroscience*, **68**, No. 4, 1097-1104 (1995).