# THE GABA-ERGIC MECHANISM OF THE EFFECT OF DIAZEPAM ON CORTICAL NEURONS

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The effect of diazepam, depakine thiosemicarbazide (TSC), and bicuculline on unit activity of the sensomotor cortex arising spontaneously and evoked by sciatic nerve stimulation was studied in unanesthetized, immobilized albino rats. Diazepam was shown to strengthen the inhibitory effects, i.e., to reduce the frequency of spontaneous discharges and to prolong postactivation depression of unit activity (the inhibitory pause). Depakine which increases the brain GABA concentration, had a similar action. Bicuculline, which blocks GABA-ergic receptors, and TSC, which reduces its concentration, had the opposite action and weakened inhibition. A two-way antagonism also was found between diazepam and bicuculline as regards their effect on unit activity. The results confirm the earlier hypothesis that diazepam can potentiate the effect of GABA on cortical neurons.

KEY WORDS: cerebral cortex; evoked and spontaneous unit activity; diazepam; GABA.

It has been shown by recording recovery cycles of evoked potentials that diazepam selectively depresses the test response; this effect of diazepam is abolished by substances with GABA-negative action, such as bicuculline and thiosemicarbazide (TSC) [5, 17]. Since changes in the amplitude of the testing potential relative to the conditioning are regarded as serving as a criterion of the relationship between excitation and inhibition [8] and in view of observations indicating a possible role of GABA as the inhibitory mediator [9], the writers have postulated [6, 17] that diazepam can potentiate inhibitory processes and that some of its effects are mediated through the GABA system. One way of testing this hypothesis is by studying the effect of diazepam on unit activity.

Since the direct action of diazepam on the cerebral cortex was demonstrated previously [2] the object of the present investigation was to study the effects of diazepam and of substances modifying the state of the GABA system (depakine, bicuculline, and TSC) on cortical unit activity.

## EXPERIMENTAL METHOD

Acute experiments were carried out on male albino rats weighing 200-300 g. Under ether anesthesia craniotomy was performed, a cannula was inserted into the jugular vein, tracheotomy was then performed, and the sciatic nerve exposed, after which the anesthetic was discontinued, the animal was immobilized with succinylcholine (3-5 mg/kg, intravenously), and artificial respiration applied. The sciatic nerve was stimulated by square pulses (300 $\mu$sec, 1-2 V) applied through a high-frequency unit from the output of a Physiovar (Alvar, France) stimulator. Unit discharges were derived by glass microelectrodes with a tip 1-4 $\mu$ in diameter from the focus of maximal activity of the primary response to sciatic nerve stimulation. The potentials were led through a cathode follower to a UBP 1-02 amplifier and then displayed on the screen of a type S1-19B oscilloscope. The data were processed in the course of the experiments on the Nokia LP-4840 (Finland) analyzer by plotting poststimulus histograms for 25-50 presentations of the stimulus (time quanta for the histograms were 2-10 msec; depending on the duration of the quantum, the epoch of analysis was 400-2000 msec). The histograms were presented by an automatic XY-writer and a digital printer.

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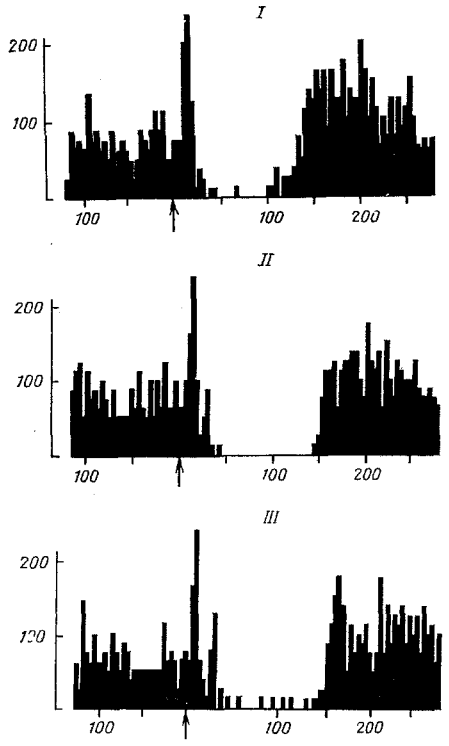


Fig. 1

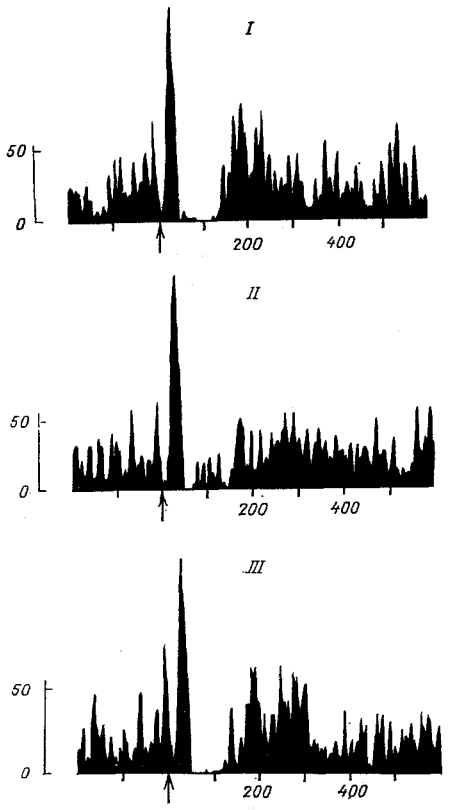


Fig. 2

Fig. 1. Effect of diazepam on unit responses to sciatic nerve stimulation in rat sensomotor cortex: I) control; II) 40 min after intravenous injection of diazepam (0.75 mg/kg); III) 90 min after injection of drug. Time of stimulation indicated by arrow. Abscissa, time, in msec; ordinate, frequency, spikes/sec.

Fig. 2. Effect of thiosemicarbazide on evoked unit activity of the rat cortex: I) control; II) 80 min, III) 180 min after intravenous injection of TSC-HCl (12 mg/kg). Legend as in Fig. 1.

During subsequent analysis the duration of the inhibitory pause and the degree of its filling, i.e., the number of spikes in it, were determined. For this purpose, the mean frequency of spontaneous activity was calculated in the prestimulus part of the histogram; the region of the histogram in which the spike frequency was below average was taken as the inhibitory pause. The number of spikes in the pause was then counted and the significance of its changes under the influence of the drugs was assessed by the nonparametric criterion of signs [1].

## EXPERIMENTAL RESULTS AND DISCUSSION

The response of neurons to sciatic nerve stimulation had several phases [11, 14, 16]: a phase of transient increase in the discharge frequency 10-15 msec after stimulation, a phase of inhibition of unit activity lasting 100-250 msec (inhibitory pause), and the phase of a late increase in discharge frequency (postinhibitory rebound), after which the original discharge frequency was restored (Fig. 1, I). Starting with doses of 0.25-0.5 mg/kg, diazepam potentiated the inhibitory component of the unit response, lengthened the inhibitory pause, and reduced the degree of its filling (Fig. 1, II, $P = 0.01$). The action of the drug was accompanied by a decrease in the postinhibitory rebound and, in some experiments, by a decrease in the spontaneous firing rate. These effects occurred after 2-5 min and reached their maximum 20-40 min after injection of the drug. Recovery of the original pattern took place 90-120 min after injection of the drug. Depakine (150-250 mg/kg, intraperitoneally), which increases the GABA concentration in the brain, also increased the duration of the inhibitory pause and reduced the degree of its filling and the spontaneous firing rate of the cells, but unlike diazepam, it had no effect on postinhibitory rebound. The effect of the drug reached a maximum 30-40 min, and

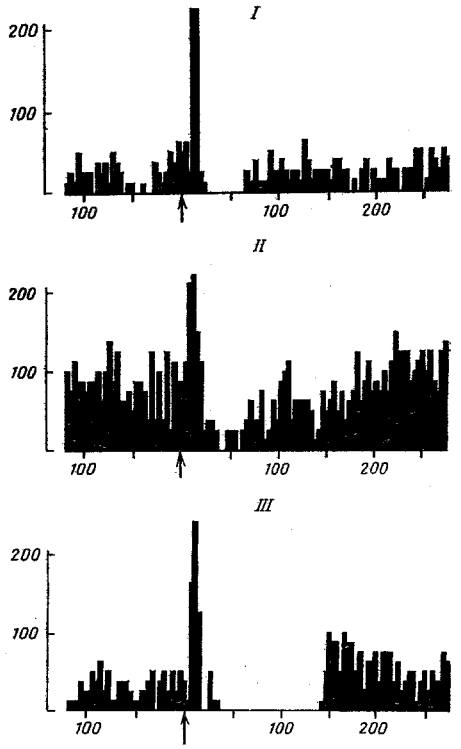


Fig. 3. Antagonism between bicuculline and diazepam in their action on cortical evoked unit activity: I) control; II) 10 min after intravenous injection of bicuculline (0.05 mg/kg); III) 10 min after intravenous injection of diazepam (0.5 mg/kg) and 20 min after injection of bicuculline. Legend as in Fig. 1.

recovery was observed 60-120 min after injection, in agreement with data in the literature for the dynamics of GABA accumulation under the influence of depakine [15]. Aminohydroxyacetic acid, another inhibitor of $\alpha$-ketoglutarate-GABA transaminase, has a similar action on evoked unit activity of the cat cortex [10].

TSC (7-12 mg/kg, intravenously), which lowers the GABA concentration in the brain, had the opposite action for it shortened the inhibitory pause and increased the degree of its filling (Fig. 2, $P = 0.01$). The action of TSC reached a maximum 80-100 min, and recovery was observed 150-180 min after injection of the drug, which corresponded to the dynamics of its behavioral effects [5]. Bicuculline, which blocks GABA-ergic receptors, had a similar effect on these parameters. It increased the spontaneous firing rate of the neurons, shortened the inhibitory pause, and increased the degree of its filling (Fig. 3, II; $P = 0.05$). The effect of the drug developed immediately after its injection and recovery took place 30 min after injection. It follows from these results that the effect of diazepam on the inhibitory pause was similar to the action of depakine and opposite to the effect of TSC and bicuculline. Furthermore, two-way antagonism was observed between diazepam and bicuculline in their effect on the inhibitory pause (Fig. 3, II, III).

The inhibitory pause of the neuronal response reflected IPSPs recorded intracellularly [13] and served as an indicator of the activity of the recurrent inhibition system [14]. Since the mediator of recurrent inhibition in the cortex is GABA [9], the results regarding the ability of diazepam and depakine to increase the duration and reduce the filling of the inhibitory pause and the opposite effect of the GABA-negative substances bicuculline and TSC on this parameter, and regarding the antagonism between diazepam and bicuculline in their action on the inhibitory pause suggests that diazepam, by potentiating the effects of endogenous GABA, also potentiates inhibitory influences exerted by inhibitory interneurons on cortical cells.

Since the changes observed in evoked activity were accompanied by changes in the spontaneous discharge frequency of the cells, it might be supposed that it is this frequency which determines the effect of the

drugs tested [3]. However, their effects occurred whether the spontaneous firing rate was raised, lowered, or unchanged. Finally, evidence has been obtained of the effect of the reticular formation and, in particular, the reticular nucleus of the tegmentum, on cortical inhibitory interneurons [4, 7]. It has, however, been shown that diazepam, even in very high doses (5-20 mg/kg), does not change the spontaneous unit activity of this nucleus [12] although, as was shown above, it inhibits cortical unit activity in doses as low as 0.25-0.5 mg/kg. These effects of diazepam on the cells of the cerebral cortex cannot thus be attributed to its effect on the reticular formation.

The results are further evidence of the cortical effect of diazepam and they confirm the earlier hypothesis [6, 17] that some of its effects are mediated by the GABA system.

## LITERATURE CITED


1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria to Assess Differences Between Two Groups of Observations in Medical and Biological Research [in Russian], Moscow (1969).
2. V. V. Zakusov and R. U. Ostrovskaya, Neirofiziologiya, 3, 582 (1971).
3. V. I. Maiorov, "Electrophysiological analysis of plastic properties of visual cortical neurons," Author's Abstract of Candidate's Dissertation, Moscow (1974).
4. S. P. Narikashvili, É. S. Moniava, and V. S. Arutyunov, Fiziol. Zh. SSSR, 52, 660 (1966).
5. R. U. Ostrovskaya and V. V. Parin, Byull. Éksp. Biol. Med., No. 5, 47 (1973).
6. R. U. Ostrovskaya, G. M. Molodavkin, R. P. Porfir'eva, et al., Byull. Éksp. Biol. Med., No. 3, 50 (1975).
7. A. Ya. Supin, Fiziol. Zh. SSSR, 54, 893 (1968).
8. H. T. Chang, J. Neurophysiol., 13, 235 (1950).
9. J. J. Dreifuss, J. S. Kelly, and K. Krnjevic, Exp. Brain Res., 9, 137 (1969).
10. Z. Gottesfeld, J. S. Kelly, and L. P. Renaud, Brain Res., 42, 319 (1972).
11. G. Y. Montplaisir and E. Sazie, Electroenceph. Clin. Neurophysiol., 35, 311 (1973).
12. M. E. Olds and J. Olds, Internat. J. Neuropharmacol., 8, 87 (1969).
13. F. Ribaupierre, M. H. Goldstein, and G. Yeni-Komshian, Brain Res., 48, 185 (1972).
14. C. Stefanis and H. Jasper, J. Neurophysiol., 27, 855 (1964).
15. S. Simler, L. Ciesielski, M. Maitre, et al., Biochem. Pharmacol., 22, 1701 (1973).
16. T. W. Stone, Naunyn-Schmiedeberg's Arch. Pharmacol., 278, 333 (1973).
17. V. V. Zakusov, R. U. Ostrovskaya, V. V. Markowitch, et al., Arch. Internat. Pharmacodyn. Ther., 21, 188 (1975).