INCREASED SENSITIVITY OF RABBIT SENSOMOTOR CORTICAL NEURONS TO GABA PRODUCED BY DIAZEPAM (MICROIONTOPHORETIC INVESTIGATION)

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Changes produced by diazepam in the effects of γ-aminobutyric acid (GABA), applied microiontophoretically to neurons of the rabbit sensomotor cortex, were investigated. Diazepam was shown to strengthen the inhibitory action of GABA on spontaneous unit activity and to prolong the effect of GABA on the duration of the inhibitory pause in unit responses to afferent stimulation and to direct cortical stimulation. Diazepam did not change unit responses to microiontophoretically applied glycine, glutamate, or acetylcholine. It is suggested that diazepam increases the sensitivity of the receptors of the postsynaptic membrane of neurons to GABA.

KEY WORDS: diazepam; γ-aminobutyric acid; glycine; glutamate; acetylcholine; microiontophoresis; sensomotor cortex.

The study of recovery cycles of interzonal responses of the cat motor cortex has shown that diazepam strengthens inhibition in the cortex [2, 12]. This phenomenon may lie at the basis of the anticonvulsant and tranquilizing action of the preparation. Muscle relaxation and ataxia produced by diazepam are evidently due to the strengthening of different types of inhibition in the spinal cord [5, 11], cerebellum [3], and striopallidary system [5].

In the modern view, presynaptic inhibition in the spinal cord and postsynaptic inhibition in supraspinal regions of the CNS are mediated by γ-aminobutyric acid (GABA) [7].

The object of this investigation was to study the effect of diazepam on inhibitory effects of GABA applied microiontophoretically directly to single neurons in the rabbit sensomotor cortex.

EXPERIMENTAL METHOD

Experiments were carried out on 15 adult rabbits (2.5-3 kg) under conditions of stereotaxic immobilization and relaxation (diplacin*, 5 mg/kg, intravenously) and artificial respiration. Action potentials of sensomotor cortical neurons were recorded extracellularly in the focus of maximal activity, located previously by single stimulation of the contralateral sciatic nerve (square pulses, 0.1 msec, 0.5-1.5 V). The cortical surface was stimulated directly through a bipolar nichrome electrode placed 2 mm caudally to the recording point (diameter of electrode 0.1 mm, interelectrode distance 0.2 mm; square pulses 0.1 msec, 5-20 V). The frequency of the stimulating pulses for afferent and cortical stimulation was 0.5 Hz.

Seven-barreled glass micropipets were used; the central barrel, filled with 3 M NaCl, served to record unit activity. The side barrels were filled with the following agents: GABA (1 M, pH 3.0), glycine (1 M, pH 3.5), acetylcholine–HCl (1 M, pH 4.0), and sodium L-glutamate (1 M, pH 7.0). Glutamate was removed from the micropipet by a current of negative polarity, all the other agents by a current of positive polarity. The

*1,3-di(β-platyneciniumethoxy)benzene hydrochloride.

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holding current was 16 nA. One of the side barrels was filled with 3 M NaCl and was used to apply a voltage of opposite sign to the cell membrane in order to prevent the polarization effect of the current removing the agent.

Spontaneous (60 sec) unit activity and activity evoked (60 sec) by stimulation of the sciatic nerve or cortical surface were recorded first. Changes in the two types of activity were then studied during microiontophoretic application of GABA or the other agents (duration of application 60 sec). Diazepam (Seduxen, Gedeon Richter) was then injected intravenously in a dose of 1 mg/kg over a period of 1 min. Spontaneous and evoked unit activities were then recorded again, as well as the changes in both types of activity under the influence of GABA. Action potentials of neurons and synchronized stimulation pulses were recorded on two tracks of magnetic tape. To analyze the data each action potential was transformed into a standard quantum of voltage; each subsequent quantum was added to its predecessor (code to analog conversion). Synchronous summation of 30 cuts of unit activity, 640 or 1280 msec long (Δt = 2.5 or 5 msec) were synchronously summed by the Neiron-I specialized computer (with the Biocode-I). As a result, the unit activity delivered to the KSP-4 potentiometer consisted of a continuous line, the slope of which relative to the time axis was directly proportional to the discharge frequency of the neuron.

EXPERIMENTAL RESULTS AND DISCUSSION

The typical effect of GABA on spontaneous sensomotor cortical unit activity is illustrated in Fig. 1A. The reduction in the gradient of the curve indicates an inhibitory action of GABA. Depression of spontaneous activity, calculated from the decrease in the ordinate of the last point of the curve, was 36% (spontaneous activity before GAPA application was taken as 100%). The strength of the applying current was chosen to be adequate for manifestation of the minimal inhibitory effect of GABA. After intravenous injection of diazepam (1 mg/kg) some decrease in the spontaneous discharge frequency was observed, but GABA applied to the cell by a current of the previous strength caused a stronger inhibitory action (82%) than before administration of diazepam (Fig. 1C).

The unit response to electrical stimulation of the sciatic nerve (Fig. 1B) consisted of an increase in the discharge frequency of the cell, against the background of which a short (30 msec) inhibitory pause could be seen (the portion of the curve parallel to the abscissa). The duration of the inhibitory pause was increased a little by GABA application (to 50 msec).

After administration of diazepam the response to afferent stimulation was reduced and the duration of the inhibitory pause increased. Application of GABA against the background of diazepam led to a marked additional increase in the duration of the inhibitory pause (up to 150 msec), to the removal of all neuronal discharges from it, and to depression of the phase of postinhibitory excitation (Fig. 1D).

The results of a similar investigation of the effect of GABA on inhibition of another neuron evoked by direct stimulation of the cortical surface are shown in Fig. 2. In response to cortical stimulation an inhibitory pause 50 msec in duration appeared in the unit activity (Fig. 2B). GABA, applied with a current of 10 nA, did not change the response of the neuron to cortical stimulation. After intravenous injection of diazepam the inhibitory pause was lengthened to 200 msec (Fig. 2D). Microiontophoretic injection of GABA 9 min after injection of Seduxen caused a further increase in the duration of the inhibitory pause to 740 msec. A marked increase in the duration of the inhibitory pause in response to GABA application still remained 41 min after the injection of diazepam, when its potentiating action on the inhibitory pause had ended (Fig. 2F). Just as in the experiments whose results are shown in Fig. 1, strengthening of the inhibitory effect of GABA on spontaneous unit activity was observed after injection of diazepam (Fig. 2A, C, E).

It is a noteworthy fact that in most experiments GABA had a long aftereffect after the injection of diazepam. This was expressed as a slow return (over several minutes) of the spontaneous and evoked unit activity to its original (before application) level after removal of the applying current (Fig. 2, C-F). Usually (without diazepam) unit activity returned to its initial level within a few seconds after discontinuing the current removing GABA.

These results are evidence of the synergic inhibition of spontaneous unit activity in the sensomotor cortex by diazepam and GABA. Diazepam also can potentiate the inhibitory action of GABA on spontaneous activity. On the other hand, diazepam increased the duration of the inhibitory pause in unit responses to afferent and direct cortical stimulation and also potentiated the prolonging action of GABA, applied microiontophoretically, on the duration of the inhibitory pause. The inhibitory pause arises as a result of active postsynaptic inhibition [8]. This inhibition has been shown to be mediated by GABA [4, 9, 10]. Diazepam thus evidently facilitates the physiological action of endogenous GABA secreted in the cortical inhibitory synapses.

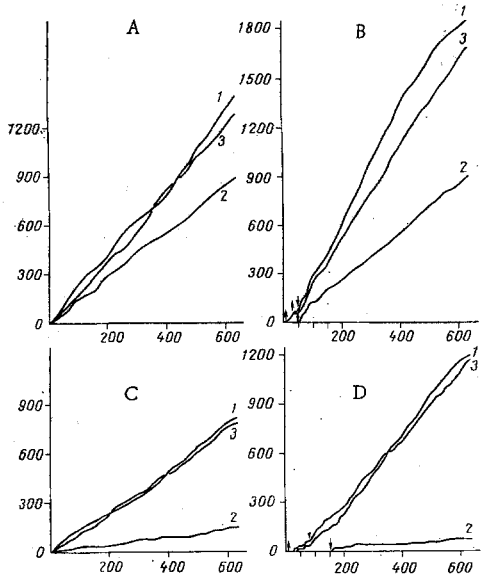

Fig. 1

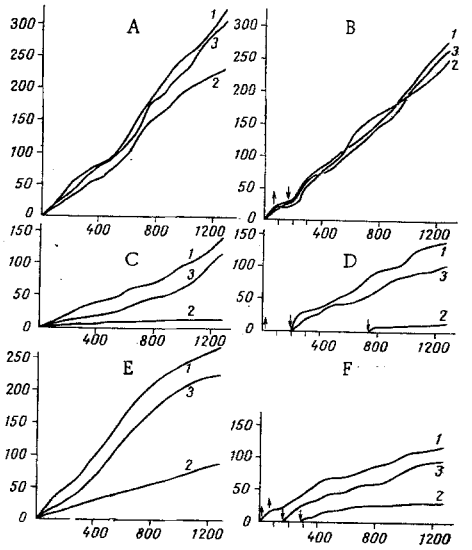

Fig. 2

Fig. 1. Effect of microiontophoretic application of GABA on spontaneous activity of a sensomotor cortical neuron and on activity evoked by afferent stimulation, before and after administration of diazepam: A) effect of GABA (25 nA) on spontaneous unit activity before diazepam; C) the same, 27 min after intravenous injection of diazepam (1 mg/kg). B) Effect of GABA (25 nA) on unit activity evoked by sciatic nerve stimulation (0.5 V) before diazepam; D) the same, 28 min after diazepam. Ordinate, number of action potentials of neuron; abscissa, time (in msec). Synchronous summation for 30 applications. 1) Unit activity before, 2) during, and 3) after GABA application. Arrow pointing upward marks beginning of inhibitory pause, arrow downward marks end of inhibitory pause. Remainder of explanation in text.

Fig. 2. Effect of microiontophoretic application of GABA on spontaneous unit activity and activity evoked by electrical stimulation of cortical surface before and after diazepam. A) Effect of GABA (10 nA) on spontaneous unit activity before injection of diazepam; C) the same 8 min after intravenous injection of diazepam (1 mg/kg); E) the same 41 min after diazepam. B) Effect of GABA on unit response evoked by electrical stimulation of cortical surface (5 V) before diazepam; D) the same 9 min after injection of diazepam; F) the same 42 min after diazepam. Remainder the same as in Fig. 1.

To explain the specificity of the intervention of diazepam in GABA-ergic inhibitory processes the sensitivity of cortical neurons to certain other mediators was investigated. Neither the inhibitory action of glycine nor the excitatory action of glutamate and acetylcholine on spontaneous cortical unit activity was found to be significantly changed after the injection of diazepam. These findings conflict with the connection between the pharmacological effects of diazepam and potentiation of the inhibitory action of glycine postulated by Young et al. [13]. They suggest that the inhibitory effect of diazepam on cortical unit activity is not due to blocking of the excitatory effect of acetylcholine and glutamate. Diazepam itself is evidently a hyperpolarizing agent capable of substantially modifying the ionic permeability of the neuron membrane.

Inhibition of spontaneous activity may thus be connected with the potentiating effect of diazepam on background inhibitory GABA-ergic influences which, in conjunction with background excitatory influences, determine the level of spontaneous unit activity at each concrete moment of time.

The prolonged aftereffect of GABA during the action of diazepam mentioned above is evidence that diazepam slows the removal of GABA from the sites of its action. However, diazepam has been shown to have no marked effect on the reassimilation of GABA by synaptosomes [1, 6]. The other mechanism of GABA inactivation, namely enzymatic transamination by α-ketoglutarate-GABA-transaminase, is blocked only by very large doses of diazepam, unsuitable for use both in the present experiments and in clinical practice [2].

The most logical suggestion from the writers' point of view is that diazepam sensitizes cortical neurons to naturally secreted GABA by increasing the sensitivity of the GABA receptors of the postsynaptic membrane and, possibly, by its effect on the molecular mechanisms determining the duration of interaction between GABA and receptor.

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ROLE OF SURFACE PHENOMENA IN THE MECHANISM OF ACTION OF ANTIARRHYTHMIC DRUGS

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Surface activity of antiarrhythmic drugs and their effect on lipid-containing interphases were studied. Compound No. 7351 (diethylaminopropyl ester of diphenylisopropylacetic acid), Fubromegan, methyldiazine, propranolol, quinidine, novocainamide, xylocaine, and trimecaine were shown to be surface active. The curves of surface activity and, in particular, of interphase activity and those of antiarrhythmic action follow parallel courses. The most active antiarrhythmic compound (No. 7351) increased the electrical conductivity of a lecithin bilayer membrane much more strongly than novocainamide.

KEY WORDS: physicochemical properties; surface activity; artificial bilayer lipid membrane; antiarrhythmic drugs.

The physicochemical and colloid-chemical properties of neurotropic drugs (local anesthetics, narcotic analgesics, etc.) are known to play an important role in the mechanism of their action [2-9]. At the same time it has been suggested that interaction with the membrane is of great importance in the mechanism of action of antiarrhythmic drugs [1, 14, 15].

It was accordingly decided to study the role of surface phenomena in the action of antiarrhythmic drugs. The connection between the pharmacological action of antiarrhythmic drugs and their surface activity and their effect on lipid-containing interphases (a lecithin bilayer) was investigated.

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