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NATURE OF POTENTIATION OF GABA EFFECTS BY BENZODIAZEPINE TRANQUILIZERS

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The most important pharmacological effects of benzodiazepine tranquilizers are due to their influence on GABA-ergic synapses of the brain [6, 14, 15]. Diazepam has been shown to potentiate presynaptic inhibition of primary afferents in the spinal cord [7] and in the cuneate nucleus of 12 cats, whereas the depolarizing [4, 10] and hyperpolarizing [10, 14] effects due to the direct action of GABA are potentiated by benzodiazepine tranquilizers.

A model according to which benzodiazepines increase the affinity of receptors for GABA allosterically [9], probably by inhibition of a protein modulator which inhibits binding of GABA with receptors [8] or by displacing it from the receptor complex and facilitating coupling of GABA receptors with chloride ionophores [2], is regarded as the molecular mechanism lying at the basis of the potentiating influence of benzodiazepine on the effects of GABA. However, these views are contradicted by data showing that benzodiazepines have no effect on the cooperativeness of interaction between GABA and the GABA receptors of nerve cell membranes in tissue culture [5].

The results described in this paper are evidence that benzodiazepines can directly influence the function of chloride ionophores in nerve cell membranes.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated perfused spinal cord of rats aged 9-15 days. Details of the method were described previously [1].

The characteristic action of chlordiazepoxide (10^{-5}-10^{-14} M) and its effect (exposure 15 min) on primary afferent depolarization or hyperpolarization of motoneurons into GABA (10^{-5}-10^{-3} M) were investigated. Synaptic transmission in the spinal cord was blocked by perfusion with a solution deficient (0.2 mM) in Ca^{++} ions and containing an excess (10 mM) of Mg^{++} ions. The effect of chlordiazepoxide on the evoked monosynaptic ventral root potential and the dorsal root potential evoked by stimulation of the dorsal root of the neighboring segment

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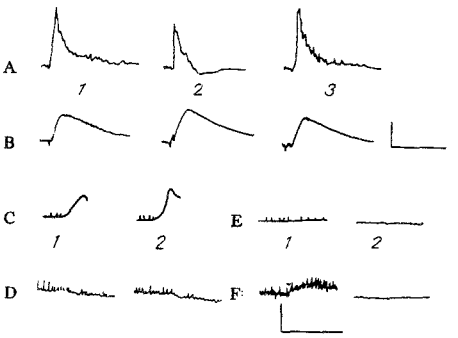

Fig. 1. Effect of chlordiazepoxide on evoked potentials of ventral and dorsal roots of isolated rat spinal cord. Potentials in ventral root L3 (A) and dorsal root L4 (B), evoked by electrical stimulation of dorsal root L3 before (1) and after (2) perfusion of spinal cord for 15 min with solution containing chlordiazepoxide (10^{-4} M), and also 15 min after rinsing (3). Electrotonic potentials arising in dorsal (C) and ventral (D) roots during perfusion of spinal cord with GABA (10^{-4} M) solution before (1) and after (2) preliminary treatment with chlordiazepoxide (10^{-4} M). Change in polarization of dorsal (E) and ventral (F) roots under the influence of chlordiazepoxide during perfusion of spinal cord with ordinary salt solution (1) and with solution containing excess of Mg^{++} ions and deficiency of Ca^{++} ions (2). Calibration: vertical, 1 mV; horizontal 100 msec (A, B) and 1 min (C-F).

(DR-DRP) also was investigated. All the evoked potentials were excited by stimulation of dorsal root L3 by square pulses of current (0.3 msec, 0.1 Hz, 2-8 thresholds) and recorded in ventral root L3 (monosynaptically) or in dorsal root L4 (DR-DRP).

In the next series of experiments dependence of the GABA-potentiating action of chlordiazepoxide (10^{-4} M) on the Cl-ion concentration in the solution with which the spinal cord was perfused was studied. The initial chloride concentration (124 mM) was increased to 165 mM by addition of 41 mM choline chloride to the solution or reduced to 83 and 42 mM by replacing the sodium chloride by an equivalent amount of sodium sulfate. The pH of the solution was kept at 7.4-7.6. The effect of chlordiazepoxide (10^{-4} M) on GABA-induced primary afferent depolarization also was studied in different concentrations of K^+ ions (0.2, 2, and 10 mM) in order to judge the relationship between the potentiating effect of chlordiazepoxide and the membrane potential.

Each version of the experiments was carried out on 4-6 spinal cord preparations. The results were subjected to statistical analysis in the usual way.

EXPERIMENTAL RESULTS

As a result of perfusion of the isolated spinal cord with solution containing chlordiazepoxide (10^{-4} M) the amplitude and duration of the monosynaptic ventral root potential was significantly reduced, but the amplitude and duration of the DR-DRP, on the contrary, were increased (Fig. 1). According to data in the literature [7, 10) these effects are the result of potentiation of GABA-ergic postsynaptic inhibition of the motoneurons and presynaptic inhibition of primary afferents, under the influence of chlordiazepoxide, respectively. Preliminary perfusion of the spinal cord for 15 min with a solution containing 10^{-4} M chlordiazepoxide in fact potentiated the depolarizing effect of GABA (10^{-4} M) on primary afferents and intensified its hyperpolarizing effect on motoneurons (Fig. 1). Chlordiazepoxide itself (10^{-4} M) did not change the level of primary afferent polarization but induced weak depolarization and intensified spontaneous activity of motoneurons. The effect of chlordiazepoxide on motoneurons was not direct, for it was not exhibited when synaptic transmission in the spinal cord was blocked (Fig. 1).

The GABA-potentiating effect of chlordiazepoxide depends on its concentration (Fig. 2). Since Hill's index was 1, interaction of chlordiazepoxide with the benzodiazepine receptors in membranes of the primary afferents and motoneurons followed the course of a monomolecular reaction, in agreement with data obtained by

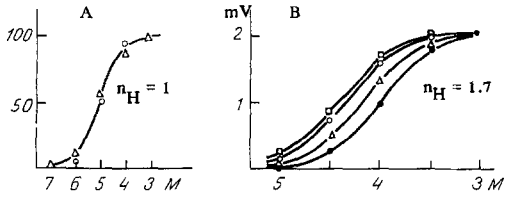

Fig. 2. Dependence of GABA-potentiating effect of chlordiazepoxide on its concentration. A) Dependence of electrotonic potentials of ventral (circles) and dorsal (triangles) roots, evoked by action of a constant concentration of GABA (10^{-4} M), on concentration of chlordiazepoxide. Abscissa, concentration of chlordiazepoxide (in M); ordinate, effect (in percent of maximal). B) Logarithm of concentration versus effect of GABA curves plotted from values of depolarization of dorsal roots induced by GABA in the absence (1) and presence of chlordiazepoxide in concentrations of 10^{-5} M (2), 10^{-4} M (3), and 10^{-3} M (4). Abscissa, GABA concentration (in M); ordinate, effect (in mV).

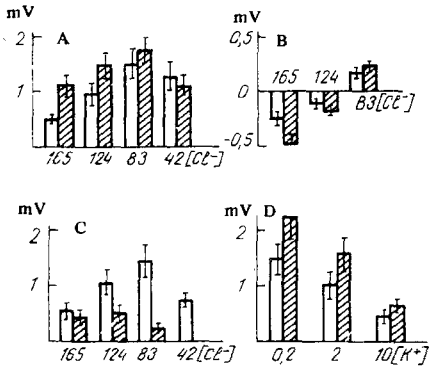

Fig. 3. Effect of changes in ionic medium on GABA-potentiating effects of chlordiazepoxide. Responses of primary afferents (A) and motoneurons (B) due to action of GABA (10^{-4}) in absence (unshaded columns) and in presence (shaded columns) of chlordiazepoxide (10^{-4} M) with different Ca^{++} ion concentrations. Primary afferent depolarization due to action of GABA (10^{-4} M) in absence and in presence of picrotoxin in concentration of 10^{-6} M (C) and with different Cl^- ion concentrations; in absence and in presence of chlordiazepoxide (D) with different K^+ ion concentrations in medium. Abscissa, concentration of ions in medium (in mM); ordinate, A, C, D, B – magnitude of depolarization (in mV).

the radioligand method [3]. Conversely, interaction of GABA with GABA receptors in primary afferent membranes took place cooperatively (Hill's index n_H = 1.7). The logarithm of concentration versus effect of GABA curves were shifted to the left parallel to the original curve in the presence of chlordiazepoxide (Fig. 2B) and, consequently, cooperativeness of interaction between GABA and GABA receptors was not changed by chlordiazepoxide. This rules out the possibility of interaction between chlordiazepoxide and the allosteric region of the GABA receptors. The fact that the maximum of the GABA effect remains constant in the presence of different concentrations of chlordiazepoxide (Fig. 2B) contradicts the view [2, 8] that benzodiazepines have an inhibitory effect on GABA-modulin function.

Meanwhile the GABA-potentiating effect of chlordiazepoxide depended essentially on the Cl^- ion concen-

tration in the solution surrounding the spinal cord (Fig. 3). The GABA-potentiating effect of chlordiazepoxide, detectable in the presence of a normal concentration of Cl^- ions (124 mM), increased with a rise (165 mM) and decreased with a fall (83-42 mM) in the concentration of these ions (Fig. 3A, B). Picrotoxin, antagonism of which with GABA is due to blockade of chloride ionophores coupled with GABA receptors [13], also depends on the chloride concentration in the medium, but its blocking effect is intensified with a fall in the Cl^- ion concentration (Fig. 3C). This suggests that the GABA-potentiating effect of chlordiazepoxide is due to its direct influence on chloride ionophores coupled with GABA receptors.

A decrease in the K^+ ion concentration to 0.2 mM or an increase in their concentration to 10 mM increased or decreased (by 50%) respectively the depolarizing effect of GABA on primary afferents (Fig. 3D), but did not change the GABA-potentiating effect of chlordiazepoxide, which at all K^+ concentrations amounted to 45-50% of the value of depolarization. Considering that the GABA-potentiating effect of chlordiazepoxide, by contrast with the effects of GABA, is independent of the initial membrane potential level (afferents), it can be tentatively suggested that chlordiazepoxide, acting on chloride ionophores, changes not so much the conductance of the single chloride channel as the duration of its open state, for the latter depends to a far lesser degree than conductance of the channel on the cell membrane potential level [11].

Chloride ionophores, coupled in the nerve cell membrane with GABA receptors, are thus the targets for the primary action of benzodiazepine tranquilizers. The latter evidently increased the duration of the open state of chloride channels which depends on the action of GABA on GABA receptors.

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