EFFECT OF FENIBUT* ON $GABA_B$-RECEPTORS OF SPINAL MOTONEURONS

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UDC 612.83.014.467:615.31:547.466.3].014.46:615.31:547.891.2

KEY WORDS: fenibut; $GABA_B$-receptors; diazepam.

The effects of fenibut are linked with its predominant action on bicuculline-insensitive GABA-ergic receptors [2]. These have been found in sympathetic nerve endings and called $GABA_B$-receptors, to distinguish them from the bicuculline-sensitive $GABA_A$-receptors [6]. $GABA_B$ receptors are located not only in the autonomic nervous system, but also in the CNS, where they modulate mediator release by axon terminals of monoaminergic neurons and also participate in the realization of spinal presynaptic inhibition [5, 8]. The idea has formed that the effects of GABA and its mimetics, mediated through activation of $GABA_B$-receptors, are unchanged by benzodiazepines [5]. However, this view is not shared by all investigators [3].

The aim of this investigation was to study interaction of fenibut with postsynaptic $GABA_B$-receptors of spinal motoneurons and the possible influence of benzodiazepines on effects mediated by $GABA_B$ receptors.

EXPERIMENTAL METHOD

Experiments were carried out on parasagittal sections of the isolated spinal cord of rats aged 7-14 days. Details of the method were described previously [1]. Electrotonic potentials (ETP) of ventral roots of segment L_4 and polysynaptic reflex discharges (PRD) of motoneurons in the same root during electrical stimulation of the dorsal root of L_3 by single square pulses of current with a duration 0.3 msec, frequency 0.1 Hz, and intensity 6-8 thresholds, were recorded by a sucrose gap technique.

In the experiments of series I the effect of different concentrations of fenibut on the level of ventral root polarization and amplitude of PRD of the motoneurons was investigated. Similar experiments were performed with GABA.

In the experiments of series II the effect of different concentrations of diazepam on ETP of the ventral roots of L_4, induced by fenibut ($2 \cdot 10^{-5}$ M), and inhibition of PRD of motoneurons recorded in this same ventral root by this GABA-mimetic, was studied.

The action of each concentration of each test substance was tested on 4 to 6 spinal cord preparations.

EXPERIMENTAL RESULTS

Unlike GABA which, in low concentrations ($2 \cdot 10^{-5}$ M), causes depolarization ETP of ventral roots, but in high concentrations ($5 \cdot 10^{-5}$-$1 \cdot 10^{-4}$ M) it has a biphasic action, causing hyper- and depolarization ETP of the ventral roots (Fig. 1), fenibut induced only slowly developing depolarization ETP of the ventral roots over the whole range of concentrations. The action of fenibut on the spinal cord was accompanied by inhibition of PRD and of spontaneous activity, recorded in the ventral roots, and was reversible in character: 3-5 min after the beginning of rinsing of the spinal cord to remove fenibut with the original salt solution, spontaneous and evoked activity in the ventral roots was completely restored.

*β-Phenyl-γ-aminobutyric acid.

Department of Pharmacology, M. Gor'kii Donetsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 698-700, June, 1985. Original article submitted October 4, 1984.

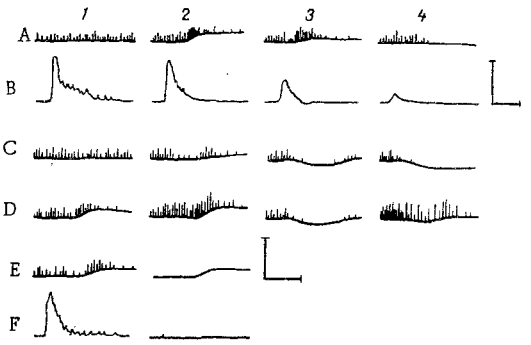

Fig. 1. ETP of ventral root L_4 and inhibition of PRD of motoneurons evoked by action of fenibut and GABA on spinal cord. A, C) Spontaneous activity in ventral root L_4 (1) and ETP in same root during action of fenibut (A), in concentrations of 10^{-5} M (2), $3 \cdot 10^{-5}$ M (3), and 10^{-4} M (4), or of GABA (C), in concentrations of $2 \cdot 10^{-5}$ M (2), $5 \cdot 10^{-5}$ M (3), and 10^{-4} M (4), on the spinal cord; B) PRD of motoneurons in absence (1) and presence of fenibut in concentrations of 10^{-5} M (2), $3 \cdot 10^{-5}$ M (3), and 10^{-4} M (4); D) ETP of ventral roots during action of $2 \cdot 10^{-5}$ M fenibut (1) and $5 \cdot 10^{-5}$ M GABA (3) respectively on spinal cord, 2, 4) potentials at 10th minute of action of picrotoxin (10^{-5} M) on spinal cord; E, F) initial ETP during action of fenibut ($2 \cdot 10^{-5}$ M) on spinal cord and DRP of motoneurons before (1) and after 45 min of superfusion of spinal cord with solution containing 14 mM Na^+ ions (2). Calibration: 1 mV, 30 sec (A, C, D, E) and 0.5 mV, 100 msec (B, F).

Preliminary (for 20 min) superfusion of the spinal cord with solution containing picrotoxin (10^{-5} M) did not change the effect of fenibut but inhibited the hyperpolarization and unmasked the depolarization ETP of the ventral roots caused by the action of GABA (Fig. 1). Preservation of the depolarization responses of the motoneurons to fenibut and GABA in the presence of picrotoxin indicates that they are effected through $GABA_B$-receptors.

Superfusion of the spinal cord with solution in which 90% of the Na^+ ions were replaced by choline, completely inhibited synaptic transmission in the spinal cord, as shown by disappearance of spontaneous activity and PRD recorded in the ventral roots, but did not change the depolarizing effect of fenibut on motoneurons (Fig. 2). This fact reflects the possibility of a direct influence of fenibut on motoneurons and is evidence of the postsynaptic localization of fenibut-activated $GABA_B$-receptors in neuron membranes. In the original investigations the localization of $GABA_B$-receptors was stated to be exclusively on presynaptic axon terminals [6], and only quite recently has it been shown that intravenous injection of the chlorinated analog of fenibut (baclofen) in a dose of 0.5-1.5 mg/kg into cats causes direct depolarization of motoneuron membranes of the phrenic nerve [10].

Motoneuron membrane depolarization induced by fenibut ought to increase excitability of the motoneurons and intensify their PRD and also spontaneous activity in the ventral roots. However, these types of activity are inhibited in the presence of fenibut (Fig. 1). Depression of PRD and of spontaneous motoneuronal activity is evidently the result of activation by fenibut of $GABA_B$-receptors located in the terminals of axons which form synaptic contacts on the dendrites and (or) soma of motoneurons. It was shown previously that baclofen disturbs mediator release from primary afferent terminals in the isolated rat spinal cord [4].

Superfusion of the isolated brain with solution containing diazepam in concentrations of 10^{-9}-10^{-6} M for 10 min was accompanied by an increase in depolarization ETP of the ventral roots, caused by fenibut ($2 \cdot 10^{-5}$M) and by more intensive inhibition of PRD of the motoneurons (Fig. 2). Judging by the character of the curves describing dependence of the potentiating action of diazepam on the effects of fenibut, interaction of diazepam with benzodiazepine receptors of spinal neurons follows a course typical of a monomolecular reaction (Fig. 2),

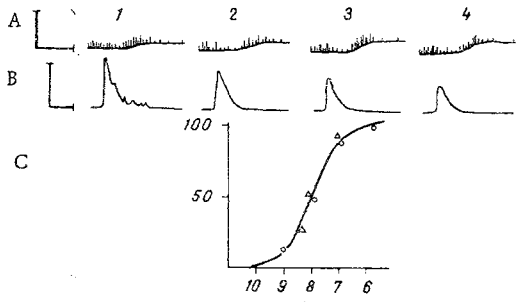

Fig. 2. Influence of diazepam on effects of fenibut. Abscissa, negative logarithms of diazepam concentration; ordinate, effect (in percent). ETP of ventral roots (A) and PRD of motoneurons (B) during action of fenibut ($2 \cdot 10^{-5}$ M) on spinal cord in absence of diazepam (I) and after 10 min of action of diazepam in concentrations of 10^{-8} M (2), 10^{-7} M (3), and 10^{-6} M (4) on spinal cord. Calibration: 1 mV, 30 sec (A) and 0.5 mV, 100 msec (B); C) effect of diazepam on ETP of ventral roots (circles) and on depression of PRD of motoneurons (triangles), induced by fenibut.

which is characteristic of benzodiazepines [7]. Values of EC_{50} for potentiation of the effects of fenibut (8-12 nM) by diazepam were very close to those (3.6-10 nM) characterizing affinity of diazepam for benzodiazepine receptors of rat brain membranes [7, 11].

These facts are evidence that $GABA_B$-receptors may be located postsynaptically and may be linked with benzodiazepine receptors. On the basis of radioligand studies, in which interrelated changes in the density of $GABA_B$- and benzodiazepine receptors were found in the mouse brain during chronic administration of fenibut and diazepam, it also was concluded that $GABA_B$- and benzodiazepine receptors are interconnected [3]. However, this connection is evidently not found with presynaptic $GABA_B$-receptors [5].

There are two possible alternative versions of interaction between $GABA_B$- and benzodiazepine receptors: either these receptors form a macromolecular complex in which the $GABA_B$-mimetic and benzodiazepine interact allosterically or activation of diazepine receptors linked with $GABA_A$-receptors potentiates effects mediated by $GABA_B$-receptors (independent interaction). The first alternative is contradicted by the absence of effect of the $GABA_B$-mimetic baclofen on binding of ^{3}H-flunitrazepam with rat brain membranes [9].

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