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CHANGES IN POSTSYNAPTIC EXCITATION PROCESSES IN THE PRESENCE OF THE SOVIET BENZODIAZEPINE DERIVATIVE PHENAZEPAM

A. V. Bogatskii,* V. V. Serdyuk,
E. I. Nazarov, and N. Ya. Golovenko

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The new psychotropic drug phenazepam inhibits spike discharges of neurons composing the visceral ganglion of the garden snail *Helix aspersa* and depresses excitatory postsynaptic potentials arising in response to application of acetylcholine to the membrane of the isolated neuron. The parameters of the electrically excitable membrane remain basically unchanged. It is suggested that one possible mechanism of the manifestation of the pharmacological action of the drug may be depression of postsynaptic excitation of the cholinergic receptor membrane.

KEY WORDS: phenazepam; excitatory postsynaptic potential; postsynaptic inhibition of excitation; acetylcholine.

Investigations of the biological activity of 1,4-benzodiazepine-2-bases (BD) have yielded an extensive literature on the subject [9]. Changes in the level and metabolism of catecholamines and acetylcholine (ACh) have been demonstrated in different parts of the brain [5, 6, 8]. Correlation has also been observed between the pharmacological activity of BD and their ability to bind with the glycine receptor [10].

On the basis of these results suggestions were put forward regarding the possible mechanism of action of BD [5, 8, 10]. The arguments supporting these hypotheses were later subjected to critical analysis, and new explanations of the various manifestations of the biological activity of compounds of this series were proposed [2, 3, 7]. In particular, it is suggested [3] that certain effects of diazepam are mediated through the γ-aminobutyric acid (GABA) system. On the basis of these data and also of the structural similarity between ACh and GABA and the possibility of competitive interaction between them for the muscarinic cholinergic receptor [4], it is natural to suggest that BD may influence excitation processes induced by ACh.

The object of this investigation was to test this hypothesis.

*Academy of Sciences of the Ukrainian SSR.

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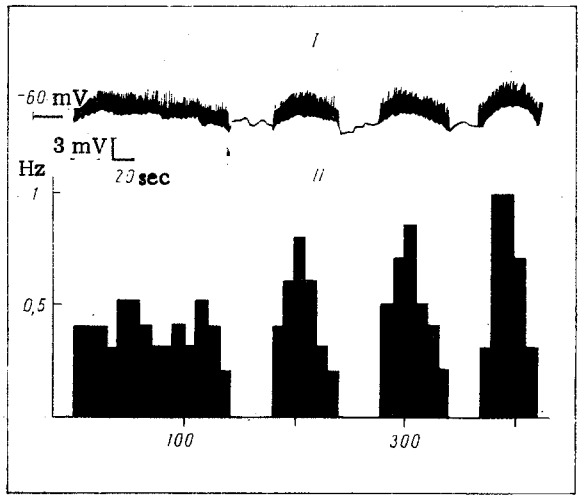

Fig. 1

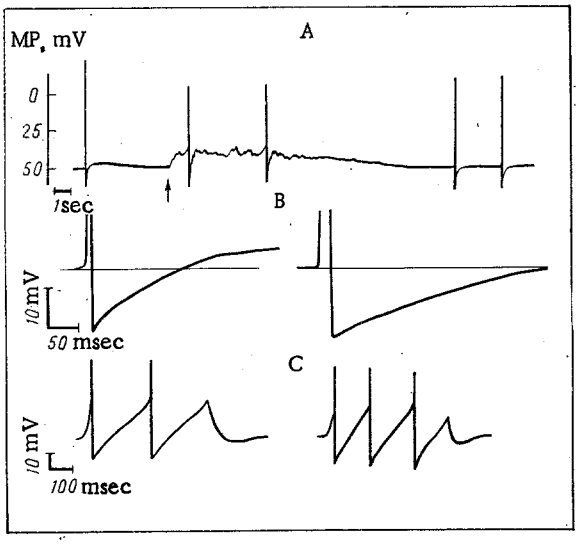

Fig. 2

Fig. 1. Effect of phenazepam on discharges of neurons of F group of visceral ganglion of *H. aspersa*. I) Changes in membrane potential under the influence of phenazepam (time between perfusion with phenazepam solution and point indicated by arrow 300 sec); II) changes in firing rate of neurons under influence of phenazepam.

Fig. 2. Effect of phenazepam on characteristics of chemically excitable soma membrane of isolated neuron of *H. aspersa*. A) Changes in membrane potential of soma of isolated neuron and inhibition of EPSP on perfusion with phenazepam solution; arrow indicates beginning of perfusion with phenazepam; B) inhibition of IPSP by phenazepam (left — control, right — response of neuron to ACh application in presence of phenazepam); C) changes in excitability of isolated neuron under influence of phenazepam (left — control cell response to intracellular electrical stimulation, right — after action of phenazepam).

EXPERIMENTAL METHOD

Neurons of the visceral ganglion of the subesophageal ring of the garden snail *Helix aspersa* were used as the test object. The isolated ring was placed in running physiological saline of the following composition (in mM): NaCl 80, KCl 4, $CaCl_2$ 7, $MgCl_2 \cdot 6H_2O$ 5; pH 7.4. The neurons of the ganglion were identified visually on the basis of whether their spontaneous spike activity was tonic in type or not, and also of the characteristics of the developing action potentials (AP).

The properties of a new member of the BD series — phenazepam, synthesized in the writers' laboratory [1] — were investigated. Because of the low solubility of phenazepam it was used as a saturated solution in 0.14 M NaCl. The concentration of phenazepam was determined polarographically at $E_{1/2} = 0.50$ mV. In the next series of experiments a model of synaptic transmission was used: The model represented the chemosensitive soma membrane of an isolated neuron taken from the upper right square of the dorsal surface of the left parietal ganglion of *H. aspersa* with a micropipet, filled with ACh solution, applied to it. The mediator was applied iontophoretically by pulses of current ($2 \cdot 10^{-8}$ A, 10^{-2} sec, resistance of micropipet 20 MΩ). Electrical stimulation was carried out through the measuring electrode by means of a bridge circuit. In all cases the membrane potential was recorded intracellularly by means of a glass microelectrode filled with 2.5 M KCl. Electrodes with a tip 0.1-0.5 μ in diameter and with a resistance of 15-40 MΩ were usually used. A UPT-2 amplifier was used and activity was recorded by the FOR-2 camera from the screen of an S-1-19B oscilloscope.

EXPERIMENTAL RESULTS

Phenazepam in a concentration of 10^{-6} M altered the discharge pattern of a group of neurons located in the middle part of the dorsal surface of the visceral ganglion of *H. aspersa*. The most typical result of the action of phenazepam is illustated in Fig. 1. The

membrane potential level showed no appreciable change as the result of extracellular perfusion with phenazepam (Fig. 1, I). Initial hyperpolarization of 3 mV was evidently not related to the cessation of the spike discharge, for later a similar picture was observed when the membrane potential was normal. The discharge of neurons of this group is known to be determined by constant postsynaptic bombardment, which gives rise to regular evoked EPSPs of above-threshold level, and this accounts for their normal tonic activity. Consequently, changes in spike activity evoked by phenazepam (Fig. 1, II) were somehow connected with a disturbance of postsynaptic excitation processes. To verify this hypothesis and fill in its details, investigations were carried out on a model of synaptic transmission. The soma of a neuron isolated from this region, which was used as the model, is extremely convenient (it has the right characteristics) for investigation of interaction between mediators and receptors of the postsynaptic membrane. For instance, iontophoretic application of the typical inhibitory mediator GABA to the correctly selected locus of the membrane usually evoked an IPSP with an amplitude of 3-5 mV, whereas ACh evoked both IPSP and EPSP for different individual isolated neurons. In this investigation only those neurons which responded to ACh application with an EPSP were chosen. Although the method of iontophoretic microapplication does not permit the quantity of mediator leaving the micropipet to be determined accurately, nevertheless the presence of a specific cell response (generation of an EPSP or IPSP) and its constancy in time do enable the relative effectiveness of preparations dissolved in the physiological saline surrounding the cell to be assessed. The adequacy of the data relating to the action of a series of cholinolytics obtained by this method and by methods of medical pharmacology [11] confirms that the experimental models described above are suitable for investigation of the mechanism of action of psychotropic drugs. As Fig. 2 shows, ACh application led to considerable and reversible depolarization of the soma membrane of the isolated neuron. Addition of phenazepam solution to the extracellular medium was accompanied by marked (of the order of 15 mV) depolarization and by the appearance of spontaneous APs. On average after 20 sec the membrane potential returned to its initial value and the membrane lost its ability to generate EPSPs in response to ACh application (Fig. 2A), and this property was not restored even after rinsing for 3 h.

The result could indicate that phenazepam is a competitive inhibitor of ACh. This explains the initial depolarization and subsequent disappearance of the EPSPs in response to ACh. The apparent increase in the sensitivity of the neuron membranes to application of the mediator after perfusion with phenazepam solution, expressed as the appearance of an AP (Fig. 2B), is due to the fact that blocking acetylcholine receptors may lead to inactivation of the conduction channels connected with them, and this increases the ohmic resistance of the membrane as a whole and so makes it more sensitive to the pulse of current which inevitably accompanies iontophoretic application. It is interesting to note that the parameters of the AP arising in response to intracellular stimulation remained basically unchanged (Fig. 2C), evidence that the normal properties of the electrically excitable membranes were preserved. It was thus shown, admittedly only for one mediator, that postsynaptic excitation can be inhibited by 1,4-benzodiazepine derivatives.

When putting forward this working hypothesis, it was assumed that there is steric conformity between ACh and phenazepam. However, the considerable differences in the chemical characteristics of these substances make this analogy highly conjectural. Only in the case of quaternization of the azomethine nitrogen atom could this analogy be made more correct from the chemical point of view. With this in mind, it is concluded that the suggested hypothesis has the right to exist but requires further verification.

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