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EFFECT OF POLYMETHYLENE- AND POLYHYDROXYETHYLENE-bis-(2-AMINO-1,3-DIAZEPINIUM) IODIDES ON CELL AND MODEL MEMBRANES

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KEY WORDS: diazepinium derivatives; end-plate potential; bimolecular lipid membranes; ionic channels.

The mono- and bisquaternary compounds widely used in clinical and laboratory practice have the property not only of interacting specifically with receptors of cholinergic synapses, but also of modifying the physicochemical characteristics of biomembranes and, in particular, their surface charge [1, 5]. It is also known that certain compounds of this class can enter the channels of chemically excitable membranes, blocking them partly or completely [6, 11]. Interaction with the recognition site of the receptor and its channel or modification of the surface charge near the channel leads to disturbance of the permeability of the activated postsynaptic membrane [6-8].

In the investigation described below, the role of the above-mentioned processes and the action of bisquaternary compounds on neuromuscular synapses were studied. Diazepinium derivatives were used as cationic heads. The choice of these heads was determined by the ability of certain diazepinium derivatives to block cationic channels and to modify the surface charge of biomembranes [10, 11].

EXPERIMENTAL METHOD

The effect of diazepinium derivatives on synaptic transmission was studied on the end-plate of the neuromuscular preparation of *Rana temporaria*. Resting potentials, action potentials, end-plate potentials (RP, AP, and EPP, respectively), and the membrane resistance of the muscle fibers were recorded by standard microelectrode techniques. Acetylcholine (ACh) was applied to the region of the end plate by a pulse of pressure from a microinjector. Kinetic parameters of interaction between the test substances and the acetylcholine receptor (AChR) were determined on the frog rectus abdominis muscle. The anticholinesterase activity of the substances was determined by measuring inhibition of acetylcholinesterase of human erythrocytes by an electrometric method [9]. Rat liver mitochondria were isolated by the method in [3]. Respiration of the mitochondria was determined polarographically. Bimolecular lipid membranes (BLM) were prepared from a solution of phosphatidylethanolamine (from Serva, West Germany) in decane (20 mg/ml), on holes in a Teflon jar. The surface potential of the BLM was measured potentiometrically [1].

The substances studied appear on the following page. All the compounds were synthesized in the Department of Chemistry of Macrocyclic Complexones, Physicochemical Institute, Academy of Sciences of the Ukrainian SSR.

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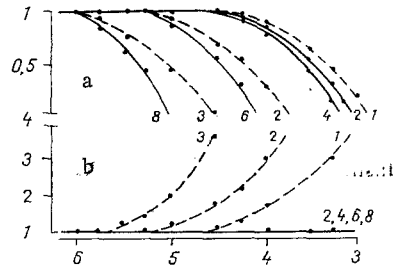


Fig. 1

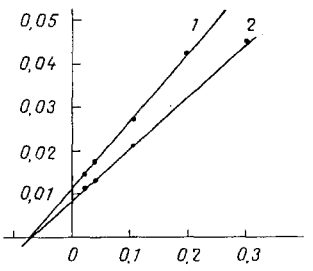


Fig. 2

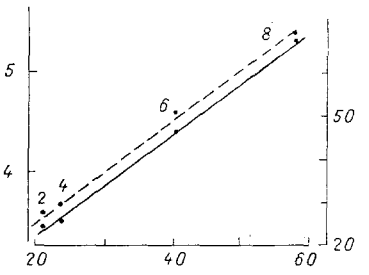
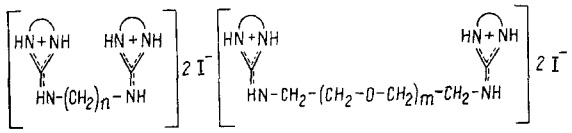


Fig. 3

Fig. 1. Dependence of change in amplitude (a) and decay time (b) of EPP on concentration. Continuous line — compound I, broken line — compound II. Numbers by curves indicate number of methylene or ester groups in chain. Abscissa, log of concentration (in M); ordinate: a) ratio between values of EPP in experiment and control, b) ratio of τ of EPP decay in experiments and control.

Fig. 2. Inhibition of contractions of frog rectus abdominis muscle by compound III (isometric conditions of measurement): 1) control, 2) in presence of 5×10^{-5} M substance III. Abscissa, reciprocal of ACh concentration (in μM^{-1}); ordinate, reciprocal of effect of ACh (in %).

Fig. 3. Correlation between action of substances I-IV on EPP (continuous line), respiration of mitochondria in state 3 (broken line), and surface potential of BLM. Numbers near curves indicate number of methylene groups in interquaternary chain. Abscissa, surface potential of BLM (substances added on one side of membrane); ordinate: on left) log of ED_{50} of inhibition of EPP, on right) **percent uncoupling** of respiration in state 3. Concentration of all substances in experiments to study effect of I-IV on surface potential of BLM and on mitochondrial respiration was 10^{-4} M.



n=2 (I); n=4 (II); m=1 (V); m=2 (VI); m=3 (VII)
n=6 (III); n=8 (IV);

EXPERIMENTAL RESULTS

All the test substances, in concentrations of 10^{-4}-5×10^{-6} M inhibited EPP evoked both by natural release of ACh during stimulation of the sciatic nerve and during iontophoretic application of the mediator to the region of the end plate. Values of RP, parameters of AP, and the membrane resistance of the muscle fibers during direct electrical stimulation remained unchanged. The character of the action of the compounds on EPP depended on the type of interquaternary chain. Inhibition of EPP by compounds I-IV was potentiated with an increase in concentration or lengthening of the interquaternary chain, and was not **accompanied by any change** in the time constant (τ) of decay. Compounds V-VII caused a sharp increase in τ of decay of the EPP, depending on the number of ethereal oxygen atoms in the chain. Simultaneously with changes in the kinetics of decay, inhibition of EPP developed (Fig. 1b). Neostigmine, an acetylcholinesterase inhibitor, potentiated the action of V-VII and weakened that of I-IV. The effects of neostigmine were several times weaker than before treatment with the test compounds and were not accompanied by changes in EPP decay. Moreover, compounds V-VII could substantially increase τ of decay even after treatment of the nerve—muscle preparation with 0.1 mM neostigmine solution or with armin*. The anticholinesterase activity of I-VII, determined from

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

50% inhibition of erythrocyte acetylcholinesterase, was 5×10^{-5}, 2.3×10^{-5}, 1.2×10^{-6}, and 2.2×10^{-7}, respectively, for I-V, and 2×10^{-4}, 5×10^{-5}, and 1.3×10^{-5} for V-VII and it was comparable with neostigmine activity.

It can be postulated on the basis of the data given above that the anticholinesterase activity of I-VII may lie at the basis of their action on the cholinergic synapse. However, it is impossible to explain the effects of the test substances described above by their anticholinesterase action, for they did not cause decurarization of the muscle, and the change in τ of decay with substances V-VII took place during complete blockade of acetylcholinesterase. All the compounds tested were able to lower EPP in the presence of 0.1 mM solutions of neostigmine or armin. Interaction between I-VII and AChR took place in different regions from ACh combining sites This is clear from the noncompetitive character of their interaction (Fig. 2; $K_i = 4.45 \times 10^{-8}$ M). It will be clear from the structure of I-VII, their cationic head has a hydrophobic component and can evidently interact with the nonpolar regions of the lipoprotein matrix of the membrane. The effect of substances I-VII on the lipid phase of the membrane was investigated with respect to mitochondria and BLM. Just like other compounds containing a guanidine group, they inhibit mitochondrial respiration in state 3, but not in state 4. Probably I-VII do not pass through the cytoplasmic membrane of muscle cells, for, since they have an uncoupling action, they would cause EPP to fall, as has been shown for 2,4-dinitrophenol [2]. It will be clear from Fig. 3 that the degree of inhibition of EPP, the degree of uncoupling of oxidative phosphorylation, and the values of the surface potential of BLM correlated closely with one another for I-IV ($r = 0.95$). The presence of correlation between the degree of inhibition by I-IV of such different processes as oxidative phosphorylation, interaction between ACh and AChR, and the surface potential of BLM indicates a similar mechanism of action of the test substances on these membranes. Lengthening of the interquaternary chain of I-IV was reflected much more strongly in the inhibition of EPP than binding with liposomes, uncoupling of phosphorylation, and changes in the surface potential of BLM (Fig. 3). Hence, it follows that interaction of I-IV with the membrane of the end plate incorporates not just a change in the surface charge of the phospholipid matrix, but also some other process specifically bound with AChR. Amino and methyl derivatives of guanidine are known to block the entrance into the ionic channel of AChR of the frog end-plate with dissociation constants of 15 and 0.5 mM, respectively [11]. I-IV probably act by a similar mechanism, and blocking of the ionic channel is due not only to steric hindrances at the entrance to the selective filter, but also to electrostatic interaction with the cation entering the channel. This conclusion follows directly from the data in Fig. 3. Differences in the effective concentration of amino- and methylguanidine from those of I-IV (Fig. 1) may arise through the increased absorptive power of the latter on account of van der Waals interactions with hydrophobic regions of AChR [4]. A contribution of electrostatic interaction of a second cationic head with negatively charged groups of the receptor or of its surroundings to the strength of binding of I-IV with the membrane likewise cannot be ruled out. The effectiveness of uncoupling of phosphorylation and of inhibition of EPP increases with compounds V-VII with lengthening of the interquaternary chain, just as for I-IV. However, no correlation could be found between their activity on the basis of these tests.

As already stated above, there are considerable differences in the action of substances I-IV and V-VII on AChR of the end-plate. A particular feature of the latter is the presence of ethereal oxygen atoms in the interquaternary chain, capable of entering into dipole–dipole interactions with positively charged ions or ionic groups. It may be that the increase in decay of the EPP during complete blockade of acetylcholinesterase is due to an increase in the mean life span of the AChR–ACh complex through the formation of dipole–dipole bonds of ethereal oxygen atoms of the interquaternary chain with certain positively charged groups of AChR. Such bonds could interfere with conformational transitions of AChR during interaction with an agonist.

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CORRELATION BETWEEN EXPERIMENTAL AND CLINICAL EFFECTIVENESS OF BENZODIAZEPINES AND THEIR AFFINITY FOR BENZODIAZEPINE RECEPTORS

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KEY WORDS: benzodiazepines; tranquilizers; prognosis; receptors.

Recently during the study of the mechanism of action of the benzodiazepines great attention has been paid to analysis of their highly specific binding with certain regions of synaptic membranes in the human and animal brain [10, 13]. With respect to certain manifestations of the action of benzodiazepines, correlation has been found between the inhibition constants and activity of these drugs experimentally [7, 9, 12]. Meanwhile, the role of binding with the receptor for realization of the tranquilizing effect of benzodiazepines when used clinically has not been adequately studied. In addition, there are no clear criteria with which to assess the experimental action of benzodiazepines [1, 14], so that their clinical effects cannot be confidently predicted.

The object of this investigation was to discover correlation between effectiveness of the benzodiazepines, according to the most representative methods of experimental study, their integral clinical effect, and their affinity for benzodiazepine receptors.

EXPERIMENTAL METHOD

To assess tranquilizers in experiments on animals, the following tests were used: conditioned defensive and food reflexes, a conflict-inducing situation, external inhibition, aggressive reactions, spontaneous motor activity, antagonism tests with metrazol and thiosemicarbazide, and potentiation of hexobarbital sleep. The methods were all described previously [2].

The comparative clinical study of the compounds was carried out by a standardized method of clinical assay of psychotropic drugs [3]. The clinical material for the investigation consisted of 360 observations on patients with various neurotic and neurosis-like states. Groups of patients receiving the various tranquilizers were standardized by diagnosis and syndrome, the severity of individual symptoms, the duration of the syndrome, and also by sex, age, and numerical composition [5].

Specific binding of benzodiazepines with the receptor was determined by the usual method [10]. Affinity for the receptor was assessed by the value of IC_{50} (the concentration of the substance in which, in a state of equilibrium, the ligand occupies half of the maximal possible number of binding sites).

Correlation was determined by calculating Spearman's coefficient of correlation [4].

EXPERIMENTAL RESULTS

The experiments showed that all tranquilizers studied, namely, phenazepam (P), lorazepam (L), nitrazepam (N), diazepam (D), oxazepam (O), and chlorodiazepoxide (C) possess high pharmacologic affinity, by all tests used (Table 1). Clinical study of the psychotropic effects of the benzodiazepines enabled the degree of therapeutic activity of the various substances in relation to particular neurotic symptoms to be established and the integral, global tranquil-

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