PHARMACOLOGICAL CHARACTERISTICS OF ACETYLCHOLINE AND L-GLUTAMATE RECEPTORS OF THE SUBESOPHAGEAL GANGLION OF THE MOLLUSK Zachrysia guanensis

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The acetylcholine (ACh) receptor systems of molluscan ganglia differ widely in their sensitivity to cholinergic compounds. As well as traditional synapses with "nicotine" specificity, and blocked by α-bungarotoxin, nicotine, and tubocurarine [7, 13], molluscan neurons also contain synapses insensitive to α-bungarotoxin, but blocked by nicotine and tubocurarine [8].

The writers showed previously that synapses of one nerve cell in the central parietal zone of the subesophageal ganglion of the mollusk Zachrysia guanensis give similar responses to microapplication of ACh and L-glutamate [12]; addition of tubocurarine, however, blocks both responses.

The aim of this investigation was to determine the characteristics of these synapses in greater detail.

EXPERIMENTAL METHOD

Experiments were carried out on a neuron in the central zone of the subesophageal ganglion of the mollusk Z. guanensis, an endemic species in the zone of Viñales (Cuba). The subesophageal ring together with nerves [10, 11] were fixed to a wax slab, placed in a chamber with a volume of 2 ml, and irrigated with physiological saline for mollusks: NaCl 80 mM, KCl 4 mM, $CaCl_2$ 7 mM, $MgCl_2$ 4 mM, Tris-HCl 10 mM, pH 7.5.

A microelectrode technique was used for intracellular recording. The electrodes, filled with 3 M KCl, had a resistance of 10-30 mΩ. The microelectrode was inserted by means of an MM-1 micromanipulator (USSR) under visual control. The electrophysiological recording system consisted of two amplifiers, an MS-4 cathode follower, VK-8 oscilloscope, and a PC-2A continuous recording chamber (from Nihon Kohden, Japan). Recordings were made on DK-5 35-mm film (from Orwo, East Germany), at a speed of 2.5-10 mm/sec. Multichannel microelectrodes (10-30 mΩ), filled with 0.5 M ACh (from Merck, West Germany), pH 8.0, or with 0.1 M sodium L-glutamate solution (from BDN, West Germany), were used for microapplication, with appropriate cathodal and anodal currents. For stimulation, pulses were applied at a frequency of 0.1-0.5 mV/sec, with a duration of 0.1-0.5 msec. In every case the current was held at 400-700 mA. No electrical effect on account of removal of the additional ion was present.

D-tubocurarine chloride was obtained from Serva, West Germany, and the atrophine sulfate and cytisine were of USSR origin. N-(p-azidophenacyl)-cytisine was synthesized as described previously [1], and the remaining preparations were of the chemically pure grade (USSR). All operations with N-(p-azidophenacyl)-cytisine were carried out in semidarkness because of instability of this compound in bright light.

EXPERIMENTAL RESULTS

Microapplication of ACh and L-glutamate to the central parietal zone of the subesophageal ganglion of Z. guanensis gave similar depolarization responses, inhibited by tubocurarine. When ACh was used, perfusion with tubocurarine completely blocked the response of the effector cell, whereas when L-glutamate was used, only a

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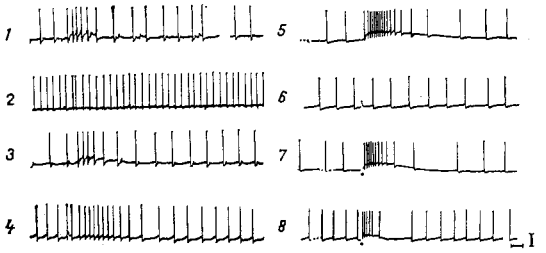

Fig. 1. Pharmacologic differences between ACh and L-glutamate receptors coexisting in the same neuron. 1, 3) Excitatory responses to microapplication of ACh and L-glutamate (100 msec) respectively, 2) blocking of response to ACh after perfusion with tubocurarine, 4) partial blocking of response to L-glutamate after perfusion with tubocurarine, 5) excitatory response to microapplication of ACh (100 msec), 6) blocking of response to ACh after perfusion with cytisine ($5 \cdot 10^{-4}$ g/ml), 7) excitatory response to microapplication of L-glutamate to the same neuron (100 msec), 8) change in response to microapplication of L-glutamate (100 msec) after perfusion with cytisine ($5 \cdot 10^{-4}$ g/ml).

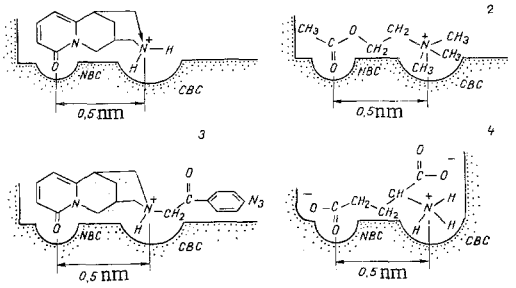

Fig. 2. Hypothetical models of recognition sites of cytisine (1), ACh (2), azidocytisine (3), and L-glutamate (4).

partial block resulted (Fig. 1). During perfusion of the neuron with cytisine (Fig. 1) both responses were completely inhibited. The alkaloid cytisine, which has a fixed three-dimensional structure, is known to have a specific action in nicotinic ACh receptors [19, 12], and it is used in medicine as a nitocinic cholinomimetic [6]. Furthermore, competitive interaction between cytisine, on the one hand, and nicotine and tubocurarine, on the other hand, has been demonstrated on a nicotinic receptor from the optic ganglia of the squid [2]. In experiments with rat diaphragm, a decrease in the response to microapplication of ACh was observed in the presence of cytisine. Microapplication of cytisine to the region of an end plate of the rat diaphragm gives a depolarization response similar in its characteristics to the response to microapplication of acetylcholine (ACh). Responses to microapplication of ACh and cytisine are abolished by tubocurarine. It has been shown by the method of radioligand analysis that cytisine has no action on ACh receptor systems with muscarinic specificity [3].

Depolarization responses to microapplication of ACh and L-glutamate are thus inhibited by cholinergic ligands with nicotinic specificity – tubocurarine and cytisine. Since the response of the effector cell takes place in two stages, namely interaction with the postsynaptic receptor system, followed by a change in ionic permeability of the postsynaptic membrane on account of functioning of ionic channels, it might be supposed that either receptors of mixed ACh-glutamate specificity are present in the nerve cell of the subesophageal ganglion of the mollusk Z. guanensis or receptor systems for ACh and glutamate are different, but the mechanisms of change of ionic permeability are similar. The possibility of a direct effect of these substances only on the channel part of the postsynaptic membrane likewise cannot be ruled out.

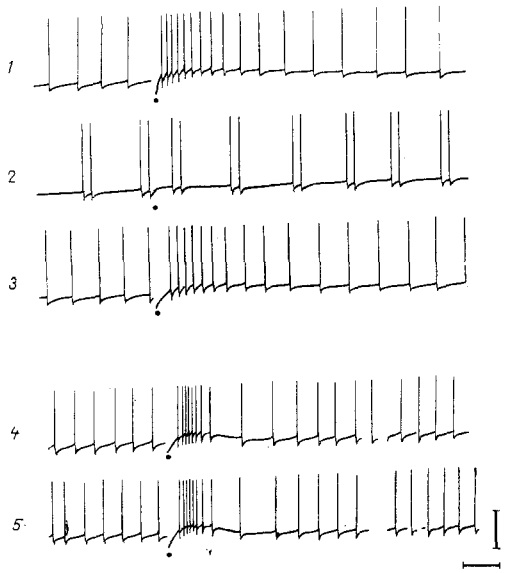

Fig. 3. Pharmacologic differences between ACh and L-glutamate receptors coexisting in the same neuron. 1) Excitatory response to microapplication of ACh (100 msec), 2) blocking of response to ACh after perfusion with azidocytisine (10^{-3} g/ml), 3) restoration of response to ACh after rinsing, 4) excitatory response to microapplication of L-glutamate (100 msec), 5) the same, after perfusion with azidocytisine (10^{-3} g/ml).

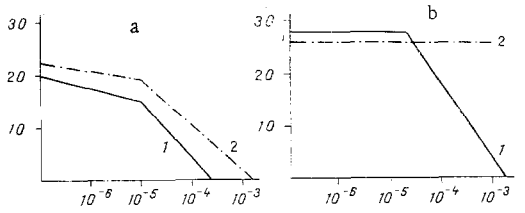

Fig. 4. Dose–response relationship for cytisine (1) and azidocytisine (2) for neuron to ACh (a) and L-glutamate (b). Abscissa, concentration (in g/ml); ordinate, voltage (in mV).

Since both tubocurarine and cytisine act on ACh receptor systems (see above), it might be considered that the two substances also acts on the glutamate system at least. Considering that the cytisine molecule is three-dimensionally rigid, similarity between recognition sites of the mixed ACh-glutamate or of ACh and glutamate receptors can be postulated. The essential components of the cytisine structure for recognition of ACh receptors [4, 5] is the presence of an ammonium group of the nitrogen atom and a carbonyl group of the α-pyridone ring. A similar group, namely the protonated α-amino group and the carbonyl group of γ-carboxyl, are also present in glutamic acid. It can therefore be postulated that receptor sites recognizing ACh, L-glutamate, and cytisine are similar in structure and that, by analogy with the structure of the recognition site of the ACh receptor, they can be represented (Fig. 2) as surfaces containing a binding center for the cationic ammonium group (CBC) and a combining center for the nucleophilic carbonyl group (NBC).

If the groups suggested above are in fact important for binding with receptors, the introduction of additional substituents into them could be reflected in their biological activity. By alkylation of cytisine with p-azidophenacyl bromide, N-(p-azidophenacyl)-cytisine (subsequently called azidocytisine), containing a three-dimensional substituent in the ammonium group, was obtained. Perfusion of the neurons with azidocytisine was

found to inhibit the response to ACh application but not to change the characteristics of the response to L-glutamate application (Figs. 3 and 4). The results demonstrate unequivocally that the receptor sites for ACh and L-glutamate are different, although they possess similar structural elements.

Further investigation of the properties of ACh and glutamate receptor systems of the subesophageal ganglion of Z. guanensis showed that both are insensitive to scopolamine, atropine, and hexamethonium.

The presence of ACh and L-glutamate receptors sensitive to cholinergic ligands with nicotinic specificity (tubocurarine and cytisine) was thus demonstrated for the first time on the basis of electrophysiological data after microapplication of ACh and L-glutamate to the central parietal zone of the subesophageal ganglion of the mollusk Zachrysia guanensis. Modification of cytosine, by introducing a three-dimensional substituent at the nitrogen atom, gives azidocytisine, which inhibits the function of the ACh receptor but does not affect the glutamate receptor. The structures of the recognition sites of the receptors are evidently closely similar and differ in the zone of recognition of the ammonium group (CBC) of endogenous ligands.

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CHARACTERISTICS OF THE PROTECTIVE ACTION OF ETHACIZINE ON THE ISCHEMIC MYOCARDIUM

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KEY WORDS: experimental myocardial infarction; ethacizine.

Ethacizine, a diethylamine analog of ethmozine, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, differs from ethmozine in its stronger and more lasting antiarrhythmic action [2], as has been confirmed by clinical trials [4, 5].

The aim of this investigation was to study the effect of ethacizine on the size of an experimental myocardial infarct and to shed light on some of the pathogenetic mechanisms of that effect.

EXPERIMENTAL METHOD

Experiments were carried out on chinchilla rabbits weighing 2.0-2.5 kg. A myocardial infarct was produced by ligation of the anterior interventricular branch of the left coronary artery in its upper third. The operation was performed under pentobarbital anesthesia (30 mg/kg, intravenously), using a trans-sternal approach to the

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