DESENSITIZATION OF BULLFROG ATRIUM MUSCLE TO THE MUSCARINIC ACTION OF ACETYLCHOLINE ON ITS ACTION POTENTIAL

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Decreases in the amplitude and duration of the action potential of bullfrog atrium muscle by the muscarinic action of ACh or carbachol, were found to be restored to a certain extent when the application of ACh or carbachol was sustained. This suggests that the muscarinic receptor-ion channel complex which is responsible for these decreases shows the desensitization to the action of ACh or its agonist.

INTRODUCTION

Transmitters released from pre-synaptic nerve terminals act on the subsynaptic membrane of target cells and induce postsynaptic potential by changing the subsynaptic membrane conductance. Such a change in the membrane conductance is thought to be caused by an activation of the receptor-ion channel complex located at the subsynaptic membrane. In neuromuscular junctions of skeletal muscle, the sensitivity of end-plate to acetylcholine (ACh) is depressed during a prolonged application of ACh or its agonists, and such a decrease of the sensitivity of membrane receptor-ion channel complex is called desensitization (Thesleff, 1955; Katz and Thesleff, 1957). This desensitization is one of most interesting nature of the receptor-ion channel complex.

Another action of transmitters is their regulatory actions on the action potential of target cells. For examples,

transmitter substances, such as ACh and catecholamine, are able to regulate the size and time course of action potentials of heart muscle or sympathetic ganglion cells (Koketsu and Minota, 1975; Kuba and Koketsu, 1975, 1976; Minota and Koketsu, 1977). Such an effect of transmitters must be exerted by acting on ion channels, which are responsible for the production of action potentials. The fact that a transmitter is able to change the configuration of action potentials, suggests that these channels compose receptor-ion channel complexes. In connection with this suggestion, question is arisen if these receptor-ion channel complexes show desensitization, like those of subsynaptic membrane producing postsynaptic potentials.

The present experiment was initiated for the purpose to test if changes in the action potentials of heart muscle, which were caused by the action of a transmitter, show desensitization. The present paper reports that changes in

the action potential of heart muscle, being caused by the action of ACh, are restored during a prolonged application of ACh, suggesting that the receptor-ion channel complex, which is responsible for these changes of action potential, shows the desensitization to the muscarinic action of ACh.

METHODS

Strips (1×7 mm in size) of quiescent muscle fiber bundles excised from the atrium of bullfrogs (*Rana catesbeiana*) were used in the present experiment. A preparation was continuously perfused with a solution during experiment and action potential were induced by applying electrical current (1 msec duration) directly to the preparation through a pair of platinum electrode. Action potentials were recorded intracellularly by use of glass microelectrode filled with 3 M KCl. In order to test the effect of a drug, superfusion medium was changed from the Ringer to the Ringer containing this drug in a desired concentration. Ionic composition of the Ringer solution is as follows: 112 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 2.4 mM NaHCO_3 , and 2.5 mM glucose. Drugs used in the present experiment was acetylcholine chloride (Wako) and carbachol (Tokyo Kasei).

RESULTS

The value of resting membrane potential recorded from present preparations was ranged between -85~-95 mV. The action potential of these preparations was induced by repetitive electrical pulse stimulations of which frequency was 0.2-0.3 Hz. An example of action potentials recorded in the present experiments is seen in record A-1

of Fig. 1. Recorded action potentials were very steady and can be recorded for more than 1 hour while muscle was impaled by a microelectrode. When ACh ($5.5 \times 10^{-7} \text{M}$) or carbachol ($5 \times 10^{-7} \text{M}$) was added to the superfusion solution (Ringer solution), the amplitude and duration of the action potential were markedly depressed within 30-60 sec (see record A-2 of Fig. 1).

An extremely interesting phenomenon which was found in the present

Fig. 1. Desensitization of action potential changes caused by the muscarinic action of ACh in bullfrog atrium muscle (resting potential, -90 mV). A: Record 1 is a control action potential recorded in the Ringer solution and records 2, 3 and 4 were recorded 1, 2, 3 min after an addition of ACh ($5.5 \times 10^{-7} \text{M}$), respectively. Record 5 was taken 3 min after withdrawal of ACh. Note the restoration of the amplitude and duration of action potential during a sustained application of ACh. B: The duration of action potentials, which was measured at the potential level of 20 % of total amplitude of control action potential, is plotted against time. The duration of control action potential is taken as 100 %. Note the increase of duration during a prolonged application of ACh.

experiment was the restoration of depressed action potential in the presence of ACh or carbachol. In other words, both the amplitude and duration of action potentials, which were decreased by the action of ACh or carbachol, were gradually increased during a prolonged application of ACh or carbachol. An example of these experiments is shown in Fig. 1. As seen in this figure, action potentials which were initially markedly depressed by ACh shows a gradual restoration to a certain extent during a sustained application of this drug; it was completely restored after ACh was withdrawn.

DISCUSSION

Decreases in the amplitude and duration of the action potential of atrium muscle by the muscarinic action of ACh or carbachol were thought to be caused by an increase of the K conductance which is responsible for the background time-independent K current (cf. Noble, 1975). According to a recent paper (Giles and Noble, 1976), however, these decreases in ACh-containing solution was explained as a result of a reduction of the slow inward current. The present experiment clearly demonstrates that the changes in both amplitude and duration of action potential of atrium muscle are restored during a prolonged application of ACh or its agonist. This suggests that the muscarinic receptor-ion channel complex, which is responsible for the changes in the action potential in ACh-containing Ringer solution, shows the desensitization to the action of ACh. Whatever this channel is for the background K

current or for the slow inward current, this muscarinic receptor-ion channel complex clearly shows the desensitization, like the nicotinic receptor-ion channel complex of the end-plate, to a prolonged application of ACh or its agonist.

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