Modification of Acetylcholine Sensitivity of Neuronal Membrane by Presynaptic Stimulation

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Abstract. The action of electrical stimulation of one of the pallial nerves on the sensitivity of the bursting RPa1 neuron of *Helix pomatia* to acetylcholine (ACh) was investigated. The depolarizing effect of ACh was significantly decreased by presynaptic stimulation. Stimulation leads also to an attenuation of the ACh-induced increase in membrane conductivity. The effect of stimulation on the ACh evoked response of the membrane was reversibly blocked by cold and was completely eliminated after long term incubation of the neuron under „in vitro" conditions.

Key words: Bursting neuron — Neuronal membrane — Presynaptic stimulation — Acetylcholine

Introduction

It was previously demonstrated that single pulse nerve stimulation induced polyphasic synaptic potentials comprising both depolarizing and hyperpolarizing phases in the burst type RPa1 neuron of *Helix pomatia L.* (Pasic et al. 1976; Salánki et al. 1979). In contrast to depolarizing and fast hyperpolarizing phases of the evoked potentials, the slow hyperpolarizing component could not be reversed even at highly negative levels of membrane potential (Salánki and Vehovszky 1981). It is known, that the same potential dependence takes place for short term hyperpolarization evoked by electrogenic sodium pump (Thomas 1972).

Conversely a close correlation has been shown to exist between acetylcholine (ACh) sensitivity and electrogenic Na-pump activity in *Helix* giant neurons: inactivation of the Na-pump increases, while its activation decreases the effect of ACh on the neuronal membrane, regardless of the factors influencing Na-pump activity (Ayrapetyan and Arvanov 1977).

It was also demonstrated that specific antagonists of ACh, such as atropine and nicotine, depressed both the EPSP and the slow IPSP evoked on the RPa1 neuron of *Helix pomatia* by nerve stimulation (Vehovszky and Salanki 1983).

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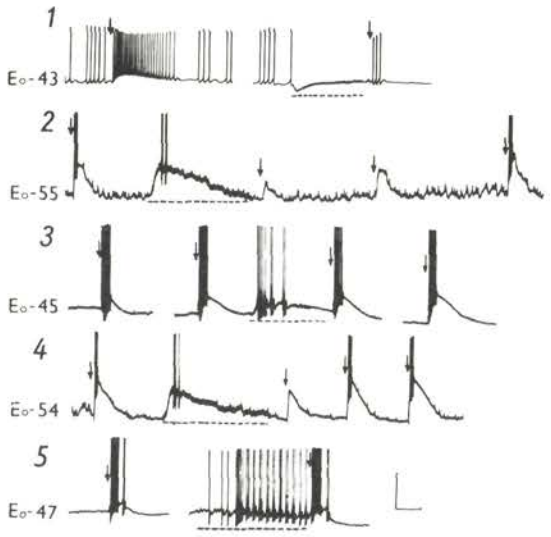

Fig. 1. Change in ACh response of the RPa1 neurone after stimulation of the left pallial nerve. Dotted lines correspond to time of stimulation ;arrows mark ACh application. 1.1. — ACh evoked depolarization and the same after stimulation evoked hyperpolarization at 23 °C; 1.2. — ACh evoked depolarization and the same (three times) after stimulation evoked depolarization at 23 °C; 1.3. — As 1.2 at 7 °C; 1.4. — As 1.2 after returning to 23 °C; 1.5. — As 1.2 90 min after penetration of the neuron by microelectrode. E_o = resting potential. Calibration: (1) — 40 mV in vertical and 15 s in horizontal, (2, 3, 4, 5) — 20 mV and 75 s respectively.

The aim of the present investigation was to clarify the nature of functional relationship between nerve stimulation and the sensitivity of the neuronal membrane to ACh, including an analysis of the possible involvement of the electrogenic sodium-pump in the effect of nerve stimulation.

Materials and Methods

Experiments were performed on the bursting RPa1 neuron located in the right parietal ganglion of the snail *Helix pomatia*.

The whole circumoesophageal ganglion was isolated and placed in an experimental chamber containing 3 ml physiological saline (NaCl 60, KCl 4, $MgCl_2$ 12, $CaCl_2$ 10, TrisHCl pH 7.6; 5 mmol/l). When isolating the ganglion a 5—10 mm long portion of the left or right pallial nerve was kept intact and was passed through a vaseline bridge into an adjacent chamber. These nerves transfer presynaptic inputs to the RPa1 neuron (Elekes et al. 1983). The nerve was stimulated by bipolar platinum electrodes with 5 V square wave pulses of 5—15 msec duration and frequency of 5 Hz.

From the RPa1 neuron the membrane potential, spontaneous activity and evoked potentials were recorded intracellularly using 2.5 mol/l KCl-filled glass microelectrodes. A bridge circuit was used to measure conductivity of the membrane (Véró 1971). The recording of electric signals was undertaken using a Helioscriptor technical recorder.

ACh was applied by pressure directly onto the neuron from a sharpened glass micropipette filled with 10^{-4} mol/l ACh solution.

Results

ACh caused depolarization and fast spiking of the neuron, while a one-two min long stimulation of the right or left pallial nerve evoked either depolarization followed by hyperpolarization or hyperpolarization without previous depolarization and spiking.

Fig. 1 shows the mode of action of nerve stimulation on the membrane potential and on the ACh-response of the neuron. As can be seen from Fig. 1.1 in this case stimulation of the left pallial nerve lead to a short term deep hyperpolarization of the neuronal membrane. If we compare ACh responses before and after stimulation it is obvious that previous stimulation significantly decreased the excitatory effect of ACh on the membrane.

It was also investigated whether the action of nerve stimulation on the effect to ACh is dependent on electrogenic Na-pump activity. Na-pump activity was inhibited, as in previous experiments, by cold and by the long term maintenance of the neuron under „in vitro" conditions (Ayrapetyan 1969). Fig. 1.2 shows that a few minutes after the penetration of the membrane at room temperature stimulation decreased the ACh response by 50 per cent. The amplitude of the ACh response was completely restored 3 min after stimulation.

Cooling markedly influenced the effect of nerve stimulation on the ACh response. In Fig. 1.3 (from left to right) ACh responses are demonstrated 2, 8 and 15 min after lowering the saline temperature from 23 °C to 7 °C. Cooling resulted in a reduction of the depolarizing effect of ACh. This was probably the consequence of cold-induced depolarization of the membrane. As the neurone was at 7 °C, the amplitude of the ACh response gradually increased. After cooling the neuron from room temperature to 7 °C nerve stimulation did not change the sensitivity of the membrane to ACh. The effect of stimulation on ACh response returned when the temperature of the saline was restored to 23 °C (Fig. 1.4).

The effect of stimulation on the sensitivity of the membrane to ACh gradually decreased as the neurone was maintained under „in vitro" conditions. The maximal stimulation-induced reduction of the ACh response took place in the first few minutes following the penetration of the membrane with the microelectrode (Fig. 1.1. and 1.2). Fortyfive minutes after the registration of cell activity (Fig. 1.4) equivalent nerve stimulation reduced the ACh response only by 30 per cent. After 90 min the stimulation had no effect on the amplitude of the ACh-evoked membrane response (Fig. 1.5), similarly to the data observed in the case of cooling.

In order to determine whether changes in membrane conductivity may be responsible for the stimulation-induced diminution of the membrane response to ACh, the membrane conductivity was measured during and after evoked poten-

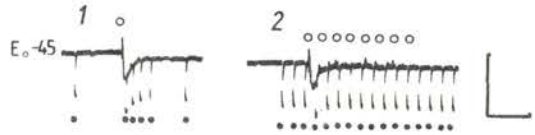

Fig. 2. Effect of stimulation on the membrane conductivity. 2.1. — Stimulation by single pulse, 2.2. — stimulation by series of pulses. Empty circles correspond to the time of nerve stimulation; full circles mark hyperpolarizing current pulse through the membrane. Calibration 20 mV and 75 s.

tials. Fig. 2 shows that a small decrease of the membrane resistance took place only in the first 1—1.5 s after stimulation, but at the moment when ACh was applied onto the neuron, membrane conductivity was already completely restored to its original value. Nevertheless, in agreement with the literature the ACh-induced membrane potential shift was accompanied by an increase in membrane conductivity for inorganic ions (Fig. 3.1). We assessed the possibility that presynaptic stimulation may influence the ACh-induced changes in membrane conductivity. Our experiments indicate that stimulation leads to a decrease in the ACh-evoked enhancement of membrane conductivity (Fig. 3.2). The depressant effect of prior stimulation on the ACh-induced change in conductivity decreases with the lifetime of the preparation (Figs. 3.3 and 3.4).

Fig. 3 also illustrates the temperature-dependence of the effect of stimulation on the ACh-induced change in membrane conductivity. As can be seen from Fig. 3.5 in the case of lowering the temperature of the saline from 23 °C to 7 °C, ACh induced a 23 per cent increase in conductance and nerve stimulation did not alter this effect.

Discussion

The present results indicate that nerve stimulation leads to a significant decrease of the sensitivity of the neuronal membrane to ACh.

It is known that artificial membrane hyperpolarization by an external current source, decreases the hyperpolarizing and increases the depolarizing membrane response to ACh. The data presented above cannot, therefore, be explained by differences between the membrane potential and the equilibrium potential for the ACh response under various conditions. What is more, ACh was applied 5—6 s after stimulation, when the membrane potential was completely restored to its previous level.

The reported data show that the membrane conductivity altered by nerve stimulation was also completely restored to its original value at the moment of ACh application. This means that the decrease of the ACh response under the influence of nerve stimulation cannot be considered to be a result of changes in passive

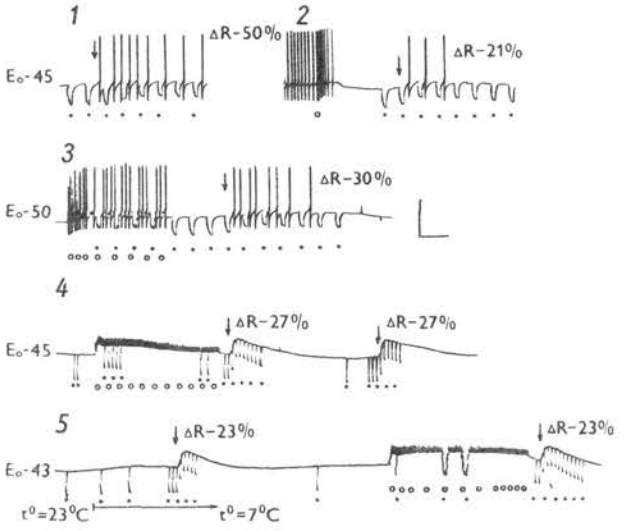

Fig. 3. Effect of stimulation on ACh-induced changes of membrane conductivity. Conventional signs are the same as in Fig. 2. ΔR — change of membrane resistance as a result of ACh application. 3.1. — Change of membrane conductivity after ACh application; 3.2. — Change of membrane conductivity to ACh application after previous nerve stimulation; 3.3. — As 3.2 but after 25 min; 3.4.— As 3.2. but after 50 min; 3.5 — Effect of cooling from 23 to 7 °C on the change of membrane conductivity to ACh application without and with previous nerve stimulation. Calibration: (3.1, 3.2, and 3.3) — 40 mV and 15 s. (3.4, and 3.5.) — 20 mV and 75 s.

permeability of the neuronal membrane for inorganic ions. The fact that stimulation leads to a lowering of the ACh-induced increase of membrane conductivity apparently indicates that a modification of the receptive properties of the membrane is responsible for the observed phenomenon.

The results showing that cold and long-term incubation of the neuron under "in vitro" conditions, respectively, eliminates the depressant effect of nerve stimulation on the sensitivity of the membrane to ACh suggest that the action of stimulation on cholinoreceptive properties of the membrane is a metabolic-dependent process. The electrogenic sodium-pump is metabolically dependent (Thomas 1972) and as shown in our previous work (Ayrapetyan and Arvanov 1979) sodium pump inactivation can result in an increase in cell volume and in a corresponding increase in the number of active ACh receptors. Although no volume changes were demonstrated as a result of strong presynaptic stimulation, the involvement of the electrogenic sodium pump cannot be excluded from the mechanism. As another possible metabolically dependent candidate, an increase in the phosphorylation state of membrane receptor proteins may be considered. This proposal is supported by the data of Nestler and Greengard (1982), showing that nerve stimulation

increases the phosphorylation of membrane proteins. On the other hand it has recently been shown that phosphorylation of the neuronal membrane by increasing intracellular ATP and cAMP concentrations were accompanied by decrease in the sensitivity of the intracellularly dialized nerve cell membrane to ACh (Arvanov and Ayrapetyan 1983).

The results presented indicate that (1) ACh sensitivity of the membrane of an RPa1 bursting neuron can be specifically depressed by presynaptic stimulation; (2) the effect of stimulation on ACh sensitivity of the membrane is a metabolically-dependent process. However, more detailed investigations are needed to determine the exact mechanism responsible for this phenomenon.

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