ANALYSIS OF THE MECHANISM OF CHANGE IN AMPLITUDE OF THE RECEPTOR POTENTIAL OF PACINIAN CORPUSCLES IN Ca^{++}-DEFICIENT SOLUTION

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The causes of the rise in amplitude of the receptor potential (RP) of Pacinian corpuscles in Ca^{++}-free solution were investigated by the external perfusion method. In Ca^{++}-free solution the amplitude of RP was shown to increase, but this increase was blocked by the addition of 10-20 mM tetraethylammonium to the solution. A temporary increase in the amplitude of RP was observed in solution with 0.2 mM 2,4-dinitrophenol. The sensitivity of the receptor membrane to mechanical stimuli was unchanged in Ca^{++}-free solution. It is suggested that there are no negative charges near the mechanically sensitive ionic channel of the receptor membrane of Pacinian corpuscles which could affect the state of the "gating" system of the mechanically sensitive channel.

KEY WORDS: mechanoreceptor; calcium ions; receptor potential.

It was shown previously that a temporary rise in amplitude of the receptor potential (RP) of Pacinian corpuscles takes place in Ca^{++}-free solution [1]. It is suggested that the change in amplitude of RP is due to the fact that in Ca^{++}-free solution there is an increase in the concentration of these ions in the axoplasm of the sensory ending, possibly on account of their liberation from the mitochondria, and also of activation of the potassium conductance of the receptor membrane, leading to an increase in the membrane potential and amplitude of RP.

To confirm this hypothesis, the results of experiments in which the receptors were acted upon by 2,4-dinitrophenol (DNP) and tetraethylammonium (TEA) are described below.

DNP is known to block processes of oxidative phosphorylation in mitochondria, with the consequent liberation of Ca^{++} from the mitochondria into the cytoplasm [3]. The addition of DNP to the solution causes an increase in the membrane potential of molluscan neurons [10] and neurons of the cat cortex [6]. The membrane potential of the photoreceptor cells of the scallop increases by 35 mV in a solution containing 0.2 mM DNP [7].

TEA ions are known to block the potassium permeability of the nerve cell and fiber membrane, with a consequent decrease in membrane potential.

The causes of the increase in amplitude of RP of Pacinian corpuscles in solution without Ca^{++} were analyzed in the investigation described below.

EXPERIMENTAL METHOD

The investigation was carried out on Pacinian corpuscles isolated from the cat mesentery. The receptors were decapsulated as far as the inner bulb and placed in a continuous-flow chamber with a capacity of 0.2 ml, kept at constant temperature, in solution flowing at the rate of 0.5 ml/min. The nerve fiber was placed in the neighboring chamber. The chambers were separated by an air gap measuring 200-300 μ. Full details of the method of recording electrical activity of the receptors were described previously [1].

DNP was added to the normal solution in a concentration of 0.2 mM and TEA ions in a concentration of 10-20 mM. Receptor discharges were blocked by 0.1% procaine solution.

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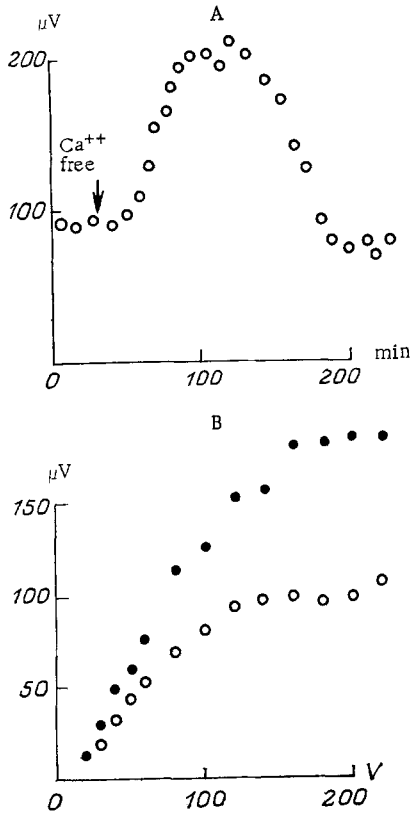

Fig. 1. Effect of Ca^{++}-free solution on amplitude of RP. A) Dynamics of changes in amplitude of RP in Ca^{++}-free solution with 10 mM EDTA; B) correlation between amplitude of RP and strength of mechanical stimulation (abscissa, relative units of V) in normal solution (empty circles) and in Ca^{++}-free solution (filled circles) at 15th minute of exposure. Duration of mechanical stimuli 3 msec.

EXPERIMENTAL RESULTS

A temporary increase in amplitude of RP of the Pacinian corpuscles took place in Ca^{++}-free solution containing 10 mM EDTA (Fig. 1A). In some experiments on photoreceptor cells an increase in the amplitude of RP also was demonstrated in a solution with a reduced Ca^{++} concentration [4, 8]. It was shown by the use of a photoemission method [12] on salivary gland cells that in Ca^{++}-free solution these ions are discharged from the mitochondria into the cytoplasm.

The present experiments showed that the amplitude of RP increased for 10-30 min both in solution with DNP and in Ca^{++}-free solution (Fig. 2). Longer exposure to DNP led to a gradual fall in the amplitude of RP or even total blocking of the electrical responses of the receptors.

If an increase in potassium conductance of the receptor membrane does in fact take place in Ca^{++}-free solution, the addition of TEA to the solution ought to block these changes. It was actually found that in Ca^{++}-free solution containing 10-20 mM TEA instead of an increase in amplitude of RP, it was reversibly reduced (Fig. 3). The duration of RP in solution with TEA increased perhaps because of a change in the resistance of the receptor membrane. The results of the present experiments are evidence in support of the view that potassium channels, activated by Ca^{++}, are present in the receptor membrane of Pacinian corpuscles, although further experiments are needed before final conclusions can be drawn.

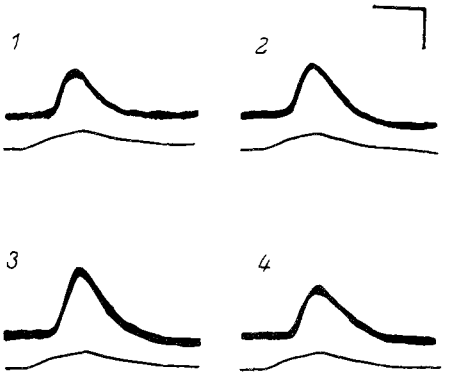

Fig. 2. Effect of 0.2 mM DNP on amplitude of RP. 1) Response in normal solution; calibration: 100 μV, 3 msec; 2-4) responses in solution with DNP at 9th, 23rd, and 29th minute.

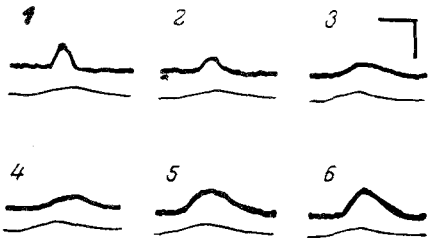

Fig. 3. Effect of Ca^{++}-free solution containing 20 mM TEA on amplitude of RP. 1) Response in normal solution; 2, 3) responses in Ca^{++}-free solution with TEA at 11th and 64th minute; 4-6) restoration of amplitude of RP in normal solution at 4th, 15th, and 20th minute; calibration: 100 μV, 3 msec.

Dependence of the amplitude of RP on the strength of stimulation in normal solution and in Ca^{++}-free solution is illustrated in Fig. 1B. Clearly in the Ca^{++}-free solution there is an increase in the maximal value of the amplitude of RP, but no difference in the location of the curves along the abscissa. This is evidence that the threshold of the receptor membrane in Ca^{++}-free solution is unchanged. It was shown recently that the threshold of the photoreceptor cells, like that of the Pacinian corpuscles, is unchanged in Ca^{++}-free solution [8]. This differs in the receptor membranes essentially from electrically excitable membranes, the threshold of which depends on the external Ca^{++} concentration. Electrically excitable membranes are considered to have fixed negative charges near the ionic channels and the effects of Ca^{++} are considered to be due to changes in the surface charge on the membrane [5, 9, 11].

Considering that the sensitivity of the receptor membrane of the Pacinian corpuscle is unchanged in Ca^{++}-free solution, this suggests that no negatively charged groups which could have an effect on the "gating" system of the channels are present near mechanically sensitive ionic channels. The reason for this may be that in the receptor membrane of the Pacinian corpuscle near ionic channels at pH 7.4 there is a high concentration of neutral lipids. It has been suggested that there are likewise no negatively charged groups in the photoreceptor membrane of the channel along which Na^{+} enters the cell [2].

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DEPENDENCE OF SINGLE MUSCLE RECEPTOR FUNCTION ON MUSCULAR ACTIVITY

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Spontaneous and evoked activity of single muscle spindles and Golgi receptors was recorded in cats anesthetized with chloralose and urethane and the effect of muscular activity evoked by direct or indirect stimulation on this activity was studied. The contractile activity of the plantaris (fast) and soleus (slow) muscles was shown to reduce the spike discharge both of muscle spindles and of Golgi receptors, and much more so in the case of muscle spindles of the fast muscles than of the slow.

KEY WORDS: single muscle receptors; muscular activity.

Investigators who have studied muscle reception in warm-blooded animals have given adequate descriptions of the basic principles governing function of single muscle receptors (muscle spindles, Golgi receptors), but no data can be found on the effect of contractile activity of a muscle on receptors located in it [4, 5]. Yet investigations in which afferent impulses are recorded from skeletal muscle receptors of cold-blooded animals have shown that during prolonged activity of a muscle the flow of afferent impulses diminishes, and the more strongly the muscle contracts, the more rapid and more marked the diminution and the faster the muscle becomes fatigued [6, 7, 10].

The object of this investigation was to study the effect of prolonged contractions of a muscle in warm-blooded animals, produced by direct or indirect electrical stimulation of the muscle, on discharges from single muscle spindles and Golgi receptors of the same muscle.

EXPERIMENTAL METHOD

Cats were anesthetized with a mixture of chloralose (5 μg/kg) and urethane (500 μg/kg) intraperitoneally. The test objects were muscle receptors (muscle spindles and Golgi receptors) of fast (plantaris) and slow (soleus) muscles before and after contractile activity of the same muscles evoked by direct or indirect stimulation.

The ventral and dorsal spinal roots were dissected and divided at level L_5-S_1; all muscles in both hind limbs were denervated except the soleus and plantaris. A weight of 200 g was attached to the tendon of these muscles, causing them to stretch and producing spontaneous activity of the receptors located in them; after attachment of the weight, the muscles contracted under isometric conditions.

To obtain impulses from single receptors the dorsal root was separated into filaments under oscilloscopic control, until impulses of equal amplitude were obtained. Activity was recorded on the UEF-5 electrophysiological system (transmission band 10-3500 Hz, sensitivity 2 mm/μV, amplifier noise 8 μV). Muscle

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