EFFECT OF TETRAETHYLAMMONIUM ON ELECTROPHYSIOLOGICAL PROPERTIES OF SMOOTH-MUSCLE CELLS OF THE PULMONARY ARTERY

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Electrical properties of single muscle cells were investigated by a microelectrode method and the contractile activity of the pulmonary artery was studied during the action of tetraethylammonium (TEA). Smooth-muscle cells in the presence of TEA were shown to be able to generate both spontaneous action potentials (APs) and APs evoked by stimulation of these cells by a depolarizing current. The appearance of the AP must evidently be connected with depression by TEA of the delayed potential-dependent potassium current, early activation of which in normal solution prevents AP development. APs in muscle cells are generated by the entry of Ca^{2+} ions into the cell and they are therefore blocked by Mn^{2+} ions. Besides the specific action of TEA, additional effects of it also are observed: a) its depolarizing action, accompanied by an increase in membrane resistance; b) stimulation of the entry of Ca^{2+} ions into muscle cells from the external medium, evoking tonic contraction of the muscle; c) the formation of a connection between the resting potential and contraction.

KEY WORDS: *pulmonary artery; smooth-muscle cells; electrophysiological properties; tetraethylammonium; calcium ions.*

Smooth-muscle cells of the pulmonary artery are known not to generate spontaneous action potentials (APs) or APs in response to sympathetic nerve stimulation or to the action of various depolarizing agents [9, 10]. Investigations have shown that the muscle cells of the pulmonary artery likewise do not generate APs in response to direct stimulation of the cells by a depolarizing current [1, 2]. What is not clear is whether the membrane of these cells possesses electrical excitability at all or whether the excitation process is masked by early activation of the delayed outward potassium current. If the second explanation is correct, APs of muscle cells would be observed by blocking the delayed potassium current, as has been shown in experiments on muscle cells of the carotid artery [7], the guinea pig stomach [6, 8], and the anococcygeus muscle of the rate [4].

In the investigation described below the action of tetraethylammonium (TEA), which blocks the outward potassium current, on electrogenesis of the muscle cells of the rabbit pulmonary artery was studied.

EXPERIMENTAL METHOD

A combined technique was used: electrical potentials of muscle cells were recorded intracellularly by means of a glass microelectrode and stimulation was applied by means of extracellular electrodes through a "sucrose gap" [2]. In some experiments, electrical potentials were derived by the double "sucrose gap" method. Membrane processes and mechanical contraction of the muscle cells were recorded simultaneously on the screen of a type S1-18 oscilloscope from the screen of which they were photographed by means of an FOR-2 photographic recorder. A recording of one of the processes under investigation also was made parallel to the above on a type KSP-4 automatic potentiometer.

The cable properties of the muscle of the pulmonary artery were taken into account when changes in the resistance of the cell membrane were determined [1]. It follows from the

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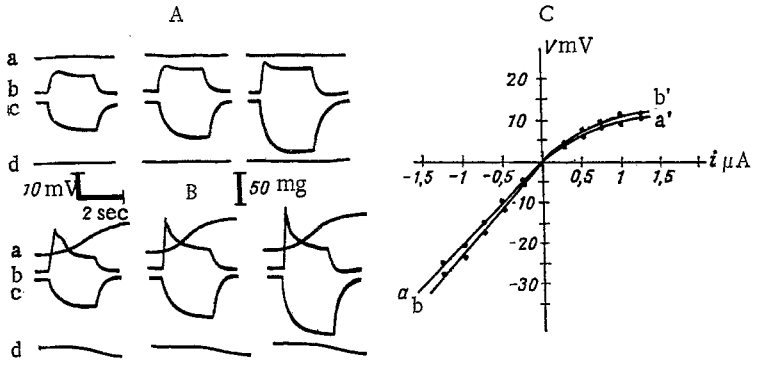

Fig. 1. Effect of TEA on volt–ampere characteristic curves of smooth-muscle cells of pulmonary artery: A) electronic potentials in normal Krebs solution; B) in response to action of 15 mM TEA. a, d) Changes in mechanical contraction of muscle in response to action of depolarizing or hyperpolarizing current respectively; b, c) catelectrotonic and anelectrotonic potentials respectively to a current of 0.25, 0.5, and 0.75 μA; C) volt–ampere characteristic curve in normal Krebs solution (a-a') and during action of TEA (b-b').

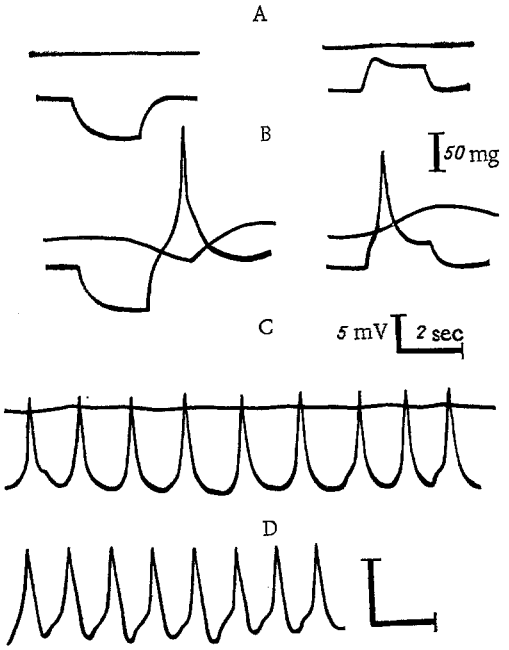

Fig. 2. Electrical and contractile activity of smooth-muscle cells of pulmonary artery in presence of 15 mM TEA; A) anelectrotonic and catelectrotonic potentials respectively in normal Kreb' solution; B) after action of TEA for 15 min; C, D) spontaneous electrical activity of muscle cells after action of TEA for 30 min. Top record shows contractile activity of muscle strip from artery.

Hodgkin–Rushton theory [6] that the ratio between the amplitude of the electronic potentials and the current evoking them in this case is proportional to $\sqrt{R_m}$, where R_m is the membrane resistance.

The composition of the Ringer–Locke solution was as follows (in mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 1.8, and glucose 5.6 to 1 liter bidistilled water. The composition of the

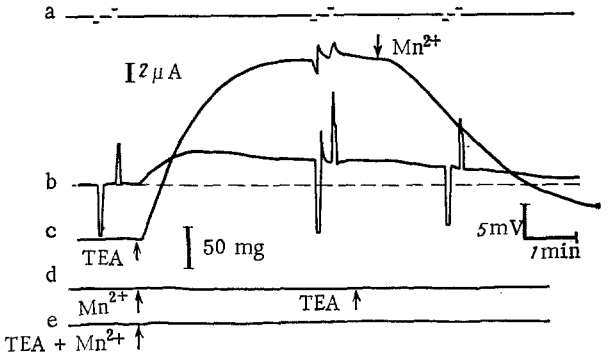

Fig. 3. Inhibitory action of Mn^{2+} ions on effect of TEA: a) marker of stimulating current relating to records b-e. Downward deflection corresponds to hyperpolarizing, upward to depolarizing current; b, c) membrane potential and mechanical contraction respectively of pulmonary artery during action of Mn^{2+} ions superposed on action of TEA. Broken line indicates original level of resting potential; d, e) mechanical contraction of pulmonary artery during action of TEA after that of Mn^{2+} and during simultaneous action of TEA and Mn^{2+}.

Krebs solution was the same as that described previously [2].

EXPERIMENTAL RESULTS

As has already been stated [1, 2], the smooth-muscle cells of the rabbit pulmonary artery have a stable resting potential (RP) of relatively high magnitude: on average 52 mV. In response to stimulation of the muscle cells by an electric current, anelectrotonic and catelectrotonic potentials arise depending on its polarity (Fig. 1A). It is clear from the volt–ampere characteristic curve that the membrane of muscle cells of the pulmonary artery has marked rectifying properties. The volt–ampere characteristic curves for hyperpolarizing and small depolarizing currents are linear. For large depolarizing currents the membrane resistance falls considerably and the volt–ampere characteristic curves lie almost parallel to the current axis (Fig. 1C, a). With a current of a certain strength a negative potential resembling local excitation arises on the catelectrotonic potential, but an increase in the strength of the stimulating current never leads to AP generation. Whatever the strength of the polarizing current, the anelectrotonic and catelectrotonic potentials that are produced are not accompanied by contraction or relaxation of the muscle strip (Fig. 1A). To activate the contractile mechanism of the muscle cells of the pulmonary artery, a change in the RP level alone is evidently insufficient.

On the addition of TEA in a concentration of 10-20 mM to the Krebs or Ringer–Locke solution depolarization of the smooth-muscle cells by 8-10 mV was observed, the electrotonic potentials were increased, and consequently, the membrane resistance also was increased on average by 55%. The observed increase in resistance of the muscle cell membrane under the influence of TEA is evidence that the main cause of TEA-depolarization is a decrease in the passive permeability of the muscle cell membrane for potassium ions.

In 60% of cells tested, during the action of TEA gradual APs arose on the catelectrotonic potentials (Fig. 1B). An anode-opening effect in the form of the appearance of an AP was often observed as an off-response to the hyperpolarizing current (Fig. 2B). In some cases in the presence of TEA the muscle cells generated spontaneous APs, the amplitude of which reached 15 mV when recorded by the microelectrode method (Fig. 2C) and 7 mV when recorded by the sucrose gap method (Fig. 2D). The appearance of an AP in the presence of TEA must evidently be connected with inhibition of the delayed outward potential-dependent potassium currents, early activation of which in normal solution prevents the development of an AP. Meanwhile TEA had virtually no effect on the permeability of the channels for the established outward currents. This is clear from the volt–ampere characteristic curves plotted for the anelectrotonic and the established values of the catelectrotonic potentials. The increase observed in the resistance of the membrane under these circumstances under the influence of TEA was not reflected in its rectifying properties (Fig. 1C).

Besides the changes described above, TEA in a concentration of 10-20 mM also induced tonic contraction of the strip of artery (Fig. 3c). In addition, under these conditions the contractile apparatus of the muscle becomes sensitive to a change in RP level in response to the action of a polarizing current. Under these circumstances a hyperpolarizing current induces relaxation of the muscle and a depolarizing current its contraction (see Figs. 1B, 2B, and 3c). These changes in mechanical contraction of the muscle persisted throughout the period of action of the polarizing current. However, besides tonic contractions, phasic contractions of the muscle also could be observed, and the latter appeared in the presence of TEA in response to both evoked and spontaneous APs (Fig. 2C).

Activation of the contractile mechanism is known to be connected with an increase in the concentration of free intracellular calcium ions. Calcium ions can enter the cell from the extracellular medium or they can be liberated from the intracellular reserves. To determine the source of these ions the action of Mn^{2+} ions on the TEA-evoked contraction was studied. As Fig. 3 shows, the addition of 2 mM Mn^{2+} ions after preliminary treatment with TEA led to relaxation of the muscle (Fig. 3c), restoration of membrane resistance (Fig. 3b), and also to partial restoration of the cell RP. Under these conditions the contractile apparatus of the muscle loses its sensitivity to a change in the RP level by the polarizing current, and APs arising on the catelectrotonic potential were inhibited. Evidently Ca^{2+} ions thus participate in AP generation for they are blocked by Mn^{2+} ions. TEA had no effect on the contractile apparatus of the muscle against the background of the action of Mn^{2+} ions or during the simultaneous action of TEA and Mn^{2+} ions (Fig. 3d, e). The results suggest that TEA increases the supply of Ca^{2+} ions to the muscle cells and thus induces tonic contraction of the muscle.

These investigations showed that, besides the well known specific action of TEA on the potential-dependent potassium channels, additional effects of it are observed in the muscle cells of the pulmonary artery, namely: a) a depolarizing action accompanied by an increase in the resistance of the membrane, which is evidently connected with a decrease in passive potassium permeability; b) stimulation of the inflow of Ca^{2+} ions into the muscle cells from the surrounding medium, causing tonic contraction of the muscle; c) establishment of a connection between RP and contraction, for an increase or decrease in RP produced by means of a polarizing current is accompanied by relaxation or contraction of the muscle respectively. In that case contraction is also induced by Ca^{2+} ions, for it is blocked by Mn^{2+} ions.

LITERATURE CITED

1. A. V. Gurkovskaya and V. A. Buryi, Abstracts of Proceedings of Symposia of the 12th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Vol. 1, Leningrad (1975), p. 211.
2. A. V. Gurkovskaya and L. P. Chaprina, Byull. Éksp. Biol. Med., No. 10, 1 (1975).
3. A. V. Gurkovskaya and M. F. Shuba, Fiziol. Zh. SSSR, No. 10, 1497 (1971).
4. K. Creed, J. Gillespie, and T. C. Muir, J. Physiol. (London), 245, 33 (1975).
5. A. Hodgkin and W. Rushton, Proc. Roy. Soc. B, 133, 444 (1946).
6. Y. Ito, H. Kuriyama, and Y. Sacomoto, J. Physiol. (London), 211, 445 (1970).
7. F. Mekata, J. Gen. Physiol., 57, 738 (1971).
8. T. Osa and H. Kuriyama, Jpn. J. Physiol., 20, 626 (1970).
9. C. Su, J. Bevan, and R. Ursillo, Circulat. Res., 15, 20 (1964).
10. C. Su and J. Bevan, Bibl. Anat., 8, 30 (1966).