EFFECT OF MANGANESE AND VERAPAMIL ON ELECTRICAL AND CONTRACTILE RESPONSES OF PULMONARY ARTERIAL SMOOTH MUSCLE CELLS TO NORADRENALIN AND HISTAMINE

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Studies of various smooth muscles have shown that the excitatory and inhibitory action of mediators is connected with a change in the passive ionic permeability of the membrane, resulting in depolarization or hyperpolarization of the muscle cells [2, 11, 12]. The excitatory action of noradrenalin (NA) on muscle cells of the pulmonary artery is manifested as depolarization of the membrane, accompanied by tonic contraction of a strip of the artery [2, 5, 7, 13, 14]. The ionic mechanism of this depolarization has not been adequately studied. Under the influence of NA and histamine the smooth muscle cells of the pulmonary artery do not generate action potentials (AP). The question arises how the contractile apparatus of the muscle cells is activated under the influence of these mediators.

The object of this investigation was to study the action of NA and histamine on the resting potential, membrane resistance, and contractile activity of the muscle cells of the pulmonary artery. The effect of Mn^{++} and verapamil, blockers of the Ca-current, on effects induced by NA and histamine also was studied.

EXPERIMENTAL METHOD

Smooth muscle cells of the rabbit pulmonary artery were used as the test object. The technique of the microelectrode investigations and of recording contraction of the muscle cells, and also the method used to stimulate them electrically were described previously [1-2].

EXPERIMENTAL RESULTS

NA in a concentration of $5\cdot10^{-7}$-$5\cdot10^{-6}$ M depolarized the membrane of the pulmonary arterial smooth muscle cells by 5-7 mV and increased its resistance on average by 30%. These changes were accompanied by tonic contraction of the muscle (Fig. 1A); within this range of NA concentrations the tonic contraction was a linear function of the logarithm of NA concentration (Fig. 1B). With an increase in the NA concentration to $2\cdot10^{-6}$-$5\cdot10^{-6}$ M, the increase in contraction was considerably reduced. In this case the membrane was depolarized by 10-12 mV. During the action of NA on catelectrotonic potentials, local potentials arose. These changes in membrane potential were accompanied by contraction of the muscle, with almost twice the original amplitude. Electrical hyperpolarization of the cell membrane led to relaxation of the muscle (Fig. 1A).

Under the influence of histamine slight depolarization and a decrease in resistance of the membranes were observed. These changes were accompanied by tonic contraction of the muscle strip, against the background of which a hyperpolarizing current caused relaxation of the muscle, but the contractile response to the depolarizing current was more than doubled (Fig. 2A). In this case also, a local potential appeared on the catelectrotonic potential. Maximal contraction was observed to histamine in a concentration of 10^{-5} M, when the membrane was depolarized by 3-5 mV. The threshold concentration of the action of histamine on the smooth-muscle cells of the pulmonary artery lay between limits of $8\cdot10^{-7}$ and $8\cdot10^{-6}$ M.

The effects of NA and histamine were considerably inhibited by Mn^{++} and verapamil, blockers of Ca channels. This inhibition was manifested as a decrease in membrane depolarization produced by NA (Fig. 1A) and histamine (Fig. 2A), by inhibition of the local potential on the catelectrotonic potential, and by considerable decrease in tonic contraction and in the response of the muscle to depolarizing and hyperpolarizing currents.

The inhibitory action of Mn^{++} is largely dependent on its concentration. The results of a study of the action of different concentrations of Mn^{++} on contraction evoked by NA (10^{-6} M), histamine (10^{-5} M), and K^{+} (40 mM) are shown

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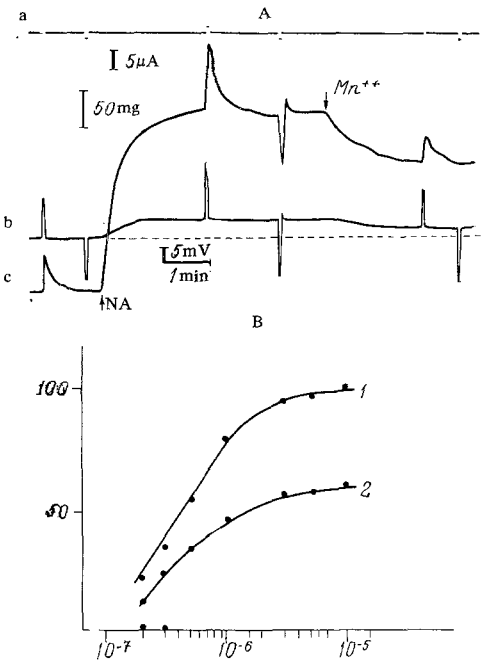

Fig. 1

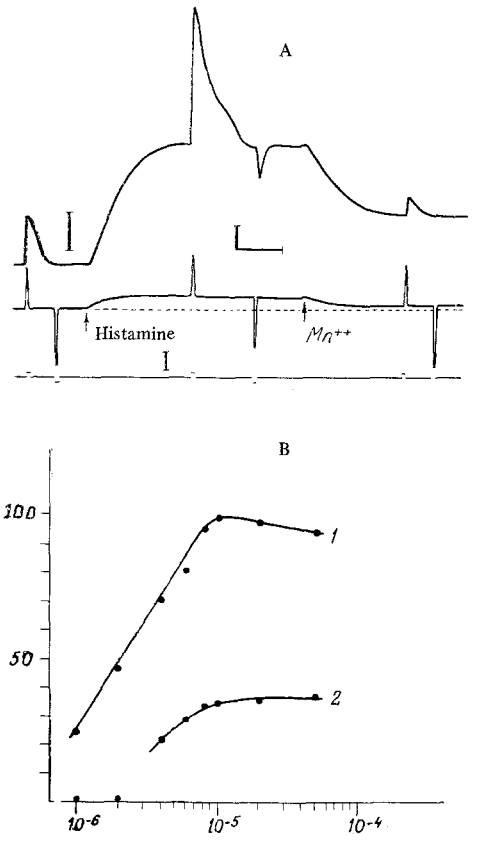

Fig. 2

Fig. 1. Effect of NA on electrical and contractile properties of pulmonary arterial smooth muscles. A: a) Marker of stimulation, upward deflection indicates depolarizing, downward hyperpolarizing current; b) membrane potential, upward deflection indicates catelectrotonic, downward anelectrotonic; c) contraction of pulmonary artery, upward deflection indicates contractile reaction to depolarizing current, downward deflection relaxation of muscle to hyperpolarizing current; B) dependence of strength of contraction on log of NA concentration in normal Ringer–Locke solution (1) and in the presence of Mn^{++} (2).

Fig. 2. Effect of histamine on electrical and contractile properties of smooth muscle of pulmonary artery. A: a) Contraction of pulmonary artery; upward deflection indicates contractile response to depolarizing current; downward deflection relaxation of muscle in response to hyperpolarizing current; b) membrane potential, upward deflection denotes catelectrotonic, downward anelectrotonic; c) marker of stimulation, upward deflection – depolarizing, downward – hyperpolarizing current; B) strength of contraction of pulmonary arterial muscle as a function of log of histamine concentration in normal Ringer–Locke solution (1) and in presence of 2 mM Mn^{++} (2).

in Fig. 3. In these concentrations the various mediators and K^+ caused maximal contraction of the pulmonary arterial muscle.

It can be concluded from these results that the ionic mechanisms of the depolarizing action of NA and histamine are different. An increase in membrane resistance observed during the action of NA may be evidence that the cause of NA-induced depolarization is a decrease in cell membrane permeability for K^+. In histamine depolarization, membrane resistance is reduced. This may be because histamine increases membrane permeability for Na^+ and, possibly, Cl^-.

Activation of mediator-induced contractions is evidently effected predominantly by extracellular Ca^{++}. However, since Mn^{++} and verapamil do not completely block the effects of NA and histamine, the possibility cannot be ruled out that during excitation of some of the cells calcium is liberated from certain intramembranous and intracellular reserves. This source of Ca^{++} in contraction induced by NA and histamine may be Ca^{++} lightly bound with the membrane [4, 6, 9, 10], on which Mn^{++} and verapamil have no action. It continues to supply the contractile apparatus with calcium even in calcium-free medium [4, 5, 10]. This source of Ca^{++} is inhibited by the addition of EDTA through a calcium-free solution. However, under these conditions also NA continues to evoke a contractile response of the pulmonary arterial muscles [5, 10]. Besides these two sources of Ca^{++}, a third source, Ca^{++} firmly bound with the membrane [8, 9] may

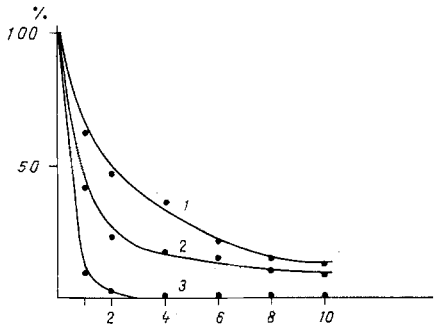

Fig. 3. Effect of different concentrations of Mn^{++} on contraction of pulmonary arterial muscle evoked by increased K^{+} concentration, histamine, and NA (maximal contraction of muscle evoked by NA, histamine, and KCl taken as 100%). Abscissa, Mn^{++} concentration (in mM): ordinate, contraction of muscles (in percent). 1) NA; 2) histamine; 3) KCl.

evidently also take part in NA-induced contractions. No satisfactory explanation of the mechanisms of liberation of Ca^{++} from these reserves during excitation of the muscle cells has yet been put forward.

Under the influence of the mediator Ca^{++} can enter the cell from the external medium in two ways. First, it can be admitted that adrenoreceptors and histamine receptors activate, i.e., open, chemosensitive Ca-channels through which Ca^{++} ions enter. This entry of Ca^{++} into the muscle cells may also perhaps make a contribution to membrane depolarization, for Mn^{++} reduces not only contraction of the muscle, but also depolarization of the cell membrane induced by NA and histamine. Another way by which Ca^{++} ions enter the cell during the action of these substances is through what are called potential-dependent slow Ca channels, which are activated by membrane depolarization induced by a change in conductance of chemosensitive Ca-, Na-, and K-channels.

The existence of these channels is confirmed by the fact that during electrical depolarization of the muscle cell membrane in normal solutions contraction develops (Figs. 1A and 2A) and ceases after the depolarizing current is switched off. This contraction is inhibited by Mn^{++} and verapamil.

The threshold of activation of electrically evoked contractions and, consequently, on the potential-dependent slow Ca-channels, lies between 3 and 5 mV of membrane depolarization.

The increase in the contractile response of the pulmonary arterial muscles to a depolarizing current against the background of the action of NA and histamine can be partially attributed to the onset, under these conditions, of a local respose due to activation of fast potential-dependent Ca-channels. As a result, the intracellular concentration of Ca^{++} ions, participating in activation of the contractile system, increases.

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