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EXCITATION-CONTRACTION COUPLING IN FEMORAL ARTERIAL SMOOTH MUSCLE IN RESPONSE TO NORADRENALIN

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KEY WORDS: noradrenalin; femoral artery; smooth-muscle cells; contractions.

Noradrenalin (NA) causes contraction of smooth-muscle cells (SMC) of the femoral artery. The contractile response of the rat [5, 9, 13] and dog [8] femoral artery has been shown to be dose-dependent on NA, the excitatory effect of which is mediated through both α_1- and α_2-adrenoreceptors [8-10].

The aim of this investigation was to study the mechanism of the excitatory action of NA on SMC of the femoral artery and the ways by which the calcium ions involved in activation of contraction enter SMC.

EXPERIMENTAL METHOD

Experiments were carried out on spiral strips (about 0.5 mm wide) of the rabbit femoral artery, using a modified single sucrose gap method [1]. Contractile activity of the muscle strips was recorded under near-isometric conditions by means of a mechanotron. Electrical potentials and contractile activity were recorded simultaneously on graph paper tape by a KSP-4 instrument and also photographically from an oscilloscope screen. The composition of the Krebs' solution was as follows (in mM): NaCl – 120, KCl – 5.9, $NaHCO_3$ – 15.5, NaH_2PO_4 – 1.2, $MgCl_2$ – 1.2, $CaCl_2$ – 2.5, glucose – 11.5. EGTA (1 mM) and $MgCl_2$ (6 mM) were added to the calcium-free Krebs' solution. The temperature of the Krebs' solution was maintained at 36°C and its pH at 7.4. Numerical values are given as the mean ± σ (σ – standard deviation); n denotes the number of observations.

EXPERIMENTAL RESULTS

In concentrations of 5×10^{-8}-10^{-5} M, NA induced dose-dependent contraction of the muscle strips of the femoral artery (Fig. 1a). The mean effective dose of NA (ED_{50}) was 10^{-7} M, almost an order of magnitude higher than for SMC of the rat femoral artery [9]. A weak tonic contraction induced by NA in a concentration of 5×10^{-8} M was not accompanied by any marked change of membrane potential, but in a concentration of 10^{-7} M, NA induced membrane depolarization to 3 mV and a marked increase in tonic contraction (Fig. 1b) compared with the previous concentration. The response of the muscle strip to NA in a concentration of 5×10^{-7} M is shown in Fig. 1c. Often against the background of depolarization, when it reached 5 mV, action potentials (AP) appeared, accompanied by phasic contractions,

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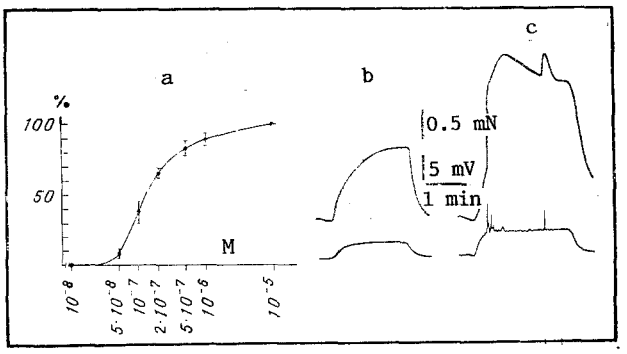

Fig. 1. Action of NA on electrical and contractile activity of smooth muscle cells of the femoral artery. a) Dependence of contractile response of muscle strip on NA concentration in surrounding Krebs' solution (response to 10^{-5} M NA taken as 100%); b, c) contractile (above) and electrical (below) responses of SMC to 10^{-7} M (b) and 5×10^{-7} M (c) NA.

superposed on the development of tonic contraction of the muscle strip. An increase in the NA concentration to 10^{-6} M led to rapidly increasing membrane depolarization by 7-9 mV and to tonic contraction of the muscle fiber (Fig. 2a). No AP developed against the background of this membrane depolarization, possibly due to inactivation of the calcium channels responsible for AP generation. An increase in the NA concentration to 10^{-5} M led to a small increase in tonic contraction of the muscle strip, although no increase in membrane depolarization was observed with this concentration.

A contraction evoked by NA was preceded as a rule by weak relaxation of the muscle strip (Fig. 1b, c). This biphasic response may be attributable to the fact that besides excitatory (α) adrenoreceptors, inhibitory (β) also are found in the femoral artery of the dog, rat, and cat [5, 10, 14].

Membrane depolarization by NA was accompanied by a decrease of resistance, the amount of which depended on the degree of depolarization. With maximal membrane depolarization of SMC, resistance was reduced by almost half (Fig. 2a). These changes in membrane potential were accompanied by increased excitability of SMC, manifested as the appearance of an anode-opening response and of AP to a catelectrotonic potential (Fig. 2a, b), accompanied by phasic contractions of the muscle strip (Fig. 2a).

NA-induced depolarization of the femoral arterial SMC, like that of SMC of other blood vessels [2, 4, 6], is evidently induced by an increase in Na- and (or) Cl-membrane permeability, for depolarization is accompanied by a decrease in resistance of the membrane. However, if the membrane is repolarized by an anelectrotonic current, its resistance is restored. This is evidence that Na (Cl)-permeability, activated by NA is voltage-dependent. The same phenomenon was shown for SMC of the pulmonary artery under the influence of NA [2].

Anelectrotonic repolarization of the NA-depolarized membrane was accompanied by a decrease of 20-30% in the contraction evoked by NA. Catelectrotonic depolarization of the repolarized membrane leads to generation of AP whose amplitude was much greater, reaching 25 mV (Fig. 2a, b). Investigations into the effect of NA on transmembrane ionic currents of SMC of the pulmonary artery under voltage clamping conditions showed that NA in a concentration of 5×10^{-6} M appreciably reduced the conductance of the potassium channels, carrying both fast inactivating and steady outward currents [2]. An increase in the excitability of SMC under the influence of NA, potentiated during anelectrotonic membrane repolarization due to removal of inactivation of calcium channels, may be linked with reduction of potassium conductance of the membrane. Investigations with the potassium current blocker TEA confirm this suggestion.

TEA in a concentration of 10 mM led to depolarization of the SMC membrane, a small increase in the electrotonic potentials, and tonic contraction of the muscle strip (Fig. 3a, b, 1). Under these circumstances TEA induced additional depolarization and a decrease in membrane resistance of SMC. These changes were rather greater than before the action

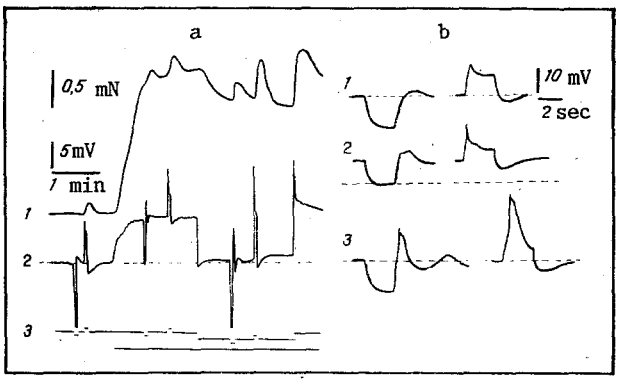

Fig. 2. Effect of analectrotonic membrane depolarization of SMC depolarized by NA (10^{-6} M) on contraction and resistance of membrane. a) Recording of contractile response (1), membrane potential, and an- and catelectrotonic potentials (2) on graph paper tape by KSP-4 instrument; 3) pulses of hyper- and depolarizing current (straight line shows time of action of NA); b) an- and catelectrotonic potentials recorded from oscilloscope screen: 1) before action of NA; 2) during depolarization induced by NA; 3) during anelectrotonic membrane repolarization of SMC, depolarized by NA. Broken line shows initial level of resting potential.

of TEA. The excitability of SMC was considerably increased, as shown by the appearance of oscillations in the form of slow depolarization waves, and phasic contractions accompanying them (Fig. 3a, b, 2).

The restoration of membrane conductance during anelectrotonic repolarization of SMC, depolarized by NA, is evidence that the primary cause of the depolarization was increased membrane permeability for Na^+, accompanied by an equivalent decrease in permeability for K^+. The developing depolarization increased activation of voltage-directed sodium conductance and activated voltage-directed potassium conductance, which prevented further membrane depolarization. Against the background of the action of TEA, which reduces potassium conductance, this effect of potassium conductance is manifested to a lesser degree, and noradrenalin depolarization therefore increases.

Against the background of anelectrotonic repolarization of the NA-depolarized SMC in the presence of TEA, the amplitude of the slow waves increased significantly (Fig. 3a, b, 3). Under these conditions the decrease in NA-induced contraction under the influence of hyperpolarization of the membrane did not take place because of summation of phasic contractions, accompanying slow depolarization waves (Fig. 3a).

NA is known to induce contraction of SMC of blood vessels in calcium-free medium also. However, release of Ca^{++} from intracellular sources takes place mainly in the presence of high concentrations of NA [2, 7].

The present investigation showed that NA in a concentration of 10^{-6} M in calcium-free medium containing EGTA induces a transient contraction, amounting to 14 ± 5.1% (n = 7) of the initial value. Further addition of NA did not cause the muscle strip to contract. In SMC of the femoral artery, just as of other blood vessels [2, 4, 7], contraction evoked by NA is activated by the presence of a raised intracellular concentration of Ca^{++} ions, mainly due to their entry from the extracellular medium.

It has been shown for SMC of different blood vessels that the excitatory action of NA is effected through activation of receptor- and voltage-controlled calcium channels, which are the principal pathway for the entry of Ca^{++} ions, involved in activation of contraction [2, 4, 11, 12], into the cells; the relative importance of these pathways of entry of Ca^{++} ions into SMC of blood vessels, moreover, may differ. The threshold of activation of uninactivated voltage-controlled calcium channels, responsible for tonic contraction of the muscle cells of the femoral artery, exceeds 5 mV [3]. NA, in a concentration of 5×10^{-8} M, leads to tonic contraction of the muscle strip without any change of membrane potential, whereas depolarization of the membrane by 3 mV, induced by the action of NA in

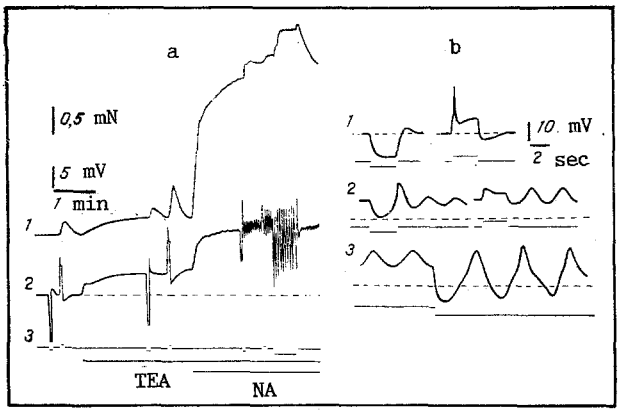

Fig. 3. Effect of 10^{-6} M NA on electrical and contractile activity of SMC during action of 10 mM TEA. a) Recording of contractile response (1), resting potential, and an- and catelectrotonic potentials (2) on graph paper tape by KSP-4 instrument; 3) pulses of hyper- and depolarizing current (straight line indicates time of action of TEA and NA), broken line shows initial level of RP); b) an- and catelectrotonic potentials recorded from oscilloscope screen: 1) before action of NA; 2) during depolarization induced by NA; 3) during anelectrotonic repolarization of membrane, depolarized by NA (rectangles below an- and catelectrotonic potentials indicate pulses of hyper- and depolarizing current; broken line indicates resting potential level during depolarization by TEA).

a concentration of 10^{-7} M, is accompanied by tonic contraction, which significantly exceeds the threshold contraction to a depolarizing current and to hyperpotassium depolarization [3]. Consequently, an increase in the intracellular concentration of Ca^{++} ions, activating the contractile response in NA in these concentrations, takes place due to the entry of Ca^{++} ions through calcium channels controlled by noradrenalin receptors. However, a small contribution to contraction induced by NA in concentrations leading to membrane depolarization above the threshold of activation of voltage-controlled calcium channels, carry in Ca^{++} ions which reach SMC through voltage-controlled calcium channels, since anelectrotronic repolarization of SMC, depolarized by NA, leads to reduction of contraction. The contribution of the voltage-controlled input of Ca^{++} to NA-induced contraction is 20-30%. This is shown also by experiments to study the effect of verapamil on contraction of SMC of the femoral artery induced by NA. Verapamil, in a concentration of 10^{-6} M, sufficient to cause complete inhibition of the voltage-controlled entry of Ca^{++} [3], inhibits contraction induced by NA by 30 ± 4.7% (n = 7). Nifedipine [13], also has an inhibitory influence on this contraction of SMC of the rat femoral artery.

The results described above may be taken as evidence that an important role in the activation of NA-induced contraction is played mainly by Ca^{++} ions entering SMC of the femoral artery through α-adrenoreceptor-controlled calcium channels and to a much lesser degree, through voltage-controlled uninactivated calcium channels.

NA thus induces AP generation against the background of weak depolarization of the SMC of the femoral artery and facilitates AP generation by these cells in response to electrical stimulation (especially against the background of TEA). It can be tentatively suggested that the modulating effect of NA on AP generation by these SMC is based on reduction of the potassium outward current.

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EFFECT OF LARGE AND SMALL DOSES OF VITAMIN E ON EXCITABILITY OF FROG CARDIOMYOCYTES

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KEY WORDS: fat-soluble vitamin E; frog cardiomyocyte; action potential; cell membrane permeability.

Fat-soluble vitamin E (tocopherol) is a component of the natural antioxidant system of the cell. According to the most widely held view [4] tocopherols protect unsaturated lipids of biological membranes against peroxidation. Since vitamin E, depending on its concentration, can exert both a labilizing and a stabilizing action on lysosomal membranes [1-3], it was decided to study the effect of different doses of tocopherol directly on the cell surface membrane itself.

By using a technique of intracellular recording of transmembrane potentials, we studied the effect of tocopherol on the resting membrane potential (RP) and on action potential (AP) generation in ventricular fibers of the frog heart.

EXPERIMENTAL METHOD

Preparations of the isolated heart of the frog Rana temporaria were used. Transmembrane potentials of the ventricular myocardium were recorded intracellularly with the aid of "floating microelectrodes." Microelectrodes with the resistance of between 5 and 15MΩ were filled with 3M KCl solution. The preparation was incubated at room temperature in a bath containing Ringer's solution, pH 7.2-7.4.

Daily for 2 weeks large (400 μg/ml) and small (16 μg/ml) doses of an ampul solution of fat-soluble vitamin E in oil (α-tocopherol) were injected intraperitoneally into the frogs. The necessary concentrations of tocopherol for intraperitoneal injection were obtained by diluting the ampul preparation in sterile olive oil as required, in accordance with data on substance 42-1087-77 in the Pharmacopoeia of the Ministry of Health of the USSR. In control experiments, 0.3 ml of the solvent (sterile olive oil, Table 1) was injected into intact frogs of group 1 in accordance with the scheme described above, and in the experiments of group 2, 1 ml of Ringer's solution was injected into intact frogs in accordance with the same schedule.

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