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COUPLING OF MEMBRANE ELECTRICAL PROCESSES AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE CELLS OF THE ANOCOCCYGEUS

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UDC 612.731.014.423

KEY WORDS: Smooth muscle cells; phasic and tonic contraction; calcium channels; blockers of the calcium current; depolarization.

A close connection is known to exist between the degree of polarization of the membrane and contraction of smooth-muscle cells (SMC) [3, 5, 6, 8]. The inflow of external calcium is considered to be the chief component in the coupling of excitation with contraction [2, 4, 6, 9]. However, according to some workers, an effect of intracellular calcium, which is connected with slow (tonic) contraction [7], is another possibility.

The object of this investigation was to study how activation of SMC of the anococcygeus muscle depends on membrane potential (MP), by the use of various agents blocking the calcium current.

EXPERIMENTAL METHOD

Experiments were carried out on strips of the rabbit anococcygeus muscle 200 μ in diameter and 1.5 cm long. Electrical activity of SMC of the anococcygeus was recorded by the double sucrose gap method [1], with simultaneous recording of contractile activity of SMC. Changes in the degree of membrane polarization were produced both by changing the external K^+ concentration and by the action of a polarizing current. The original Ringer-Locke solution (35°C) was of the following composition (in mM): NaCl 154.0, KCl 5.1, $CaCl_2$ 2.2, $NaHCO_3$ 1.8, glucose 5.6. Changes in the external K^+ concentration were produced by removal of KCl or addition of the dry salt to the Ringer-Locke solution.

EXPERIMENTAL RESULTS

In most cases the anococcygeus muscle cells possess spontaneous electrical and contractile activity. Accordingly, experiments were carried out on muscle strips in which spontaneous activity was weak or completely absent, for such activity would complicate the course of the investigation.

A depolarizing current of 10 μA, 100 msec in duration, evoked action potentials (AP), but a depolarizing current (1 μA) led to the appearance of an anelectrotonic potential (AET) in the muscle cells (Figs. 1a, b: K_0, A_0). The action of a hyperpolarizing current on SMC of anococcygeus normally does not cause relaxation of the muscle, regardless of the strength of stimulation used (Fig. 1a, b: A_0). The AP of anococcygeus SMC con-

Department of Neuromuscular Physiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 392-395, April, 1980. Original article submitted April 4, 1979.

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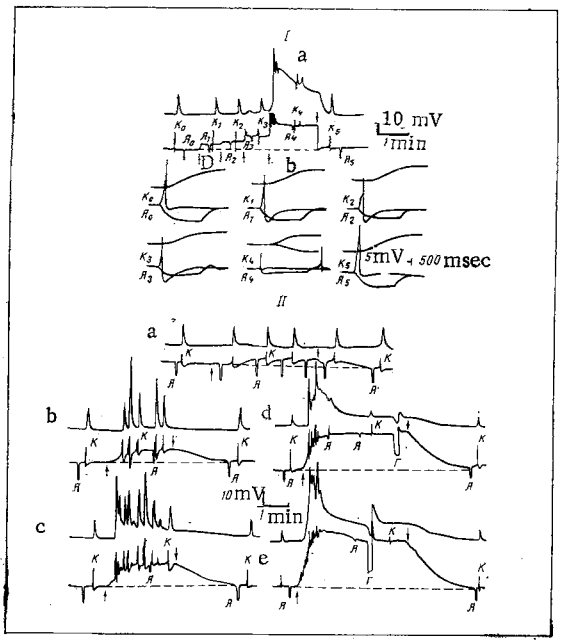

Fig. 1. Effect of polarizing current (I) and various external potassium concentrations (II) on electrical and contractile activity of anococcygeus SMC. Ia, b: K_0A_0) normal conditions; $K_1A_1 - K_4A_4$) during depolarization of SMC; K_5A_5) after removal of depolarization. IIa) $[K^+]_0 = 0$; IIb-e) with 15, 30, 60, and 120 mM K^+, respectively, in Ringer-Locke solution. Here and in Figs. 2 and 3: top tracing shows contraction, bottom tracing electrical response of SMC. K and A) action of cathode and anode of polarizing current on muscle cells. D) depolarization of SMC membrane during action of cathode of polarizing current of varied strength and duration. Arrows indicate beginning and end of membrane depolarization.

sists of monophasic potentials with an amplitude of 20-30 mV and a duration of 50-100 msec. To each spike there is a corresponding phasic contraction (Fig. 1a, b: K_0).

Depolarization of the anococcygeus muscle cell membrane by the action of the cathode of a polarizing current of up to 10 mV was not accompanied by AP generation or by a change in muscle tone (Fig. 1, Ia). However, with a shift of MP by about 12-13 mV from normal, spike potentials and a very small increase in tonic contraction of the muscle cells appeared. A further increase in the degree of depolarization of SMC led to an increase in the frequency of AP, which was accompanied by a high-amplitude phasic contraction. The magnitude of the contractile response in this case was determined by summation of the individual phasic contraction. During the prolonged action of a depolarizing current on the muscle cells, AP was inhibited and the degree of muscle contraction gradually diminished. After the depolarizing current had acted for about 2 min stabilization of tone of SMC was observed (Fig. 1, IA). In all the experiments, membrane depolarization was accompanied by a decrease in resistance of the membrane and a decrease in evoked APs and phasic contractions of the anococcygeus SMC (Fig. 1, Ia, b). Whereas under normal conditions, as stated above, hyperpolarization did not cause changes in contraction of the muscle cells (Fig. 1, Ia, b: A_0), with an increase in muscle tone in response to hyperpolarization of the membrane, relaxation of SMC took place (Fig. 1, Ia, b: A_4). With discontinuation of cathodal depolarization of the membrane, recovery of AP, muscle tone, and the response of the anococcygeus SMC to the action of the polarizing current was observed.

In the next series of experiments dependence of the contractile activity of the anococcygeus SMC on the level of membrane polarization was studied during a change in the external K^+ concentration. Removal of K^+ from the Ringer-Locke solution caused very slight depolarization (5 mV), the appearance of spikes with con-

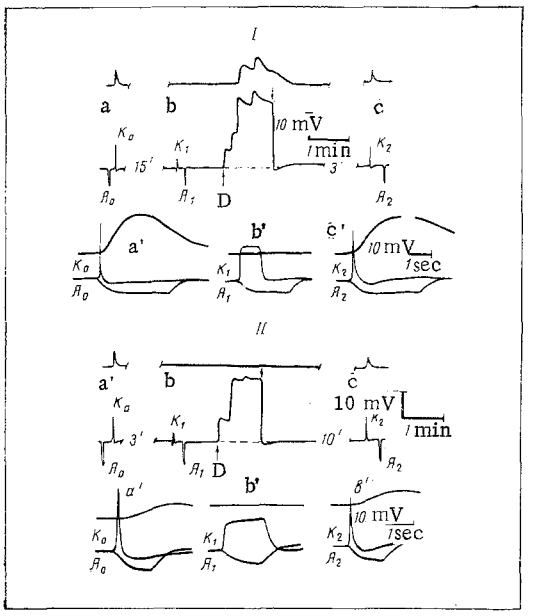

Fig. 2. Action of Ma^{++} (I) and Cd^{++} (II) on electrical and contractile activity of anococcygeus SMC. I, II a,a') action of anode and cathode of polarizing current on anococcygeus SMC under normal conditions. I, IIb, b') in Ringer-Locke solution containing 10 mM Mn^{++} and 1 mM Cd++, respectively. I, IIc, c') rinsing the SMC with normal Ringer-Locke solution.

siderable after-hyperpolarization, and an increase in membrane resistance. No tonic contraction was observed under these circumstances (Fig. 1, IIa).

An increase in $[K^+]_0$ to 15 mM in the Ringer-Locke solution led to depolarization of the membrane by 5 mV, to the appearance of APs in the form of separate volleys of discharges accompanied by high-amplitude phasic contractions and to a decrease in membrane resistance (Fig. 1, IIb). Muscle tone in this case was unchanged. With an increase in $[K^+]_0$ to 30 mM, further membrane depolarization (13 mV), an increase in the frequency of AP and of phasic contraction, and a decrease in membrane resistance were observed. Muscle tone increased appreciably (Fig. 1, IIc). A further increase in $[K^+]_0$ to 60 and 120 mM led to even more marked changes in electrical and contractile activity of the anococcygeus SMC. For instance, the action of K^+ in these concentrations caused depolarization of the muscle cell membrane by 18 and 25 mV, respectively (Fig. 1, IId, e). The contractile response of SMC under these circumstances consisted of a fast increase of contraction (phasic component), on account of summation of individual phasic contraction, and a slow contraction (tonic component), which persisted even after inhibition of AP and was unchanged in the course of action of potassium depolarization. Under these conditions the action of strong hyperpolarizing current caused relaxation of the muscle (Fig. 1, IId, e). The resistance of the SMC membrane in the presence of a high $[K^+]_0$ concentration fell almost to zero.

The action of agents blocking the calcium current on electrical and contractile activity of the anococcygeus SMC is illustrated in Figs. 2 and 3. The experiments show that the action of MN^{++} (10 mM) and Cd^{++} (1 mM) on SMC led to inhibition of spikes and of phasic contractions of the muscle cells. The resting potential and membrane resistance were unchanged (Fig. 2, I, II b, b': K_1, A_1). During prolonged and high-amplitude cathodal depolarization (over 20 mV), despite the presence of Mn^{++} in the Ringer-Locke solution, contraction of the strip was observed (Fig. 2, Ib). Under the influence of Cd^{++}, however, both the phasic and the tonic components of contraction of SMC were completely suppressed regardless of the degree and duration of cathodal depolarization (Fig. 2, IIb, b'). Rinsing the strips with normal Ringer-Locke solution completely restored the electrical and contractile response of the anococcygeus SMC (Fig. 2, I, IIc, c').

The results of investigation of the effect of potassium depolarization of contractile activity of the anococcygeus SMC under normal conditions (I) and with Ca^{++} present in the external solution (II) are given in Fig. 3. The SMC membrane was depolarized by 40 mM $[K^+]_0$. As Fig. 3, II shows, the increase in $[K^+]_0$ in the

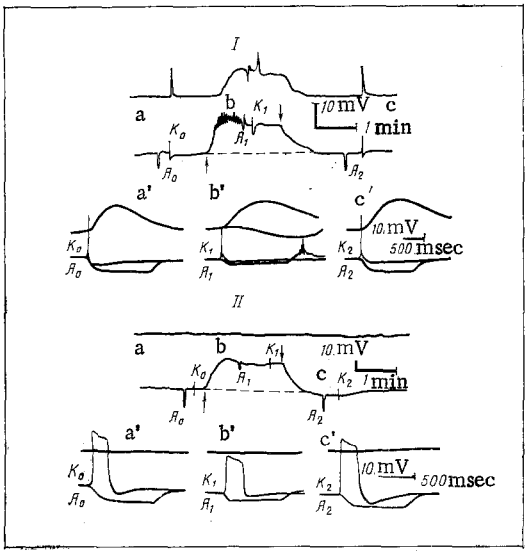

Fig. 3. Action of polarizing current and potassium depolarization on electrical and contractile activity of anococcygeus SMC under normal conditions (I) and with Cd^{++} in external solution (II). I, IIa, a') action of anode and cathode of polarizing current on muscle cells under normal conditions and in Ringer-Locke solution containing 1 mM Cd^{++}. I, IIb, b') during the action of potassium depolarization on SMC under normal conditions and with the addition of Cd^{++} to Ringer-Locke solution. I, IIc, c') electrical and contractile activity of SMC during action of polarizing current after removal of potassium depolarization.

Ringer-Locke solution in the presence of Cd^{++}, just as under normal conditions (Ib), caused membrane depolarization (IIb). Under these circumstances, however, neither AP generation nor contraction of the strip was observed. Application of the depolarizing current in this case, both before potassium depolarization (Fig. 3, IIa, a') and during its course (Fig. 3, IIb, b'), was not accompanied by AP and phasic contractions of the muscle cells. The resistance of the SMC membrane was reduced during potassium depolarization, just as under normal conditions.

The results thus suggest that the contractile system of the anococcygeus SMC is activated in two ways: by the AP and by steady membrane depolarization.

The fact that under the influence of Mn^{++} and Cd^{++}, which block the calcium current, both AP and phasic contraction are inhibited indicates that the AP are calcium in nature and that phasic contraction are evoked by the same Ca^{++} ions which participate in AP generation and enter the muscle cells through fast potential-dependent Ca-channels. Since Cd^{++} blocks tonic contraction but reversibly, it can be postulated that the tonic components of contraction is also activated by extracellular Ca^{++} ions which, however, enter the muscle cells through the so-called slow potential-dependent Ca-channels, which are activated by slow depolarization of the muscle cells.

Hence, both phasic and tonic contraction in the anococcygeus muscle cells are evoked mainly by extracellular Ca^{++}.

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EFFECT OF DESYMPATHIZATION ON PLATELET AGGREGATION AND BLOOD COAGULATION POTENTIALS IN RATS

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UDC 612.115-06:612.89

KEY WORDS: adhesion and aggregation of platelets; desympathization; hemostasis.

During formation of a blood clot, besides plasma components of blood coagulation cells also take part, principally the blood platelets. Adhesion and aggregation of platelets under the influence of collagen, thrombin, adrenalin, prostaglandins, ADP, and certain other agents can create a basis for the formation of an intravascular thrombus [9, 10]. There is no doubt that the nervous system also participates in the regulation of intravascular blood clotting. Dysfunction of the sympathetic or parasympathetic nervous system leads to changes in hemostasis [12, 13].

In previous investigations the writers showed that an experimental model of intracardiac thrombosis can be created [14]. This model was obtained in rats deprived of their sympathetic peripheral innervation since the time of birth. In stress situations the desympathized animals died, due to the formation of large thrombi in the chambers of the atria [6, 13, 14].

The object of the present investigation was to study the effect of chemical desympathization on platelet aggregation and the role of platelets in thrombus formation in desympathized rats.

EXPERIMENTAL METHOD

Noninbred rats were desympathized with the aid of guanethidine. If this substance is injected into newborn animals, they develop irreversible degeneration of sympathetic ganglion cell bodies [8, 14]. The following groups of animals were used: 1) partially desympathized rats receiving guanethidine for two weeks after birth. About 25% of uninjured neurons remained in the stellate ganglia of these animals; 2) rats with complete desympathization, receiving guanethidine for four weeks after birth. Only 0.5% of neurons were preserved in the stellate ganglia of these animals [3, 14]. ADP-induced aggregation was determined in both groups of rats on reaching the ages of 1.5, 2.5, and 4 months, and thrombin-induced aggregation was studied in the completely desympathized animals (CDS) only, at the ages of 1.5 and 2.5 months.

Blood for investigation was taken from the jugular vein and treated with 3.8% sodium citrate solution in the ratio of 9:1. The aggregating power of the platelets was determined by the method described in [7]. ADP (Reanal, Hungary) or thrombin (Sigma), in a final concentration of 10 μM and 0.1 unit, respectively, were used to induce aggregation. The recalcification time of the animals' blood was determined with the N333 coagulograph, and the thrombin time and adrenalin concentration were also measured [5]. The sensitivity of the vas deferens to adrenalin also was investigated by determining the apparent dissociation constant (K) of the adrenalin–adrenoreceptor complex, which is the reciprocal of sensitivity [1].

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 395-397, April, 1980. Original article submitted April 20, 1979.

0007-4888/80/8904-0405$07.50 © 1980 Plenum Publishing Corporation