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FAST CALCIUM ENTRY ACTIVATION IN DENERVATED SMOOTH MUSCLE OF THE CAT NICTITATING MEMBRANE

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UDC 612.731.06:612.81.089.64].019:599.742.27

KEY WORDS: denervation; hypersensitivity; action potential; calcium channels; calcium channel blockers

Severing the connection between efferent neurons and the target cell leads to structural and functional changes and, in the case of smooth-muscle cells (SMC), to changes in the contractile system and excitable membrane. Similar changes are found in tonic fibers of striated muscle after denervation [5, 9]. In SMC, just as in many other objects (neurons, glands, striated muscle) denervation leads to increased sensitivity to neurotransmitters and hormones [8]. It was shown previously [1] that, besides causing a shift of the dose — effect curve and of expression of the contractile and membrane response, the denervated SMC of the cat nictitating membrane can generate action potentials (AP). Generation of agonist-mediated AP is not characteristic of innervated SMC of this particular object, and this makes them similar to SMC of large arteries, whose work is concerned with pharmacomechanical coupling between excitation and contraction (E–C). Meanwhile we know that a large group of SMC (in the intestine and ureter) generate AP in the innervated state and have an electromechanical type of E–C coupling [3, 6]. Denervation evidently leads to a change not only of sensitivity, but also of E–C coupling. This explains the importance of a study of the ionic mechanisms of AP generated in SMC with the pharmacomechanical type of E–C coupling after denervation.

EXPERIMENTAL METHOD

Experiments were carried out on the inferior smooth muscle of the cat nictitating membrane, isolated in noninbred animals weighing 2.5 kg. A surgical denervation model was used. After isolation of the nictitating membrane, the lower layer of the smooth muscle was carefully dissected free from the connective tissue and cut into strips 20 mm long and 0.5 mm wide. After isolation the strips were incubated in Krebs' solution (in mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, glucose 11.5 (pH 7.4); 95% O_2 and 5% CO_2. Contraction was recorded by a mechanical to electrical transducer of original design [2]. An isotonic sucrose solution was prepared by dissolving chemically pure sucrose in bidistilled water, and hyperpotassium solutions were prepared by adding the dry salt to Krebs' solution. Calcium-free solutions contained 2 mM EGTA and 10 mM $MgCl_2$ to stabilize the cell membrane. Replacement of calcium by barium was carried out on an equimolar basis. Sodium was replaced by Tris-HCl. In experiments to study the effect of inorganic calcium blockers, the phosphate-bicarbonate buffer was replaced by HEPES (10 mM). Noradrenalin (NA) was obtained from Merck, West Germany, nifedipine from Arzneimittelwerke, East Germany, and Tris and HEPES from Sigma, USA.

Central Research Laboratory, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 579-580, May, 1989. Original article submitted January 14, 1988.

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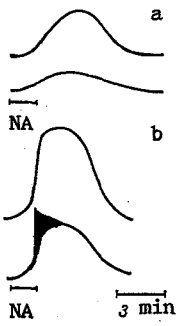

Fig. 1. Contractile (top curves) and membrane (bottom curves) responses of innervated smooth muscle of cat nictitating membrane to application of 100 μM NA (a) and smooth muscle of cat nictitating membrane after denervation to application of 0.1 μM NA (b).

EXPERIMENTAL RESULTS

A characteristic feature of innervated SMC of the cat nictitating membrane was the development of a maximal contractile response to NA in a concentration of 100 μM without any significant change of membrane potential and AP generation (Fig. 1a). Depolarization of the cell membrane by high potassium chloride concentrations (120 mM) led to AP generation on the ascending phase of the slow depolarization wave. Tetraethylammonium in a concentration of 10 mM led to membrane depolarization and AP generation. Phasic contractions corresponded to single AP.

After surgical denervation, at all times of the investigation spontaneous AP generation was observed in SMC of the cat nictitating membrane. NA, in a concentration of 0.1 μM, and potassium chloride, in a concentration of 30 mM, led to AP generation on the ascending phase of the slow depolarization wave (Fig. 1b). The ionic nature was assessed on the basis of criteria suggested previously [17].

The amplitude of AP depended on the extracellular calcium concentration (Fig. 2). In potassium-free solution no AP were generated, but they could be if the calcium was replaced by barium (Fig. 3). The amplitude of spontaneously generated AP was potentiated by the addition of barium chloride to normal Krebs' solution. Addition of barium led to AP generation in innervated SMC, evidently due to the higher penetrating power of barium ions through calcium channels [4]. Replacement of sodium in the external solution did not change evoked AP generation.

Organic and inorganic blockers of voltage-dependent calcium channels inhibited AP, whether spontaneously generated or evoked by NA or potassium chloride. Verapamil, in a concentration of 10 mM, inhibited spontaneous activity and reduced the frequency of AP generation, evoked by NA and potassium chloride. Complete blockage of spike activity was observed after incubation for 15-20 min with the blocker. This concentration of verapamil inhibits the phasic and tonic components of contraction evoked by potassium chloride.

Nifedipine, as a more selective blocker of voltage-dependent calcium channels, blocked AP without going through the phase of reduction of the frequency of AP generation. The effective concentration of nifedipine was 0.1 μM. Inorganic calcium blockers (in our experiments, Mn and Ni) blocked AP in a concentration of 2 mM without changing the amplitude of slow depolarization evoked by NA.

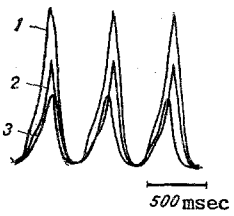

Fig. 2

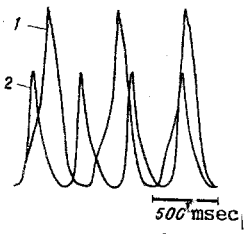

Fig. 3

Fig. 2. Dependence of amplitude of spontaneous AP on extracellular calcium concentration. 1) 2.5 mM, 2) 1.2 mM, 3) 0.7 mM.

Fig. 3. AP generation following replacement of extracellular calcium by barium. 1) 2.5 mM Ca + 0 Ba; 2) 1.0 mM Ba + 0 Ca.

AP generation in the innervated strips in the presence of high potassium chloride concentrations and during blockade of potassium conductance by tetraethylammonium, probably through lowering of the membrane potential, confirms the presence of a fast calcium entry system in SMC with a pharmacomechanical type of E–C coupling, and that the conditions of its possible activation are controlled by the sympathetic nervous system.

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