EFFECT OF CALCIUM, BARIUM, AND MANGANESE IONS ON ELECTROPHYSIOLOGICAL PROPERTIES OF SMOOTH-MUSCLE CELLS OF THE PORTAL VEIN

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Smooth muscle cells (SMC) of the rat portal vein were studied by the double sucrose bridge technique. Replacement of calcium (Ca^{++}) by barium (Ba^{++}) ions in equimolar amounts caused depolarization, a marked increase of tone, and an increase in the frequency of spontaneous electrical activity of the SMC. The effect of a polarizing current was weakened. The addition of 0.3 mM Ca^{++} to calcium-free Krebs' solution did not lead to significant changes in the electrical and contractile properties of the SMC. Rinsing the SMC with Krebs' solution containing 0.3 mM Ba^{++} was followed by slight depolarization and an increase in tone of the SMC. The response of the SMC to a polarizing current differed only a little from normal. The addition of 1.25 mM Ba^{++} to Krebs' solution containing 1.25 mM Ca^{++} caused hyperpolarization, a decrease in tone, an increase in the frequency and amplitude of the spontaneous electrical activity and phasic contractions, an increase in evoked activity, and a very small increase in resistance of the membrane. The addition of manganese ions to Krebs' solution, whether with Ca^{++} or with Ba^{++}, caused complete inhibition of the electrical and contractile responses of the SMC.

Evidence has now been obtained that calcium ions (Ca^{++}) not only participate in the control of membrane conductance of smooth muscle cells (SMC) of blood vessels, but possibly they also actively generate excitation [3, 4, 6]. There is evidence in the literature that the Ca^{++} ions in SMC are evidently not specific and can be replaced to some extent by other ions of the alkaline earth metals, such as barium (Ba^{++}) and strontium (Sr^{++}) ions [2, 5, 8].

For these reasons it was decided to investigate the effects of Ca^{++}, Ba^{++}, and manganese (Mn^{++}) ions on the electrophysiological properties of SMC in the portal vein.

EXPERIMENTAL METHOD

Isolated strips of rat portal vein 15-20 mm long were used as the test object. Experiments were carried out by the double sucrose bridge technique [1] in Berger's modification [7]. Contractions were recorded from the testing part of the chamber with the aid of a type 6MKhIS mechanotron. The testing solutions used for these investigations were described previously [4]. To study the effect of Mn^{++} which, as several workers have shown, blocks the calcium channels [2, 8], $MnCl_2$ was used in a concentration of 0.5 mM. To study the specific effects of Ca^{++} on SMC, Ca^{++} was replaced by Ba^{++}. Different concentrations of Ba^{++} (2.5, 1.25, 0.3 mM) were used in the experiments. The temperature of the testing solutions was kept constant at +35°C.

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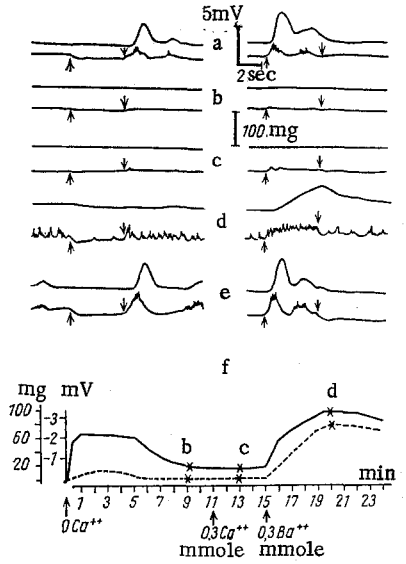

Fig. 1. Effect of calcium-free solution (0 Ca^{++}) and of 0.3 mM Ca^{++} and 0.3 mM Ba^{++} in Krebs' solution on SMC in the portal vein: a) normal, b) after action of 0 Ca^{++} for 9 min; c) after action of 0.3 mM Ca^{++} for 4 min; d) after action of 0.3 mM Ba^{++} for 5 min; e) rinsing SMC with normal Krebs' solution; f) graph showing changes in membrane potential and tone of SMC during this experiment. In this and subsequent figures the left half shows electrical and mechanical activity in response to a hyperpolarizing current; the right half the same to a deep polarizing current. Top line – contractile; bottom line – electrical activity of SMC. Continuous line on graph represents membrane potential, broken line tone of SMC. Strength of current 0.5 μA.

EXPERIMENTAL RESULTS

As in the previous investigation [4], removal of Ca^{++} from the Krebs' solution caused depolarization of the membrane and inhibited the spontaneous electrical and contractile activity of the SMC. Inhibition of evoked electrical and contractile activity and a decrease in the membrane resistance (the development of anelectrotonus) also were observed under these conditions (Fig. 1a, b).

The action of 2.5 mM Ba^{++} in Krebs' solution in the absence of Ca^{++} on the SMC is shown in Fig. 2A. Clearly the action of Ba^{++} was accompanied by rapid depolarization of the membrane and a marked increase in tone of the SMC, reaching a maximum during the first minutes of action of the Ba^{++} (Fig. 2A, g). The subsequent action of Ba^{++} led to a very small decrease in membrane potential followed by its stabilization at a certain level. The tone of the SMC under these circumstances was almost unchanged, but after 8-9 min of exposure of the SMC to Ba^{++} a decrease in general tone was observed although there was no change in the membrane potential at this time. The spontaneous electrical activity diminished during the action of the solution with Ba^{++}, and the amplitude of the spikes fell sharply. The action of a depolarizing current during the first 6 min of action of the Ba^{++} solution on the SMC caused a decrease in spontaneous electrical activity, accompanied by marked phasic contractions which were more prolonged but shorter in amplitude than normally (Fig. 2A, b, c). At this time the action of the hyperpolarizing current was accompanied by the development of slight anelectrotonus and considerable relaxation of the SMC (Fig. 2A, b, c). During exposure of the SMC to Krebs' solution with Ba^{++} the effect of the polarizing current weakened. By about the 10th minute of exposure to Ba^{++}, for instance, the depolarizing current evoked an almost imperceptible change in spontaneous electrical activity, accompanied by an ill-defined contraction. In that case, although the hyperpolarizing current did not induce the development of anelectrotonus, nevertheless inhibition of spontaneous electrical activity was observed and the response of relaxation of the SMC was less marked in this case than at the beginning of action of Ba^{++} (Fig. 2A, d). Rinsing the SMC with normal Krebs' solution led to restoration of the membrane potential, the tone of the SMC, and also their response to the action of the polarizing current (Fig. 2A, e, f, g). Subsequent tests showed that this powerful excitatory action of Ba^{++} is reduced only if the ratio between Ba^{++} and Ca^{++} has a certain value, or with a decrease in the Ba^{++} concentration in the solution.

As Fig. 2B shows, the action of Krebs' solution with 1.25 mM Ca^{++} and 1.25 mM Ba^{++} was accompanied by an initial decrease in membrane potential and an increase in the general tone of the SMC, reaching maximal values after exposure to this solution for about 2-3 min (Fig. 2B, e). However, subsequent exposure of the SMC to solutions containing Ca^{++} and Ba^{++} led to a marked decrease in the tone and to hyperpolarization. An increase was observed in the amplitude and duration of the spontaneous electrical and contractile activity (Fig. 2B, a, b). The action of a depolarizing current caused an increase in the frequency of spontaneous electrical activity, accompanied by the high-amplitude phase of the contractile response of the SMC. Stopping the current did not inhibit spontaneous electrical activity. The action of the hyperpolarizing current led to the development of anelectrotonus of slightly larger amplitude than normally although it was not accompanied by relaxation of the strip. Stopping the hyperpolarizing current led to a marked anode-breaking response with a rather longer latent period than normally, and this also was accompanied by a high-amplitude contractile response of the SMC (Fig. 2B, c, e). Rinsing the SMC with normal Krebs' solution led to restoration of the electrical and contractile properties of the SMC (Fig. 2B, d, e).

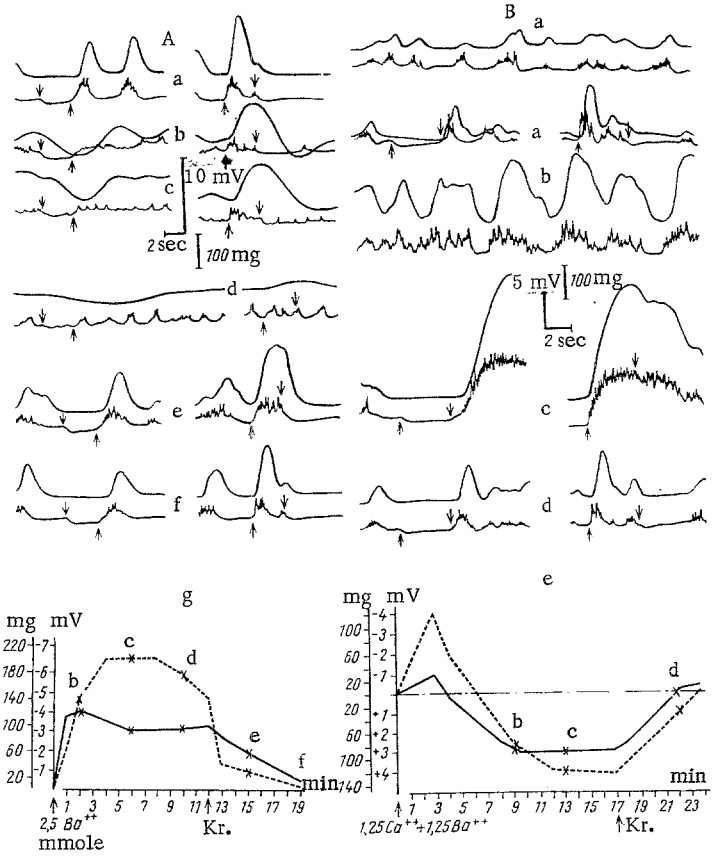

Fig. 2

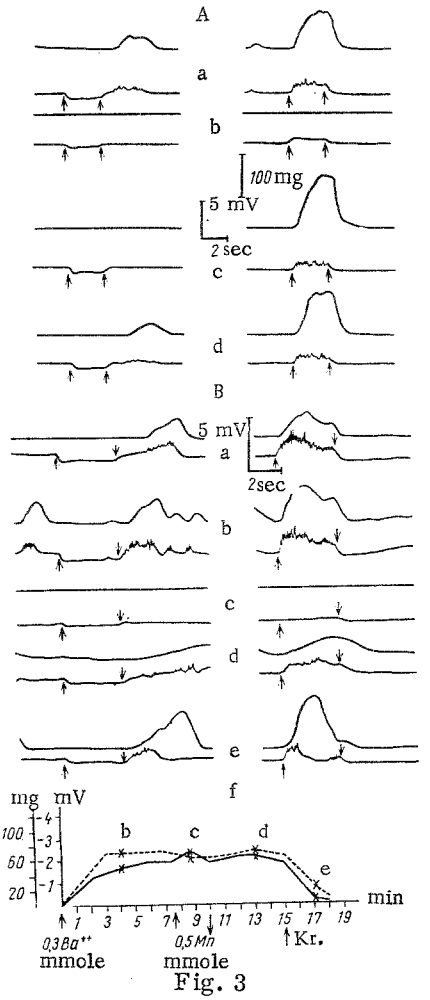

Fig. 3

Fig. 2. Effect of 2.5 mM Ba^{++} (A) and 1.25 mM Ca^{++} + 1.25 mM Ba^{++} (B) in Krebs' solution on SMC of the portal vein. A: a) normal; b, c, d) after the action of 2.5 mM Ba^{++} for 2, 6, and 10 min respectively; e, f) after rinsing SMC with normal Krebs' solution for 3 and 7 min, respectively. B: a) spontaneous electrical and contractile activity of SMC under normal conditions; b and c) the same after exposure for 9 and 13 min respectively to 1.25 mM Ca^{++} + 1.25 mM Ba^{++}; d) after rinsing SMC with normal Krebs' solution for 5 min. Graphs below show changes in membrane potential and tone of SMC during experiments. Strength of current 0.8 μA.

Fig. 3. Effect of 0.5 mM Mn^{++} in Krebs' solution on SMC of the portal vein: A) normal Ca^{++} concentration, B) Ca^{++} replaced by Ba^{++} (0.3 mM Ba^{++}). A: a) normal; b) after exposure to 0.5 mM Mn^{++} for 2 min; c and d) after rinsing SMC with normal Ringer-Locke solution for 3 and 6 min, respectively. B: a) normal; b) after exposure for 4 min to 0.3 mM Ba^{++} instead of Ca^{++}; c) after exposure to Mn^{++} for 1 min; d) after exposure to Krebs' solution without Mn^{++} for 3 min; e) after rinsing SMC with normal Krebs' solution for 2 min; f) graph showing changes in membrane potential and tone of SMC in this experiment. Strength of current 0.5 μA.

Investigation of the effect of minimal concentrations of Ba^{++} gave the results shown in Fig. 1d, f and Fig. 3B, b, d, f. As will be clear from Fig. 1c, the addition of 0.3 mM Ca^{++} to the calcium-free Krebs' solution did not cause significant changes in the membrane potential and tone of the SMC, disregarding the appearance of a weak electrical response to the action of the depolarizing current, unaccompanied by contraction. Meanwhile replacement of the 0.3 mM Ca^{++} in the Krebs' solution by 0.3 mM Ba^{++} caused frequent spontaneous electrical activity, stable depolarization of the membrane, and an increase in tone of the SMC (Fig. 1f and Fig. 3B, f); the action of the depolarizing current under these circumstances was accompanied by an increase in the frequency of spontaneous electrical activity and a marked contractile response, whereas exposure to the hyperpolarizing current led to slowing of spontaneous electrical activity, the development of anelectrotonus, and slight relaxation of the SMC. Stopping the current was followed by an anode-breaking response (Fig. 1d and Fig. 3B, b).

Since in calcium-free Krebs' solution Ba^{++} ions in a certain concentration maintained electrical and contractile activity of the SMC this suggested that Ca^{++} ions are not strictly specific. This hypothesis also was confirmed by the experiments to study the action of Mn^{++} on SMC.

The experiments with Mn^{++} showed that, just as in the tests with Krebs' solution containing Ca^{++} (Fig. 3A, b), the addition of Mn^{++} to the Ba^{++} solution led during the first few minutes to complete inhibition of the electrical and contractile responses of the SMC (Fig. 3B, c). Removal of Mn^{++} from the Krebs' solution restored the excitability of the SMC (Fig. 3B, d). Exposure of the SMC to Krebs' solution with a normal concentration of Ca^{++} completely restored the electrical and contractile properties of the SMC of the portal vein (Fig. 3B, e, f).

Consequently, Ba^{++} can not only replace Ca^{++} in the processes responsible for electrical and contractile activity of the SMC, but it can also evidently utilize the same channels for this purpose as Ca^{++}.

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