109. *Electrical Phenomenon in Rabbit Papillary Muscle during Ba⁺⁺-Induced Contracture*

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There is numerous evidence that the barium ion (Ba⁺⁺) has similar electrical and mechanical actions as the calcium ion (Ca⁺⁺) on various types of muscle.¹⁾⁻³⁾ However, Ba⁺⁺-induced contracture, which has recently been used for the study of mechanical properties of heart muscles,⁴⁾ has never been analyzed from the view point of electrical-mechanical interaction. In this study, the effects of Ba⁺⁺ on the action potential and contraction were examined in order to obtain information about the mechanism of Ba⁺⁺-induced contracture in rabbit papillary muscle.

Methods. Right ventricular papillary muscles were obtained from 7 rabbits (1.5–2 kg in body weight). Immediately after the chest cavity was opened, the beating heart was quickly removed and placed in a dissection bath containing Tyrode's solution. The right ventricle was cut open to expose the papillary muscle and select a long and thin muscle appropriate for the study. The muscle was tied at the tendinous and ventricular wall end with 6-0 surgical silk thread. It was then excised from the ventricle and transferred to a small lucite chamber. The nontendinous end was mounted on a cork block with stainless steel pins. The tendinous end of the preparation was tied to a tungsten wire. The other end of this wire was connected to an isometric tension transducer (Kulite semiconductor BG-50) on a micromanipulator which could be moved to adjust the muscle length. The preparation, thus mounted in the lucite chamber, was superfused with Tyrode's solution gassed with a mixture of 98% O₂ and 2% CO₂ at a temperature of 24°C. The muscle was stimulated with platinum plate electrodes by using electrical pulses of 5 msec duration and a voltage of about 50% above threshold. The stimulation rate was 30/min. The action potentials were obtained using a conventional microelectrode technique. Together with tension they were recorded simultaneously on a pen-recorder (Watanabe, Linearcorder Type WTR 331) and an oscilloscope (Tektronix Type 565).

All muscles were studied after a stabilization period of more than 2 hr, during which the muscle was loaded with a resting ten-

sion of about 0.5 g and stimulated at a rate of 30/min under isometric condition. Throughout the experiments, the muscle remained in the constant length at which the twitch tension was maximum.

Results. To obtain a steady contracture with no spontaneous twitch, the muscle was first incubated in a Ca^{++} -free Tyrode's solution.⁴⁾ After the twitch contraction had been markedly diminished, an amount of BaCl_2 was added to the Ca^{++} -free Tyrode's solution so that the required concentration of Ba^{++} could be attained. Fig. 1 shows a representative action potential change and tension change which were observed during the process of inducing Ba^{++} -contracture.

Fig. 1. Electrical and mechanical events during the process to induce Ba^{++} contracture. See text for explanation.

Fig. 2. Relationship between external Ba^{++} concentration and the strength of contracture. Contracture tension is expressed as the percent of twitch tension at a stimulation rate of 30/min in normal Tyrode's solution. Three different symbols represent the data obtained from three different preparations.

Immediately after the solution was changed from a normal Tyrode's solution to a Ca^{++} -free Tyrode's solution, twitch tension began to decrease and continued to do so. The resting potential and the duration of action potential decreased concurrently with the decrease of twitch tension. After 18 min of superfusion in Ca^{++} -free Tyrode's solution the resting potential decreased from -84 mV (resting potential in normal Tyrode's solution) to -65 mV. Shortly after the Ba^{++} was added to the Ca^{++} -free Tyrode's solution, the muscle started to produce a contracture. During the rising phase of contracture spontaneous small twitches were superimposed on the contracture in most of the preparations studied, as shown in Fig. 1. However, the muscle soon lapsed into a state of stable contracture which continued for hours. At this state, the resting potential was markedly decreased (to about -30 mV). No action potential was observed. During the rising phase of contracture spontaneous pacemaker-like action potentials were often observed.

The strength of contracture depends on the concentration of Ba^{++} . Fig. 2 shows the relationship between the external Ba^{++} concentration $[\text{Ba}^{++}]_o$ and the sustained contracture tension obtained from 3 different preparations. The minimum concentration of Ba^{++} needed to produce a contracture was about 0.1 mM. The tension increased with increasing Ba^{++} concentration, and plateaued over a concentration of about 4 mM. The resting potential was depolarized to the level of about -30 mV with the application of 0.1 mM Ba^{++} . With increasing Ba^{++} concentrations, the depolarization level slightly increased (about -20 mV at 5 mM Ba^{++}).

Both of these electrical and mechanical events induced by Ba^{++} were reversed when the bath solution was switched back to the normal Tyrode's solution.

When 0.5 mM manganese (Mn^{++}) or 4 $\mu\text{g}/\text{ml}$ verapamil was added to Ba^{++} -Tyrode's solution, in which the muscle was in a state of steady contracture, the muscle tension immediately started to relax toward the resting level. However, the resting potential showed almost no change.

When Ba^{++} was added to the normal solution (which contains Ca^{++}), the strength of contracture induced by Ba^{++} was greatly suppressed and always accompanied by spontaneous pacemaker-like action potentials and twitches.

Discussion. The results described above indicate that Ba^{++} has two remarkable actions: The activation of contractile system and the depolarization of membrane potential. The former suggests that Ba^{++} enters the cell across the membrane and acts upon the contractile system. This possibility is supported by the direct finding⁵⁾ that

in the rabbit ventricle there was a clear accumulation of Ba^{++} within the tissue during the Ba^{++} -induced contracture. According to Ebashi and Endo's report,⁶⁾ Ba^{++} can interact with cardiac troponin, though it is 32 times less sensitive to Ba^{++} compared to Ca^{++} . In the absence of Ca^{++} , Ba^{++} can substitute for Ca^{++} in activating the contractile system.

The depolarization of membrane potential was previously reported to be primarily due to the decrease of K^+ conductance.^{1),2)} This explains well the significant depolarization when Ca^{++} in the normal Tyrode's solution was replaced by Ba^{++} . Although the inward depolarizing current could be carried by Ba^{++} , it may not contribute to the depolarization. This is supported by the finding that Mn^{++} and verapamil, which are known to block the influx of Ba^{++} ,¹⁾ produced the relaxation of contracture, but did not show any statistical change in membrane potential. The fact that there was still marked depolarization, in spite of the nearly complete relaxation of contracture after the application of Mn^{++} or verapamil, seems to suggest that the mechanism of producing the depolarization is not related to that of the Ba^{++} influx across the membrane. Furthermore, Ba^{++} influx may be able to take place without the presence of action potential since the contracture continued to develop gradually even after twitch contraction had completely disappeared (Fig. 1).

The present observation that Ba^{++} produced pacemaker-like action potentials in the Ca^{++} containing solution is consistent with the findings in cat papillary muscle.³⁾ This suggests that Ca^{++} and Ba^{++} act antagonistically on the change of K^+ permeability since Ca^{++} is known to increase K^+ conductance.⁷⁾

Since muscle in Ba^{++} contracture has already been proved to have different mechanical characteristics from those of muscle in the rigor,⁴⁾ such a long-lasting Ba^{++} contracture is worthy of further study, particularly in regard to their relationship to the Ca^{++} sequestering system such as the sarcoplasmic reticulum and mitochondria.

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