Separable Spike and Plateau Action Potentials and their Roles in Contraction of Frog Ventricle* (34123)

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Evidence has accumulated which shows that the cardiac action potential is composed of fast spike and slow plateau components (1). Recently, Paes de Carvalho, Hoffman, and Langan (2) pointed out that the slow component can exist and propagate even when the fast component seems to be absent. In terms of ionic movements across the cell membrane (3), the fast spike is considered to be a Na^+ action potential and the slow plateau is mainly ascribable to a decrease in K^+ conductance of the membrane. However, there is also considerable literature indicating that in cardiac muscle a part of the membrane current during depolarization is carried by Ca^{2+} ions (4, 5). Recent studies conducted with myocardial cells of warm- and cold-blooded animals by means of current- or voltage-clamp methods strongly support this view (6). The present article will also support these concepts and bring out the point that the spike potenial triggers the plateau response which may relate to Ca^{2+}-influx, Ca^{2+}-release, and consequent contraction of the myocardium.

Methods. A small muscle strip (about 1 × 3 mm) dissected from the lower half of the frog ventricle and held at room temperature (22–25°) was used in these experiments. The membrane potential, applied depolarizing current, and tension were recorded by means of a two-compartment chamber method similar to that employed by Kamiyama and Matsuda (7). The membrane potential was measured between an intracellular and extracellular microelectrode. Only cells located within 0.1 mm from the barrier plate were penetrated. This permitted observation of maximum potential change in the membrane as well as preventing dislodgement of the intracellular microelectrode by muscle contraction. Tensions developed in the muscle were recorded by means of a strain gauge (Grass, FT10C). The rather massive depolarizing currents (0.01–1.2 mA) were passed between large Ag–AgCl electrodes at either end of the chamber, and the responses were examined before and under the action of tetrodotoxin, Mn^{2+}, and hypertonic Ringer solution containing glycerol, which are known to inhibit the Na^+ permeability (8), Ca^{2+}-influx (5, 9), and function of transverse tubules (11, 12) respectively.

Results. Administration of Mn^{2+} (10–20 m*M*) produced first a gradual diminution of contraction and of rate of rise of plateau potential as expected (5). Next, a sudden disappearance of the plateau as well as the contraction occurred, leaving solely a spike potential behind. Strengthening of the massive depolarizing current, however, could still elicit an all-or-nothing type of plateau and contraction (Fig. 1A and B). Although the contractility was markedly depressed at this stage, it could be seen that twitch contraction occurred only with appearance of the plateau. Existence of two different thresholds, one for spike generation and the other for plateau potential, was demonstrated and can be seen most clearly in Fig. 1C, D, and E. When the membrane was depolarized to a critical level (—50 ~—55 mV), first the spike potential alone was generated; when the spike potential was elevated by stronger depolarizing current reducing membrane potential to about —5~0 mV, the plateau re-

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sponse was triggered. These two critical potentials were always observed not only in case of gradual strengthening of the current from a subthreshold level (Fig. 1A–D) but also in case of weakening from a suprathreshold level (Fig. 1E). Thus, arbitrary separation and reunification of the spike and plateau potentials was possible and repeatable as many times as wanted.

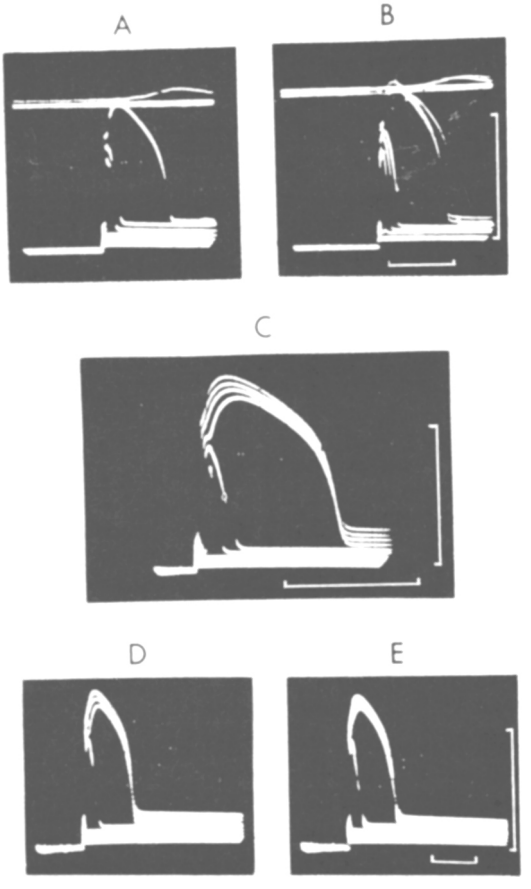

FIG. 1. Separated spike and plateau responses of the frog ventricle under the effects of 10 m*M* Mn^{2+}. Depolarizing currents of 4-sec duration and different intensities were applied at 10-sec intervals using the separation chamber method. Each figure shows superimposed records of more than 10 successive responses. A and B: Simultaneous recordings of tension (upper tracings) and membrane potential (lower tracings); note that contraction occurred only with appearance of the plateau. C, D, and E: Records of transmembrane potentials, showing two thresholds, one for spike generation and one for the plateau response. Two thresholds were always seen not only in case of gradual strengthening of depolarizing current (A–D), but also in case of gradual reductions from maximum (E). Note slow rate of rise of the plateau potential. Calibrations are 100 mV and 1 sec.

Tetrodotoxin (10^{-7}–10^{-6} g/ml) was found to eliminate this spike component of the response. When the same concentration of tetrodotoxin was applied without Mn^{2+}, first a decrease in rate of rise of the spike potential (5) and then a marked elevation of threshold occurred, and finally, an elimination of all-or-nothing membrane behavior. A partially graded slow plateau-like response could still be produced by application of strong depolarizing currents. Similar graded slow responses were observed in a Na^+-deficient and Ca^{2+}-excess solution. Thus, it became clear that the spike is definitely a Na^+ action potential while a part of the plateau is a non-Na^+, probably a Ca^{2+} potential. It must be noted, however, that even under the influence of a high concentration of Mn^{2+} the main part of plateau remained unchanged (Mn^{2+}-insensitive part, the plateau response in Fig. 1) although the initial and terminal parts were greatly inhibited (Mn^{2+}-sensitive part). The rising phase of the plateau was slowed markedly (5) and prolongation of the falling phase, which is commonly produced by application of a long depolarizing pulse (10), disappeared.

Howell and Jenden (11) and Eisenberg (12) demonstrated that on the frog "skeletal" muscle fibers a Ringer solution containing 400 m*M* glycerol selectively destroyed the transverse tubules, and subsequently Gage and Eisenberg (13) showed that this solution eliminated contractility as well as the after-negativity of the action potential without affecting its spike potential. Therefore, we attempted to determine how the same solution would affect the excitation-contraction coupling events of the myocardium in the presence or absence of Mn^{2+} (10 m*M*), hoping that Mn^{2+} ions would eliminate the Ca^{2+}-influx through the surface cell membrane and that the hypertonic solution would eliminate the tubular function, if such existed.

The hypertonic solution without Mn^{2+} caused a conspicuous shortening and lowering of the plateau which resulted in a short triangular action potential, by affecting the main part of plateau potential, and caused a corresponding shortening and depression of contractility. These findings were consistent with those of earlier workers (14) who conducted studies with sucrose or NaCl hypertonic solutions. In the glycerol hypertonic Ringer solution, however, neither complete elimination of the plateau nor of contractility was possible without Mn^{2+} even after 90-min immersion or after rapid washing with normal Ringer solution. The washing even produced a complete or considerable recovery of the plateau potential and contractility respectively. Thus, the cardiac muscle appeared markedly different in character from skeletal muscle, in which destruction of the tubular system (11, 12) as well as uncoupling of the excitation and contraction (13) are known to occur merely after reimmersion of the muscle in normal solution. The disparity in results is probably explained by the ultrastructure of the frog ventricle which is significantly different from that of the skeletal muscle; there is a paucity of transverse- and sarcotubules in frog ventricle despite the abundance of intercellular clefts (15).

The hypertonic solution with Mn^{2+} could produce a marked elevation of thresholds and even a complete elimination of the plateau potential. Typical results are illustrated in Fig. 2, in which the effects of hypertonic Ringer solution containing 400 m*M* glycerol were examined in the constant presence of 5 m*M* Mn^{2+}. Figure 2A, B, and C shows a series of recorded tracings which were obtained successively after the addition of hypertonic solution. After a marked shortening of the plateau and appearance of a short triangular action potential due to the hypertonicity, a distinct notch in the rising phase of action potential was produced initially (Fig. 2A). Next, the shortened plateau decreased in size and suddenly disappeared, leaving the first spike component almost unchanged (Fig. 2B), Thus, the hypertonic solution with Mn^{2+} appeared to eliminate all of the slow plateau components. However, when the intensity of depolarizing current was greatly increased, the plateau response was found still elicitable in this solution (Fig. 2C). Records in the lower row of Fig. 2 show similar but more marked effects of a hypertonic Ringer solution containing 800 m*M* glycerol and 10 m*M* Mn^{2+}. As before, a partial separation of the spike from the plateau (Fig. 2D), generation of isolated spike with weak depolarizing currents and of spike and plateau separated by a distinct dip when stronger current was applied (Fig. 2E) were observable. The deepness of the dip between spike and plateau was difficult to explain as a membrane phenomenon; however, it was soon recognized that the slow plateau-like response contained a spike component in its rising phase as indicated by the arrow (Fig. 2E). Thus, it became apparent that the first spike failed but that this second spike, summating with the falling phase of the first, was able to generate the real plateau response. Finally, prolonged immersion in this solution produced a complete absence of the plateau component even for extremely strong depolarizing pulses (Fig. 2F). Although after long immersion when these strong pulses were applied the generated spike potential itself appeared rather rounded, neither trace of a dip phenomenon nor a sudden addition of plateau to the spike was observed. A weak sustained contraction was produced by the strongest pulse; however, this was not due to the appearance of a plateau potential, but simply to the strong electrotonic depolarization of the membrane, because the tension developed after the action potential had ended and diminished only after cessation of the depolarizing current.

Rapid reintroduction of normal Ringer solution caused a reappearance of the spike potential accompanied by a plateau response, but soon these two components became difficult to separate. Recovery of contractility, however, was only partial, probably because of the destruction of the tubular system or other cellular elements by a marked swelling after shrinkage of individual cells (16).

Discussion. Although electron microscopic examination of change in the ultrastructure

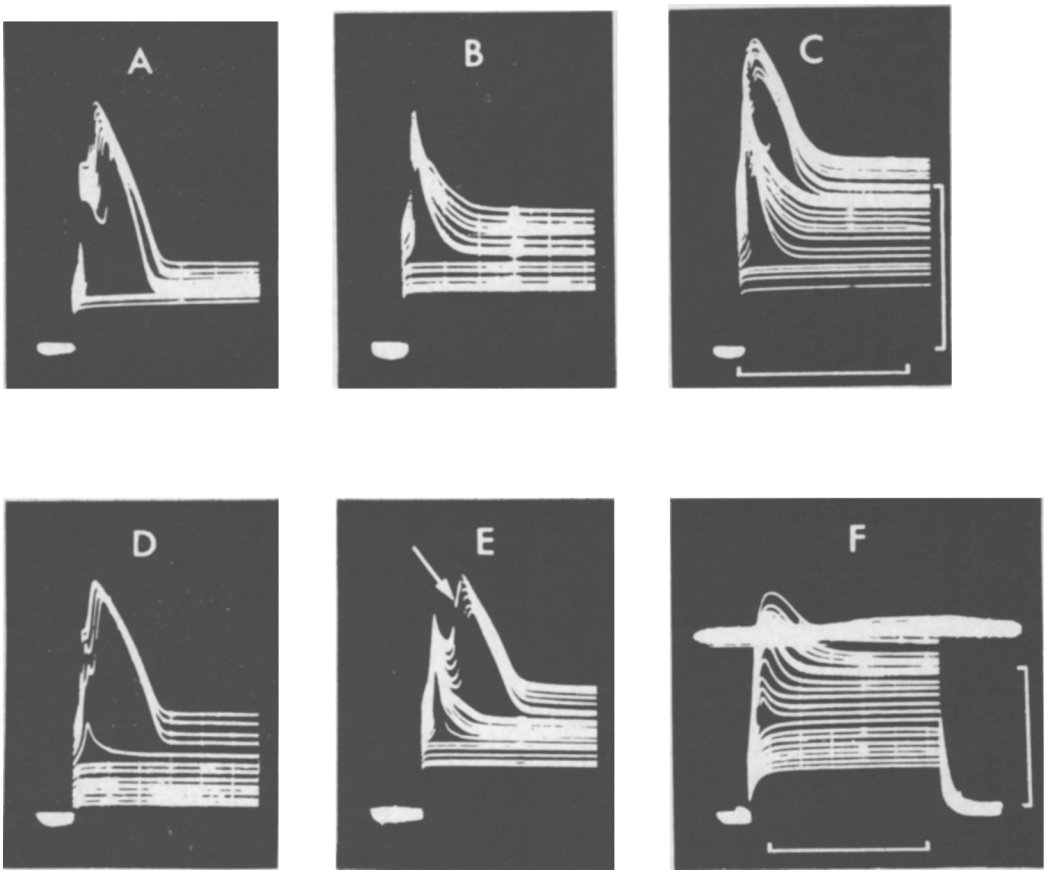

FIG. 2. Isolation of spike potential from plateau response of the frog ventricle in hypertonic Ringer solutions containing glycerol and Mn^{2+}. Experimental procedures the same as in Fig. 1, but responses were elicited with 1.3-sec duration pulses. A, B, and C: A series of records of the membrane potentials obtained 3, 8, and 25 min after addition of hypertonic Ringer solution containing 400 m*M* glycerol in presence of 5 m*M* Mn^{2+}. D, E, and F: The same but at 2, 12, and 25 min after introduction of hypertonic solution which contained 800 m*M* glycerol and 10 m*M* Mn^{2+}. Note the appearance of a distinct notch in the rising phase of the action potential (A and D), isolated spike responses for weak depolarizing pulses (B, C, and E), and spike and plateau responses for strong pulses (C and E). In (F) tension of the muscle is also recorded in upper trace. Further explanations are given in the text. Calibrations, 100 mV and 1 sec.

produced by conditions described is essential to ultimate certainty, the above results lead us to a tentative conclusion, which is as follows. When the membrane is depolarized to a critical level, that of the spike threshold, a Na^+ spike which is sensitive to tetrodotoxin is generated. The sodium-dependence of this initial spike has been demonstrated by others (17). When this spike potential reaches the second threshold, that for plateau generation, the plateau response is triggered. This plateau potential, however, is composed of two subcomponents, Mn^{2+}-sensitive part and Mn^{2+}-insensitive part. The Mn^{2+}-sensitive part can be considered to be due to Ca^{2+}-influx through the surface cell membrane (5), while the Mn^{2+}-insensitive parts might relate to a decrease in K^+ conductance [anomalous rectification (3)], or even to Ca^{2+}-release from some storage sites because it also elicited a contraction and was eliminated by a hypertonic glycerol solution. The existence of two different thresholds after Mn^{2+}, one for spike and the other for plateau gener-

ation, the selective elimination of the spike component by tetrodotoxin and the abolition of the plateau (Mn^{2+}-insensitive part) by the hypertonic solution, which naturally causes a widening of the intercellular clefts, strongly suggest that the spike and plateau originate from different parts of the cell membrane. At any rate, relationships between the Mn^{2+}-insensitive component of the plateau, function of Ca^{2+} storage sites, and decrease in K^+ conductance of the membrane present problems in urgent need of solution.

Summary. The compound nature of the ventricular muscle action potential was demonstrated. Two thresholds, one for spike and the other for plateau generation were observed in the frog ventricles under action of Mn^{2+}, and even more clearly in hypertonic Ringer solutions containing glycerol and Mn^{2+}. Twitch contractions occurred only with appearance of the plateau. Tetrodotoxin eliminated the spike, leaving a plateau-like response behind. The Na^+-spike is thought normally to trigger the plateau response which relates somehow to Ca^{2+}-influx and release and thus to the initiation of contraction.

1. Goto, M., Tamai, T., Abe, Y., and Kawata, H., Kyushu J. Med. Sci. **12**, 177 (1961); Hoshiko, R., and Sperelakis, N., Am. J. Physiol. **201**, 873 (1961); Antoni, H. and Delius, W., Pflügers Arch. Ges. Physiol. **283**, 187 (1965); Matsuda, K., Kamiyama, A., and Hoshi, T., *in* "Electrophysiology and Ultrastructure of the Heart" (T. Sano *et al.* eds.) Bunkodo, Tokyo (1967).

2. Paes de Carvalho, A., Hoffman, B. F., and Langen, W. B., Nature **211**, 938 (1966).

3. Weidmann, S., Elektrophysiologie der Herzmuskelfaser (Huber, Bern, 1956); Hoffman, B. F., and Cranefield, P. F., "Electrophysiology of the Heart", McGraw-Hill, New York (1960); Brady, A. J., and Woodbury, J. W., J. Physiol. **154**, 358 (1960); Noble, D., J. Physiol. **160**, 317 (1962); Deck, R. A., and Trautwein, W., Pflügers Arch. Ges. Physiol. **280**, 63 (1964).

4. Lüttgau, H., *in* "Electrophysiology of the Heart" (B. Taccardi *et al.* eds.) Macmillan (Pergamon), New York (1965); Niedergerke, R., and Orkand, R. K., J. Physiol. **184**, 291 (1966); Niedergerke, R., and Orkand, R. K., J. Physiol. **184**, 312 (1966).

5. Hagiwara, S., and Nakajima, S. J Gen. Physiol. **49**, 793 (1966).

6. Reuter, H., Pflügers Arch. Ges. Physiol. **287**, 357 (1966); Reuter, H., J. Physiol. **192**, 479 (1967); Gargouil, Y-M., Rougier, O., Coraboeuf, E., and Vassort, G., Proc. Intern. Union Physiol. Science **7**, 150 (1968).

7. Kamiyama, A. and Matsuda, K., Jap. J. Physiol. **16**, 407 (1966).

8. Narahashi, T., Deguchi, T., Urakawa, N., and Ohkubo, Y., Am. J. Physiol. **198**, 934 (1960); Narahashi, T., Moore, J. W., and Scott, W. R., J. Gen. Physiol. **47**, 965 (1964); Coraboeuf, E., and Vassort, G., J. Electrophysiol. **1**, 19 (1968).

9. Nonomura, Y., Hotta, Y., and Ohashi, H., Science **152**, 97 (1966).

10. Goto, M. and Brooks, C. McC., Federation Proc. **27**, 303 (1968); Goto, M., and Brooks, C. McC., Proc. Intern. Union Physiol. Science **7**, 164 (1968).

11. Howell, J. N. and Jenden, D. J., Federation Proc. **26**, 553 (1967).

12. Eisenberg, B. and Eisenberg, R. S., Science **160**, 1243 (1968).

13. Gage, P. W. and Eisenberg, R. S., Science **158**, 1702 (1968).

14. Sperelakis, N., Hoshiko, T., Keller, R. F., and Berne, R. M., Am. J. Physiol. **198**, 135 (1960); Goto, M., Abe, Y. and Kawata, H., Kyushu J. Med. Sci. **12**, 187 (1961).

15. Staley, N. A. and Benson, E. S., J. Cell Biol. **38**, 99 (1968); Kisch, B., Exptl. Med. Surg. **19**, 104 (1961); Scheyer, S. C., Anat. Record **136**, 273 (1960).

16. Girardier, L., *in* "Electrophysiology of the Heart" (B. Taccardi *et al.*, eds.) Macmillan (Pergamon) New York (1965).

17. Reuter, H. and Beeler, G. W., Science **163**, 399 (1969).

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