Loss of the Plateau
of the Cardiac Action Potential
in Hypertonic Solutions

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ABSTRACT The effect of hypertonicity on the electrical properties of vertebrate myocardial cells was studied in ventricular muscle fibers of guinea pig, cat, frog, and chicken. The latter two species do not have a T-tubule system, whereas the former two do. In hypertonic solutions (2 X isotonic) produced by addition of sucrose or excess of NaCl, cell diameter decreased and there was a slight hyperpolarization and decrease in action potential overshoot. In guinea pig and cat, the hypertonic solution caused a decrease in input resistance and the plateau of the action potential to disappear in some of the cells; contractions of the entire ventricle also became depressed. These effects were reversed by returning the muscle fibers to isotonic solution. Addition of 5 mM SrCl\textsubscript{2} to the hypertonic solution also caused the plateau component and contraction to reappear. In frog and chick cells, loss of the plateau component and contraction never occurred in hypertonic solution, and input resistance increased. Urea and glycerol hyperosmolarity (2 X) caused no loss of the plateau component or contraction. If the frog and chicken ventricular, and guinea pig atrial myocardial cells (all of which lack T tubules) were to serve as an adequate control for possible effects of hypertonicity on the surface membrane and on contractile proteins, then the results suggest that swelling of the T tubules of mammalian myocardial cells leads to loss of the plateau component.

INTRODUCTION

Sperelakis et al. (1960) have shown that the plateau component of the frog cardiac action potential disappears in solutions made strongly hypertonic (∼3.7 X isotonicity) using sucrose, mannitol, NaCl, or Na\textsubscript{2}SO\textsubscript{4}. Short-duration spikes remained, and these often occurred as high frequency bursts accompanied by sustained contractions. In some instances, phasic contractions disappeared although spikes continued. With prolonged hypertonic perfusion, all excitability was lost. Sometimes a "slow wave" differentiated from the
plateau and resulted in separation of the action potential into two components: an initial spike and a later slow wave separated by a prominent "notch." These effects were very rapid in onset, and could be readily reversed by reperfusion with isotonic Ringer solution. During hypertonic perfusion, the population of myocardial cells was not affected homogeneously, as evidenced by the fact that, adjacent to spontaneously excited cells, quiescent cells or cells firing at some fractional rate were sometimes found. Satisfactory explanation for the plateau separation or loss has never been given.

The present experiments represent an extension of these earlier experiments. The effects of a lower degree of hypertonicity (2 X isotonic) have been examined on the electrophysiology of ventricular myocardial cells from several vertebrate species, some of which have a transverse (T) tubule system and some of which do not. Frog ventricular (Staley and Benson, 1968; Rubio and Sperelakis, 1971), chicken ventricular (Sommer and Johnson, 1969; Sperelakis, unpublished observations), and guinea pig atrial (Sperelakis and Rubio, 1971 a) cells do not contain a T-tubule system, whereas ventricular cells of the guinea pig (Sperelakis and Rubio, 1971 a) and cat (Sperelakis, Rubio, and Redick, 1970; Sperelakis and Rubio, 1971 b) do. It has been demonstrated that vertebrate and invertebrate myocardial cells behave as nearly perfect osmometers in hypertonic solution (Page and Storm, 1966; Sperelakis and Rubio, 1971 b; Sperelakis, 1971) and that the T-tubule system of cardiac (Girardier, 1965; Sperelakis, 1971; Sperelakis and Rubio, 1971 a, b) and skeletal (Huxley et al., 1963; Freygang et al., 1964; Sperelakis and Schneider, 1968) muscle fibers swells in hypertonic solutions. The present results demonstrate that the plateau component of the mammalian ventricular action potential disappears (reversibly) in many cells of hearts bathed in solutions made hypertonic to an extent that does not produce plateau loss in myocardial cells without a T-tubule system, specifically, frog and chick ventricular muscle and guinea pig atrial cells.

METHODS

Hearts isolated from guinea pigs (weighing 300 g each), cats (3 kg each), frogs (Rana pipiens), and 16–18-day old embryonic chicks were studied. Right papillary muscles from guinea pig and cat hearts, atrial sections from guinea pig, and strips of ventricle from frog and chick hearts were used. The muscles were suffused in a chamber (3 ml) at a flow of about 2-6 ml/min. The suffusing Tyrode solution for guinea pig, cat, and chick muscles consisted of (in millimoles per liter): 149.3 Na+, 4.7 K+, 0.9 Ca++, 0.4 Mg++, 140.3 Cl-, 14.3 HCO3-, and 7.8 glucose. The frog Ringer solution contained: 114 Na+, 1.88 K+, 0.92 Ca++, 115.84 Cl-, 1.88 HCO3-, and 7.8 glucose. For the hyperosmotic experiments, the solutions were made 2 X normal osmolarity by adding sucrose, NaCl, glycerol, or urea: 327 mosmol/liter for the Tyrode solution and 285 mosmol/liter for the frog Ringer solution. Sr++ was added to the various solutions in the experiments indicated as SrCl2. All solutions were bubbled with 95 %
O_2–5% CO_2; their pHs during the experiments were 7.3–7.4 (Tyrode) and 7.0–7.2 (frog Ringer). Guinea pig, cat, and chick experiments were conducted at 36°C and frog experiments at 24°–26°C.

Bipolar external electrical recording was done using fine stainless steel wires spaced about 1 mm apart and draining the muscle bath during 30-sec recording periods. The potentials were amplified and displayed on a Grass polygraph (Grass Instrument Co., Quincy, Mass.). Isometric contractions were simultaneously recorded on the polygraph by means of a Grass force-displacement transducer. The frequency of stimulation (Pt wire on either side of the muscle) in the experiments was 36/min.

Intracellular recording was done by the hanging microelectrode technique previously described (Hermsmeyer and Sperelakis, 1970). The microelectrodes, which were filled with 3 M KCl by a vacuum boiling process, had resistances of 5–15 MΩ and tip potentials of 3 mv or less. Membrane potentials (E_m) were amplified for display on a Tektronix 565 oscilloscope (Tektronix Inc., Beaverton, Ore.) using a W-P Instrument M-4 negative capacitance, current injection preamplifier (W-P Instruments, Inc., Hamden, Conn.). Input resistance was measured by applying constant current pulses (3 sec duration and a few nanoamperes) through the intracellular microelectrode. The input resistance is equal to the resulting change in E_m divided by the magnitude of the injected current. Simultaneous phase plane display (V vs. dV/dt, where V is voltage and t is time) of action potentials (Jenerick, 1963; Sperelakis and Shumaker, 1968) was accomplished using a Tektronix operational amplifier. The phase planes were oriented so that the voltage axis was parallel to that of the V-t display, and a positive dV/dt was given by a deflection to the right. The phase planes were retouched to show only the rising phase of the action potential (i.e., positive deflections).

RESULTS

Sucrose and NaCl Hypertonicity The striking finding in hypertonic solutions was the reversible disappearance of the action potential plateau of some of the ventricular myocardial cells in guinea pig and cat hearts (Figs. 1 and 2). In contrast, the action potential plateau of frog (70 cells) and chick (43 cells) ventricles and guinea pig atria (20 cells) did not disappear under these conditions (Fig. 3 and 4). The criterion used to define loss of the plateau component was the loss of the shoulder on the action potential, i.e., no inflection point on the falling phase. Thus, the myocardial cells that have a T system (guinea pig and cat ventricle) show a loss of action potential plateau in some cells, whereas myocardial cells from guinea pig atria and from animals without a T system (frog and chick) do not show loss of the plateau component in any of the cells. About 30% of the (101) cat cells and 90% of the (120) guinea pig ventricular cells sampled showed plateau loss during the peak effect of the hypertonicity, which usually occurred within 20–30 min. In any given cell, the loss of the plateau component usually occurred in progressive stages. Initially, there was a decrease in duration without significant change in configuration, which was followed by a progressive geometrical change to a nearly triangular shape. The
final stage of the transition was a change to the configuration in which the entire falling phase of the action potential had a concave upward shape. The plateau loss was reversible; i.e., the plateau component of all cells sampled returned upon reexposure to isotonic solution. Sometimes (in about 30% of those cells observed) the plateau component of an impaled cell returned even during continued suffusion (up to 1 hr) with hypertonic solution.

The plateau of the action potential reappeared in guinea pig and cat ventricular cells 10–50 min after the muscles were returned to isotonic solution (Figs. 1 C, F, and 2 C, F). In experiments in which there was a single, long-term impalement, the same cell that had lost its plateau in hypertonic solution was observed to recover it in isotonic solution. The return of the plateau was abrupt, occurring by a progressive change in shape back to nearly control conditions over a period of about 15 sec. In experiments in which several cells were sampled during the recovery period, all cells sampled eventually exhibited plateau components. There was no appreciable change in action potential configuration on return to isotonic solution in guinea pig atria and in
Figure 2. Disappearance of the action potential plateau of guinea pig (A-C) and cat (D-F) ventricular myocardial cells in NaCl hypertonic solution and its reappearance in isotonic solution. Conventional and phase plane displays of action potentials given in control (A, D), 2 X NaCl (B, E), and recovery (C, F) solutions. Calibrations for voltage and time apply to all photos; the $dV/dt$ calibration in (A) applies to (A)-(C), and that in (D) to (D)-(F).

Figure 3. Lack of disappearance of the action potential plateau in frog (A-C) and chick (D-F) ventricular myocardial cells in sucrose hypertonic solution. Conventional and phase plane displays of action potentials given in control (A, D), 2 X sucrose (B, E), and recovery (C, F) solutions. Calibrations for voltage and time apply to all photos; the $dV/dt$ calibration in (A) applies to (A)-(C), that in (D) to (D)-(F).
frog and chick ventricle (Figs. 3 and 4) either in single or in multiple impalement experiments.

Cells from three of the four species (all but the chick cells) showed a slight hyperpolarization and a decrease in action potential overshoot ($E_{ov}$) 5–10 min after exposure to hypertonic solutions (Fig. 5). Durations (measured at half-amplitude) of the guinea pig and cat ventricular cell action potentials were, of course, greatly decreased by hypertonicity; the decrease was less marked in the case of the frog (Fig. 6). In the case of the chick, there was very little change in action potential duration in sucrose hypertonic solution, and the mean duration actually increased in NaCl hypertonic solution. External electrical recording of the electrocardiogram (ECG) from guinea pig or cat papillary muscle generally showed R-T interval shortening or disappearance of the T wave, consistent with the intracellularly recorded plateau loss. External recording from frog and chick ventricular strips showed either no change or an increase in the R-T interval. The input resistance ($r_{in}$) changed differently in those cells having and those not having T tubules (Fig. 6). Input resistance decreased in guinea pig and cat ventricle in contrast to a significant increase in frog and chick. Maximum rate of rise ($+V_{max}$) of the action potential did not significantly change in ventricular myocardial cells from any of the

![Diagram](image-url)

**Figure 4.** Lack of disappearance of the action potential plateau of frog (A-C) and chick (D-F) myocardial cells in NaCl hypertonic solution. Conventional and phase plane displays of action potentials given in control (A, D), 2x NaCl (B, E), and recovery (C, F) solutions. Calibrations for voltage and time apply to all photos; the $dV/dt$ calibration in (A) applies to (A)-(C), and that in (D) to (D)-(F).
four species (Table I). A high degree of variability of maximum $dV/dt$ was observed among the cells sampled in each muscle.

Contraction decreased in guinea pig and cat papillary muscle in hypertonic solution at the same time (after 10–30 min) that the action potential plateau was disappearing. The decrease in developed tension was gradual and cor-

![Graph showing resting potential ($E_m$) and action potential overshoot ($E_{ov}$) in control isotonic, 2 × sucrose hypertonic, and 2 × NaCl hypertonic solutions for frog, chick, guinea pig, and cat ventricular myocardial cells. Cells became slightly hyperpolarized and $E_{ov}$ decreased in three of the four species. A star above the bar indicates a difference from the control significant at the 5% confidence level.](image)

responded to the progressive reduction in plateau component. The contractions in guinea pig papillary muscle decreased until they became undetectable, and in the cat they decreased to about 20% of control levels. Associated with the decreased contraction, there was a gradual but significant increase in diastolic tension. In contrast, the frog and chick hearts in the hypertonic solutions developed about 60 and 80%, respectively, of control tension, with no
significant increase in diastolic tension. On return to isotonic solution, there was a recovery of force production. At the same time that the plateau of the guinea pig and cat action potential reappeared, contraction returned, i.e.,

plateau and contraction always returned simultaneously in each muscle. Contractile force never completely returned to more than 70% of control levels, the incomplete recovery being most pronounced (<50%) in the cat. Diastolic tension also did not completely decrease back to control levels during recovery in isotonic solution.

Figure 6. Summary of data giving means and standard errors of duration of the action potential (at half amplitude) and input resistance ($r_{in}$) in control isotonic, 2× sucrose hypertonic, and 2× NaCl hypertonic solutions. Input resistance increased in the hypertonic solutions in frog and chick, but decreased in guinea pig and cat. Data are included from all cells sampled, whether they showed plateau loss or not. Star denotes statistical significance, as in Fig. 5.
Reversal of Plateau Loss by Sr

Addition of 5 mM Sr++ to the hypertonic bathing solution caused reappearance of the action potential plateau and contraction in guinea pig and cat ventricular myocardial cells within 15 min (Fig. 7). As in the case of recovery in isotonic solution, contraction reappeared simultaneously with the action potential plateau. Sr++ did not detectably alter \( E_m \) or \( r_{1a} \). Frog and chick action potentials did not change significantly when 5 mM Sr++ was added to hypertonic suffusion solution at comparable times. In all four species, addition of the Sr++ to the hypertonic solution produced a noticeable increase in contraction in 15 min or less. No decrease was observed in diastolic tension in response to addition of Sr++.

Urea and Glycerol Hyperosmolarity

Neither urea nor glycerol, used as rapidly penetrating molecules to test the effect of hyperosmolarity (2 X normal) on the cells, caused loss of the action potential plateau in guinea pig or cat hearts. Instead, sometimes the duration of the action potential actually increased. Although contraction was decreased during exposure to glycerol hyperosmotic solution by 5–50% in guinea pig and by 0–30% in cat myocardium, the decrease was not as great and recovery was more complete (to nearly control levels) after return to isosmotic solution. In both guinea pig and cat papillary muscles, contractions actually became more vigorous in urea hyperosmolarity. Because quick return to isosmotic Ringer solution, after equilibration in Ringer solution made 2.4 X hyperosmotic with glycerol, presumably disrupts skeletal muscle T tubules (Howell and Jenden, 1967), the same treatment was specifically tried on guinea pig and cat ventricular myocardia; however, no loss of action potential plateau or contraction was ever observed. There was no pronounced increase in diastolic tension. The action potential plateaus and contractions of frog and chick myocardia were not

| Animal       | Control (isotonic) | Sucrose | NaCl  |
|--------------|--------------------|---------|-------|
| Guinea pig   | 216±12 (15)        | 204±10  (9) | 189±19 (5) |
| Cat          | 225±32 (15)        | 197±23  (15) | 168±16 (3)  |
| Frog         | 20±1 (14)          | 20±3  (5) | 29±7 (4)   |
| Chick        | 186±21 (8)         | 167±20 (8) | 170±25 (3) |

Mean ± se. The numbers in parentheses give the number of animals. Guinea pig, cat, and chick values were measured at 36°C and frog values at 25°C. There were no significant differences (at the 5% confidence level) in the hypertonic solutions compared to the control isotonic solutions.
greatly affected by urea or glycerol hyperosmotic treatment or by the rapid return to isosmotic solution. Significant changes in resting $E_m$, $E_{Os}$, $r_{in}$, or $+V_{max}$ were never observed for any of the four species in these solutions.

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**DISCUSSION**

The present experiments, designed to investigate electrical properties of myocardial T tubules, provide the observation that cells with T tubules (guinea pig and cat papillary muscle) showed loss of the action potential plateau in hypertonic solutions which did not similarly affect cells without T tubules. Electron microscopic studies of cells exposed to similar experimental conditions showed that the T system volume was markedly increased. Such ultrastructural observations have been made in both cardiac (Girardier, 1965; Sperelakis, 1971; Sperelakis and Rubio, 1971a, b) and skeletal (Huxley et al., 1963; Freygang et al., 1964; Sperelakis and Schneider, 1968) muscles. For example, cat ventric-
ular cells decreased in diameter from 16.6 to 10.6 μm, while tubule diameter increased from 1050 to 5010 Å in 2 × sucrose hypertonic solution (Sperelakis and Rubio, 1971 b).

Various investigators have previously reported that electrical properties of skeletal muscle T-tubule membranes are different from those of surface membrane. For example, the change in input resistance of frog sartorius fibers in hypertonic solutions (with swollen T tubules) containing or lacking Cl suggests that the T-tubule membrane lacks gcl in contrast to the surface membrane, where gcl is greater than gK (Sperelakis and Schneider, 1968). A T-tubule membrane having a large gK with no appreciable gcl is also indicated by experiments on cells in which the T tubules are disrupted by glycerol osmotic shock (Eisenberg and Gage, 1969). T-tubule swelling may thus be an indication that myocardial T-tubule membranes have different conductance properties from surface membranes.

Membrane electrical changes recorded in hypertonic solutions were explicable on the basis of size changes. The decrease in cell diameter (and thus in cell volume) in hypertonic solution may account for the hyperpolarization and decreased Eov because [K+]i and [Na+]i should be increased. If each cell behaved as a perfect osmometer, the volume would decrease to 50% and the surface area would decrease to 71% of the control value. A decrease in surface area might explain the increase in input resistance in frog and chick myocardia. The decrease in ria in guinea pig and cat papillary muscle in the present experiments was probably due to the increased surface area as a result of T-tubule swelling, which more than balanced the decrease in surface area due to cell shrinkage. The electrophysiological effect of lateral cell separation in hypertonic solutions is unknown. The +Vmax would not be expected to change much if the hyperpolarization and the increased [Na+]i were offsetting changes. Hypertonicity (2 × isotonic using sucrose) also markedly slows velocity of propagation in cat, chick, and frog cardiac muscles (Sperelakis et al., 1970) and the effect probably results from depressed transmission across the intercalated disks because of the observed increase in gap width (Sperelakis and Rubio, 1971 b).

Since the classical experiment of Huxley and Taylor (1958), the T tubule has been implicated in the inward spread of activation (see review by Peachey, 1968). Recently, Costantin (1970) has reported evidence that a regenerative activation of the T-tubule membrane, rather than passive decremental conduction, is responsible for inward spread; isolated fibers, voltage clamped to contraction threshold with brief depolarizing pulses, showed only axial myofibril shortening in low Na+ solution in contrast to only superficial shortening in tetrodotoxin (TTX)–containing solution. Direct electrical stimulation of skinned skeletal muscle fibers (surface membrane peeled away) also suggests that the internal membrane system is capable of a regenerative response
(Costantin and Podolsky, 1967). This internal membrane system has also been shown to be capable of regenerative Ca++ release (Ford and Podolsky, 1970). That the T tubules are responsible for the excitation-contraction (E-C) uncoupling in hypertonic solutions reported by Hodgkin and Horowicz (1957) was suggested by the demonstration of disruption of the T tubules and simultaneous loss of contraction upon quick return of the skeletal muscle fibers to isotonic solution, following equilibration in a solution made hyperosmotic with glycerol (Howell and Jenden, 1967). Other experiments also imply an actively conducted event in the T tubules. Taylor and Rüdel (1970) have suggested that shortening may partly interrupt the inward spread of the activating signal, perhaps due to a pinching off of the T tubules near the core. In analogy to the Huxley-Taylor experiments on skeletal muscle, myocardial local stimulation experiments showed that contraction always spreads longitudinally for at least a few sarcomere lengths (all-or-none effect) in mammalian ventricular fibers (Müler, 1966). This contraction pattern, combined with the observation of longitudinally running (axial) tubules which interconnect with the T tubules (Sperelakis and Rubio, 1971a), also suggests regenerative activation of the T-system membrane.

Any Ca++ moving through the T tubules during the action potential could contribute to E-C coupling. Voltage-clamp experiments indicate that inward Ca++ current ($I_{Ca}$) flows during the cardiac action potential, especially during the plateau, and there is evidence for a slow as well as a fast ion conductance activation (Beeler and Reuter, 1970a). The $I_{Ca}$ during the action potential contributed to E-C coupling only after a delay of several beats in Na+-containing solution. In Na+-free solution, however, tension reached its maximum as quickly as $I_{Ca}$ was fully activated (Beeler and Reuter, 1970b). The slow channel admits Ca++ and/or Na+, depending on the species (Rougier et al., 1968; Garnier et al., 1969). If activation of the T-tubule membrane does involve Ca++, it could contribute to E-C coupling.

If swelling of the T tubules is causing loss of the action potential plateau by interfering with a regenerative membrane response, the observed data would have an explanation which is based on regenerative activation of T-tubule membrane and the participation of coupling Ca++ in the action potential plateau. Several circumstances of the present experiments would be consistent with such an interpretation. First, the loss of plateau occurred at this tonicity only in cells with T tubules. The explanation for the plateau loss must thus include attention to a phenomenon which fails to occur in chick, frog, or even guinea pig atrial cells, none of which have T tubules. Another consideration is the effect of the increased intracellular ionic strength on the contractile proteins. The possibility that loss of contraction specifically in guinea pig and cat ventricular fibers was due to a higher sensitivity to ionic strength effects is unlikely because addition of Sr++ to the hypertonic solutions caused simulta-
neous return of the plateau and contraction. Vereecke and Carmeliet (1971) have demonstrated that Sr++ readily carries the inward current during the plateau phase of the cardiac action potential. This same Sr++ current causes strong contractions which have properties of Ca++-activated contractions (Verdonck and Carmeliet, 1971). Pappano and Sperelakis (1969) have reported that Sr++ can carry inward current during the action potential more readily than Ca++ in cultured chick heart cells. Sr++ has been shown to cause propagated action potentials in normally nonregenerative crustacean skeletal muscle (Werman and Grundfest, 1961) and Limulus cardiac muscle (Rulon et al., 1971). Thus it is conceivable that swelling of the T tubules could cause inability of the Ca++ conductance mechanism to be activated while still allowing Sr++ conductance activation and the corresponding contractions. On the other hand, ionic strength attenuation of contractile protein activation (Gordon and Godt, 1970) is probably the explanation for the smaller decrease in contraction which was observed in cells without T tubules.

If the slow channels participating in the cardiac action potential plateau were localized to the T tubules, there would also have to be an explanation of the plateau (which can also be lost) in cells without T tubules. For example, frog ventricle loses its plateau component under conditions of hypertonicity (Sperelakis et al., 1960), or in Mn++ (Goto and Brooks, 1969). These cells with no T system must have their slow, as well as fast, activation channels in the surface membrane. These slow channels would also be expected to be susceptible to ionic blocking agents and other agents which have been generally observed to be effective. The use of hypertonic solutions to cause T-tubule swelling in myocardial cells which have them has thus provided an interesting loss of plateau of the action potential which seems specific and well correlated with contraction loss. It may be that subsequent experiments will be able to use this special technique for producing plateau loss to further elucidate the mechanism of E-C coupling.

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