Two Inward Currents in Frog Atrial Muscle

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ABSTRACT The double sucrose-gap voltage-clamp technique was applied to frog atrial tissue to investigate the ionic currents responsible for the action potential in this tissue. Membrane depolarization elicited two distinct components of inward current when the test node was exposed to normal Ringer solution: a fast inward current and a slow inward current. The fast inward current appeared to be carried by sodium ions, since it was rapidly abolished by exposure of the fiber to Na^+-free solution or tetrodotoxin but persisted on exposure to Ca^{++}-free solution. In contrast, in the majority of the preparations the slow inward current appeared to be primarily carried by calcium ions, since it was abolished on exposure of the fiber to Ca^{++}-free solution but persisted on exposure to Na^+-free solution. Action potential data supported the voltage-clamp findings. The normal action potential shows two distinct components in the upstroke phase: an initial rapid phase of depolarization followed by a slower phase of depolarization reaching the peak of the action potential. Abolition of the fast inward current resulted in abolition of the initial rapid phase of depolarization. Abolition of the slow inward current resulted in abolition of the slow phase of depolarization. These data support the hypothesis that two distinct and different ionic mechanisms contribute to the upstroke phase of the action potential in frog atrial tissue.

INTRODUCTION

During the last two years, there has been increasing evidence from voltage-clamp experiments on a variety of cardiac preparations that two distinct components of inward current are elicited by membrane depolarization. These experiments have demonstrated that depolarization elicits an inward current which is (*a*) rapidly activated and inactivated, (*b*) dependent on extracellular sodium concentration, (*c*) abolished by application of tetrodotoxin, and (*d*) inactivated by sustained depolarization (Rougier et al., 1968; Mascher and Peper, 1969; Beeler and Reuter, 1970 *a*; Besseau and Gargouil, 1969; Ochi, 1970). This current has generally been designated as the *fast inward current* and the consensus is that it is carried by sodium ions and is responsible for the

fast upstroke phase of the cardiac action potential. In some cardiac preparations (atrial and ventricular), an inward current remains after reduction or abolition of the fast inward current by various experimental maneuvers, e.g. application of tetrodotoxin, reduction in extracellular sodium concentration, or inactivation with a conditioning depolarization. This inward current has a higher threshold (i.e., requires a greater depolarization) than the fast inward current, is activated and inactivated more slowly than the fast inward current, is relatively insensitive to tetrodotoxin, and is abolished by application of manganese ions (Rougier et al., 1969; Mascher and Peper, 1969; Beeler and Reuter, 1970 *b*; Besseau and Gargouil, 1969; Ochi, 1970). This current has been designated as the *slow inward current* and the consensus is that this current is responsible for the peak and the plateau of the cardiac action potential in ventricular and atrial cardiac tissue.

The ion responsible for the slow inward current appears to be variable. In dog ventricular trabeculae (Beeler and Reuter, 1970 *b*) and sheep ventricular trabeculae (Mascher and Peper, 1969) the slow inward current appears to be primarily carried by calcium ions, since it is abolished by removal of extracellular calcium ions but not markedly affected by removal of sodium ions. In contrast, in frog atrial tissue (Rougier et al., 1969), rat ventricular fibers (Besseau and Gargouil, 1969), and guinea pig myocardium (Ochi, 1970), it appears that both sodium ions and calcium ions contribute to the slow inward current, since the current persisted when the fiber was bathed in either sodium-free or calcium-free solution.

Alternative explanations have been given to the voltage-clamp data purported to demonstrate the existence of a slow inward current (Johnson and Lieberman, 1971). The cardiac preparations which have been subjected to voltage clamp are composed of a large number of relatively small diameter cells with the majority of the cell membranes connected to the periphery of the bundle through the narrow clefts between the tightly packed cells. It is reasonable to assume that the equivalent circuit for such a preparation would be that of an external resistance in series with the cell membranes. Indeed, data to support this conclusion have recently been presented for dog ventricular trabeculae (Beeler and Reuter, 1970 *a*) and frog atrial tissue (Tarr and Trank, 1971). A consequence of such an equivalent circuit is that the potential of the cell membranes is not under ideal voltage-clamp control. Experiments on squid axon have demonstrated that multiple peaks of inward current can result from nonuniform spatial control of membrane potential (Cole and Moore, 1960; Taylor et al., 1960). Johnson and Lieberman argue that the slow inward current in cardiac muscle is an artifact due to inadequacies in the spatial and temporal control of membrane potential. They suggest that the membranes of the peripheral cells are responsible for the fast inward current, and that the membranes of the deeper cells less accessible to voltage control are responsible for the slow inward current.

This paper describes the results of voltage-clamp experiments on frog atrial tissue. Evidence to support the following conclusions is presented. First, two distinct inward currents contribute to the generation of the action potential. Second, the fast inward current is carried by sodium ions. Third, the slow inward current is carried by either calcium or sodium ions depending on the preparation: the majority of preparations have slow inward calcium currents. Fourth, tetrodotoxin selectively abolishes the fast inward current without significantly affecting the slow inward current. Fifth, manganese inhibits both the fast and slow inward currents.

METHODS

The experiments were performed on thin bundles of frog (*Rana catesbeiana*) atrial muscle. The double sucrose-gap voltage-clamp technique as applied to cardiac muscle and the general experimental procedure have been discussed extensively in the previous paper (Tarr and Trank, 1971).

In early experiments (experiments before A121669) the normal Ringer solution had the following composition in millimoles per liter: NaCl, 111; KCl, 5.4; $CaCl_2$, 1.8; $NaHCO_3$, 1.8. This solution was bubbled with 95% O_2–5% CO_2 to maintain a constant pH. In those experiments the "Na^+-free" solution was obtained by replacing the NaCl with choline chloride. In later experiments (experiments after A121669), tris (hydroxymethyl) amino methane (10 mM) was used as the buffer for the Ringer solution; the pH of this Ringer solution was adjusted to 7.0 at 25°C by adding 12.4 N HCl. In the latter experiments, the Na^+-free solution was obtained by replacing the NaCl with Tris-HCl. No difference was observed in the results obtained with the two low sodium solutions, and no distinction will be made in discussing the data from these experiments. The Ca^{++}-free solution was obtained by omitting $CaCl_2$ from the above Ringer solutions. For solutions containing tetrodotoxin (TTX), TTX was added to the Ringer solution to obtain a TTX concentration of 100 nM. For solutions containing $MnCl_2$, $MnCl_2$ was added to the Ringer solution to obtain concentrations of 1, 3, or 10 mM.

The preparation was stretched such that mechanical activity of the fiber within the test node associated with depolarizing clamp pulses did not alter the magnitude or geometry of the test node.

RESULTS

Two Inward Current Components

When the voltage-clamped region (test node) was exposed to normal Ringer solution, two distinct inward currents could generally be distinguished during stepwise depolarizing clamp pulses. A typical family of membrane currents is shown in Fig. 1. The inward current consisted primarily of the fast inward current at a small depolarization (40 mv), whereas at a large depolarization (80 mv) the slow inward current was most prominent.

A reasonable test to exclude the possibility that the two current components were artifacts due to an inhomogeneous voltage clamp would be to demon-

strate that either current component could be selectively abolished without significantly affecting the other current component. The selective abolition of the fast inward current after exposure of the test node to TTX is shown in Fig. 2. A similar selective abolition of the fast inward current after removal of Na^+ is shown in Fig. 3. In both cases the fast inward current was rapidly abolished (within 5 min after application of the test solution) and the slow inward current was not significantly affected. The selective abolition of the slow inward current after removal of Ca^{++} is shown in Fig. 4. The slow inward current was rapidly abolished and the magnitude of the fast inward

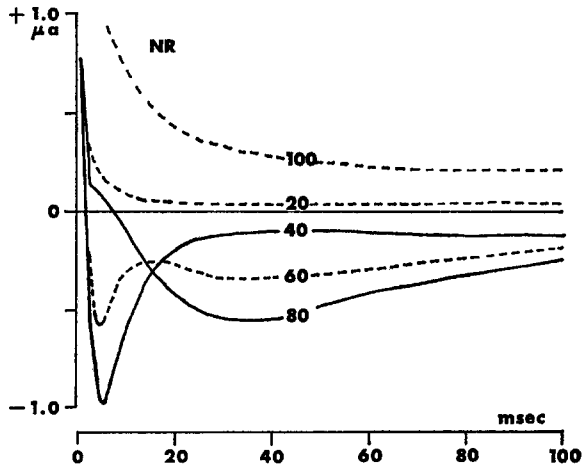

FIGURE 1. Family of membrane currents associated with step depolarizations under voltage-clamp conditions. Numbers associated with each trace indicate the amount of depolarization from the holding potential in millivolts. Ordinate: current in microamperes; abscissa: time in milliseconds. The holding current is considered as zero current: inward current designated as negative, outward current as positive. Exp. C073069.

current was slightly increased after removal of Ca^{++}. The slow inward current returned rapidly upon restoration of the extracellular Ca^{++}.

Slow Inward Calcium Current

The above findings are representative of the majority of fibers. That is, removal of Ca^{++} abolished the slow inward current, whereas the removal of Na^+ slightly suppressed but did not abolish the slow inward current.

The effect of removal of Ca^{++} on the current-voltage relationship of the slow inward current is shown in Fig. 5. It is apparent that no inward current was present after removal of calcium ions.

The effect of removal of Na^+ on the current-voltage relationship of the slow inward current is shown in Fig. 6. In this preparation, only slow inward

current was present when the test node was exposed to normal Ringer solution. Subsequent removal of Na^+ simultaneous with application of TTX did not significantly affect the magnitude of the slow inward current. The shift

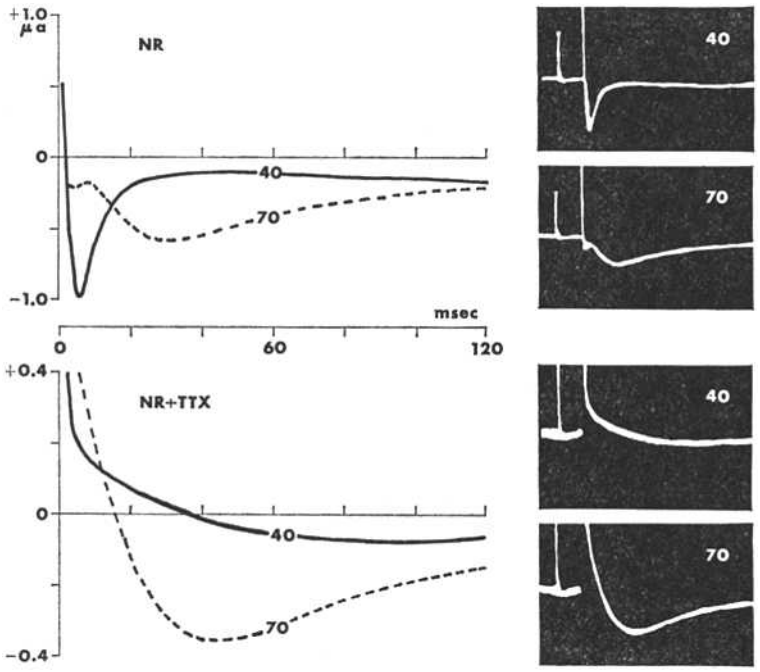

FIGURE 2. Effect of tetrodotoxin (TTX) on the fast and slow inward current components. Step depolarizations of 40 and 70 mv from a holding potential of −70 mv. The magnitude and temporal relationships of the currents are shown in the superimposed tracings at the left: normal Ringer solution (upper left); normal Ringer plus TTX (lower left). The actual recordings corresponding to the tracings are shown at the right of the respective tracings: NR (right upper half); NR + TTX (right lower half). The spike of outward current preceding each recording of the membrane current associated with the step depolarization resulted from a pulse of small magnitude used to obtain a measure of the external series resistance (Tarr and Trank, 1971). This resistance is proportional to the width of the test node and is, therefore, a measure of the voltage-clamped membrane area. The recordings in NR + TTX (right lower half) were at a higher gain than those in NR (right upper half) and the increased spike magnitude does not reflect an increase in membrane area. Exp. C073069.

of the current-voltage curve along the voltage axis is due to the removal of Na^+, since this shift is not apparent after application of TTX alone.

The above data suggest that in these preparations the slow inward current was primarily a calcium current. The effects of Na^+ or Ca^+ removal on the maximum values of the slow inward current from these preparations are given in Table I (Ca^{++} current).

The effects of removal of Ca^{++} on the action potential are demonstated in Fig. 7. The effects appeared to fall into two categories. In some preparations, removal of Ca^{++} abolished both the slow phase of depolarization and the plateau phase of the action potential (see left half of Fig. 7). In other preparations, removal of Ca^{++} abolished the slow phase of depolarization of the action potential although a long-duration response (plateau) was still obtained (see right half of Fig. 7). Voltage-clamp data on these preparations (no voltage-clamp data was obtained on Exp. B071869, upper left of Fig. 7)

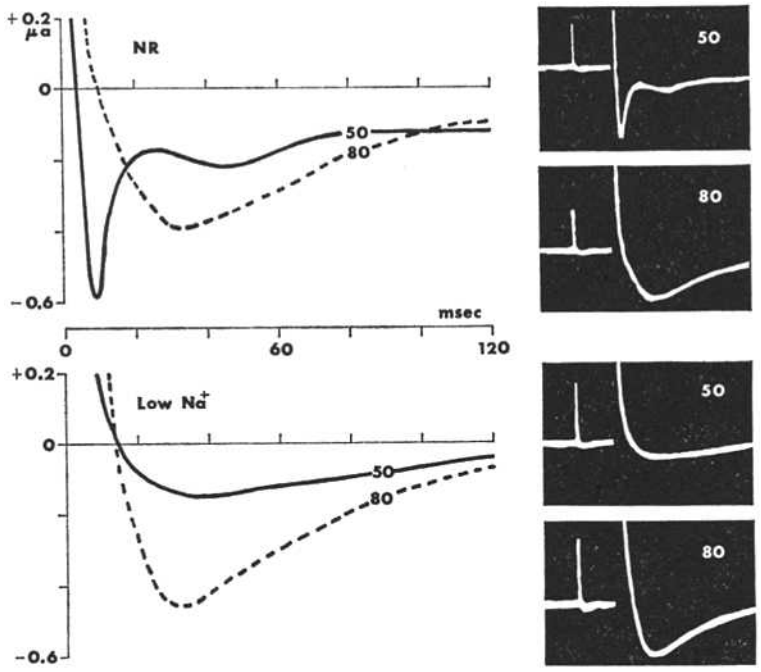

FIGURE 3. Effect of removal of sodium ions on the fast and slow inward current components. Step depolarization of 50 and 80 mv from a holding potential of −65 mv. Exp. B070269.

demonstrated that removal of Ca^{++} indeed abolished the slow inward current (see Table I, and Fig. 4).

The effects of removal of Na^{+} on the action potentials are demonstrated in Fig. 8. Following exposure of the test node to Na^{+}-free solution, the threshold of the action potential was markedly increased compared to when the test node was exposed to normal Ringer solution. This increased threshold was due to the abolition of the fast phase of depolarization (top half of Fig. 8). In contrast, the slow phase of depolarization and plateau remained in Na^{+}-free solution. The effect of Na^{+} removal on the slow phase of depolarization is best demonstrated by comparison of the action potentials before and after

removal of Na^+ from a Ringer solution containing TTX (bottom half of Fig. 8). Voltage-clamp data on these preparations demonstrated that removal of Na^+ did not significantly affect the magnitude of the slow inward current (see Table I).

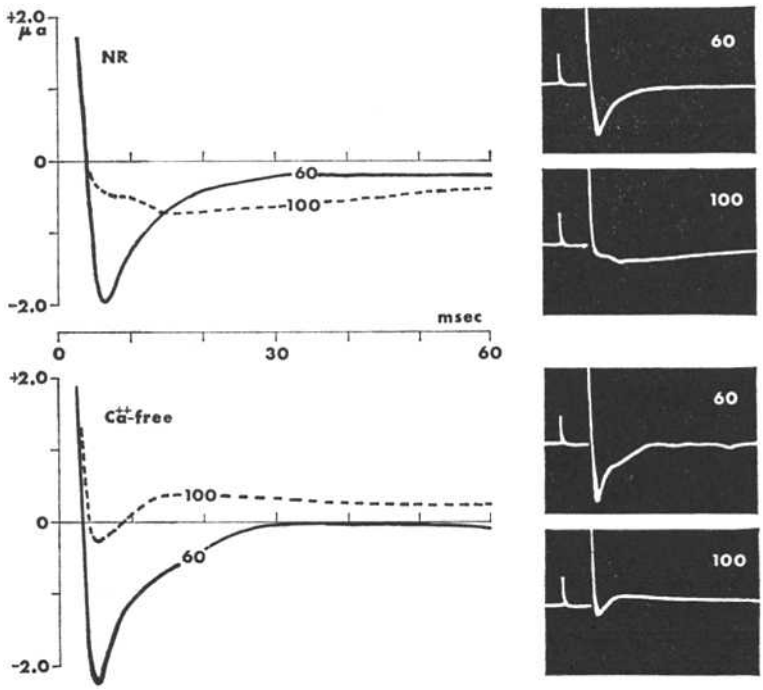

FIGURE 4. Effect of removal of calcium ions on the fast and slow inward current components. Step depolarizations of 60 and 100 mv from a holding potential of −80 mv. Exp. C061969.

Slow Inward Sodium Current

In some preparations, the slow inward current appeared to be primarily a sodium current, since it was abolished by removal of Na^+ and persisted in Ca^{++}-free solution.

The effect of removal of Na^+ on the current-voltage relationship of this slow inward sodium current is shown in Fig. 9. It is apparent that no inward current was present following removal of Na^+.

The effect of removal of Ca^{++} on the current-voltage relationship of the slow inward sodium current is shown in Fig. 10. In this preparation, only slow inward current was present when the test node was exposed to normal Ringer solution. This slow inward sodium current persisted after exposure of the test node to Ca^{++}-free Ringer solution containing TTX.

The above data suggest that in these preparations the slow inward current

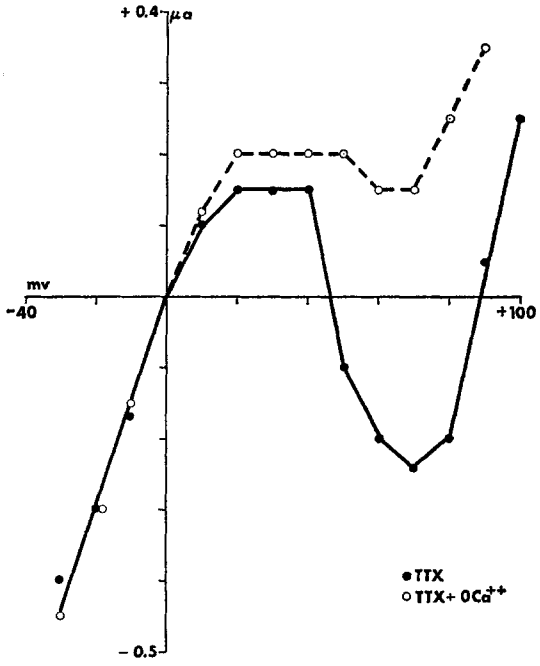

FIGURE 5

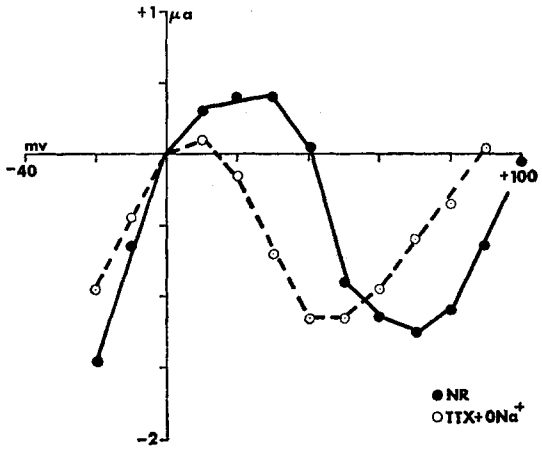

FIGURE 6

FIGURE 5. Effect of removal of calcium ions on the current-voltage relationship of the slow inward calcium current. Ordinate is the current in microamperes: outward currents are positive values, inward currents are negative values. Abscissa is the magnitude of the step change in clamp potential from the holding potential of −110 mv: positive values indicate depolarization, negative values indicate hyperpolarization. Current-voltage curves were constructed as follows. Currents associated with depolarizations are either maximum values of inward current or minimum values of outward current; currents associated with hyperpolarizations are currents 50 msec after the step change in clamp potential (i.e., at approximately the same time as the peak of the inward current associated with membrane depolarizations). Closed circles: current-voltage curve when test node was exposed to Ringer solution containing TTX. Open circles: current-voltage curve during exposure of the test node to Ca^{++}-free Ringer containing TTX. Exp. B061769.

FIGURE 6. Effect of removal of sodium ions on the current-voltage relationship of the slow inward calcium current. Current-voltage curves were constructed as follows. Currents associated with depolarizations are either maximum values of inward current or minimum values of outward current; currents associated with hyperpolarizations are currents 150 msec after the step change in clamp potential. Closed circles: current-voltage curve when test node was exposed to normal Ringer solution (only slow current was present). Open circles: current-voltage curve during exposure of the test node to Na^{+}-free Ringer containing TTX. Holding potential was −110 mv. Exp. B122369.

was primarily a sodium current. The effects of Na^+ or Ca^{++} removal on the maximum values of this slow inward current are given in Table I (Na^+ current).

TABLE I

EFFECT OF Na^+ OR Ca^+ REMOVAL ON MAXIMUM VALUE OF SLOW INWARD CURRENT

	Sodium			Calcium	
Exp.	Normal	Low	Exp.	Normal	Zero
Ca^{++} current					
A070269	−0.33	−0.29	C060569	−0.22	+
B070269	−0.49	−0.58	D060669	−0.20	+
C070369	−0.23	−0.18	B061369	−0.16	+
C070769	−0.75	−0.59	A061669	−0.75	+
D070769	−0.29	−0.26	B061869	−0.50	+
C070969	−0.40	−0.17	B061969	−0.22	+
E070969	−1.65	−1.00	C061969	−0.80	+
B121169	−0.08	−0.06	E062069	−0.25	+
A121869	−0.90	−0.23	A062369	−0.25	+
A122269	−0.60	−0·50	A072269	−0.19	+
B122269	−1.10	−0.40	B072569	−0.28	+
C122269	−0.16	−0.17	B061769	−0.23	+
B122369	−1.25	−1.15	A071169	−0.28	+
B012070	−0.40	−0.25	B071169	−0.11	+
C012270	−0.80	−0.65	A033170	−1.05	+
D012370	−0.41	−0.44	B050670	−0.16	+
B041770	−1.00	−0.70		(−0.35)	
A042270	−0.40	−0.31			
A042470	−0.32	−0.16			
C042970	−0.65	−0.40			
B050170	−0.45	−0.33			
A050770	−0.33	−0.38			
	(−0.59)	(−0.42)			
Na^+ current					
C070269	−0.90	+	C071869	−0.85	−1.60
B020969	−1.25	−0.08	C072369	−1.05	−0.60
B121069	−0.55	+	B072869	−1.10	−2.10
A123069	−0.11	+	F072469	−0.65	−0.48
	(−0.70)			(−0.91)	(−1.20)

Values are in μa; a + sign indicates that the current was outward.
Average values are given in parentheses.

The effects of removal of Na^+ or Ca^{++} on the action potentials from this type of preparation are demonstrated in Fig. 11. Removal of Na^+ (upper half of Fig. 11) abolished both the upstroke and peak of the action potential. Voltage-clamp data on this preparation confirmed that both the fast and slow components of inward current were abolished after the removal of Na^+

(Table I). The effect of removal of Ca^{++} is shown in the lower half of Fig. 11. In this preparation the action potential had an extremely slow upstroke phase with a reasonably normal plateau phase when the test node was exposed to

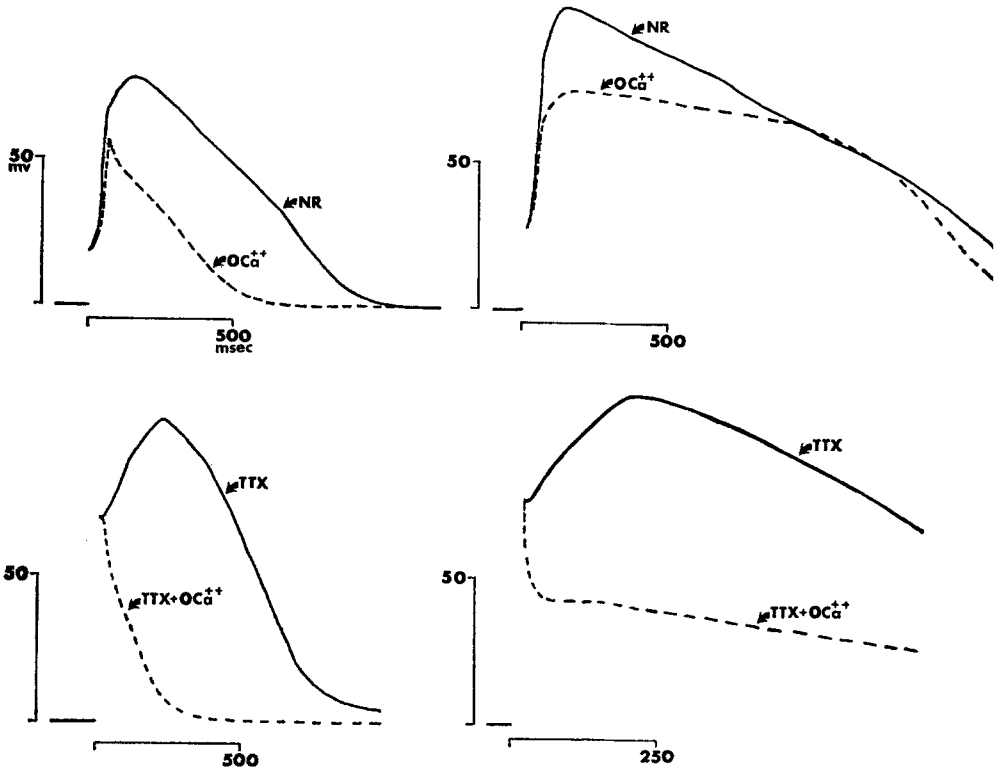

FIGURE 7. Effect of removal of calcium ions on the action potential from fibers with slow inward calcium currents. Action potentials in left half of figure are representative of preparations in which removal of Ca^{++} abolished both the peak and plateau phase of the action potential. Exp. B071869 (top left); Exp. A033170 (bottom left). Action potentials in the right half of the figure are representative of preparations in which removal of Ca^{++} abolished the peak of the action potential although a long-duration response (plateau) was still obtained. Exp. C061969 (top right); Exp. B050670 (bottom right). Each action potential tracing begins at the termination of the stimulus. Action potentials from fibers exposed to normal Ringer solution are compared to the action potential during exposure of the test node to Ca^{++}-free Ringer solution in the top half of the figure. Bottom half of figure compares action potentials before and during exposure of the test node to Ca^{++}-free Ringer solution containing TTX.

normal Ringer solution. Under voltage-clamp conditions only a slow inward current was recorded (current-voltage curve given in Fig. 10). This slow inward current persisted after exposure of the test node to Ca^{++}-free Ringer solution containing TTX, and there was no marked effect on the action potential (Fig. 11).

Selectivity of TTX

It was demonstrated in Fig. 2 that exposure of the test node to TTX abolished the fast inward current, whereas the slow inward current persisted. An attempt was made to determine if TTX at a concentration of 100 nM had any significant effect on the magnitude of the slow inward current.

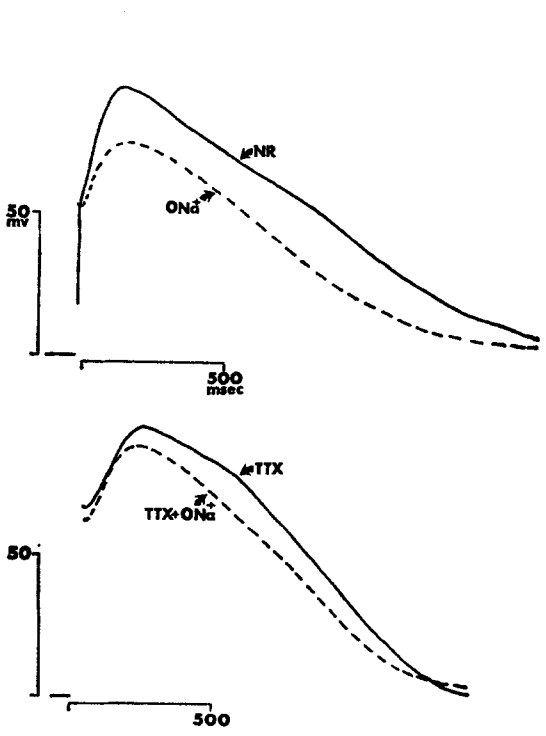

FIGURE 8

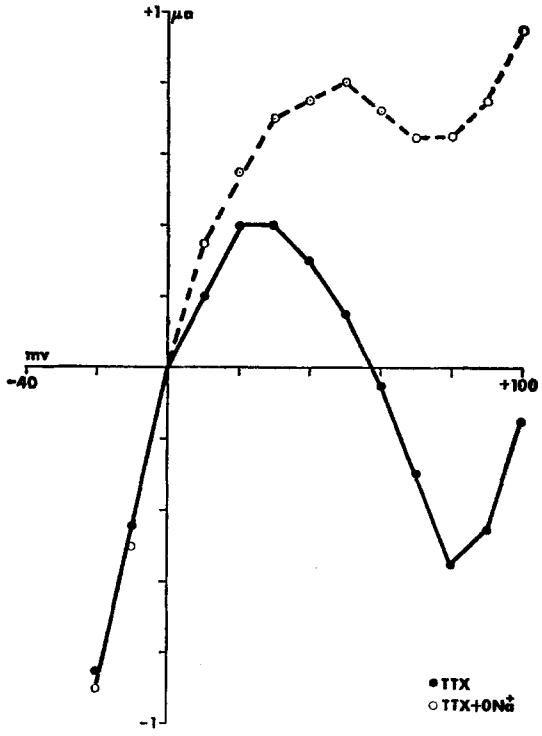

FIGURE 9

FIGURE 8. Effect of removal of sodium ions on the action potential from fibers with slow inward calcium currents. Top half of figure (Exp. D070769) compares the action potentials from a fiber before and after removal of Na^+ from the Ringer solution bathing the test node. Bottom half of figure (Exp. A050770) compares action potentials before and after removal of Na^+ from a Ringer solution containing TTX. Each action potential tracing begins at the termination of the stimulus.

FIGURE 9. Effect of removal of sodium ions on the current-voltage relationship of the slow inward sodium current. Current-voltage curves were constructed as follows. Currents associated with depolarizations are either maximum values of inward current or minimum values of outward current; currents associated with hyperpolarizations are currents 100 msec after the step change in clamp potential. Closed circles: current-voltage curve when test node was exposed to Ringer solution containing TTX. Open circles: current-voltage curve during exposure of the test node to Na^+-free Ringer solution containing TTX. Holding potential was −120 mv. Exp. B121069.

Three types of experimental situations contributed to this analysis. In preparations where distinct fast and slow currents were present when the test node was exposed to normal Ringer solution, the effect of TTX was evaluated

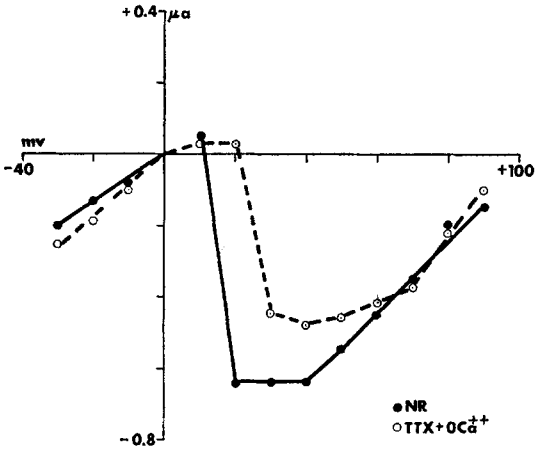

FIGURE 10

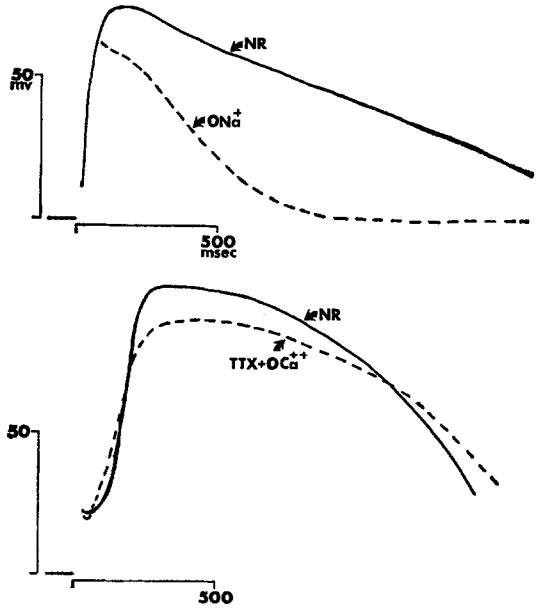

FIGURE 11

FIGURE 10. Effect of removal of calcium ions on the current-voltage relationship of the slow inward sodium current. Current-voltage curves were constructed as follows. Currents associated with depolarizations are either maximum values of inward current or minimum values of outward current; currents associated with hyperpolarizations are currents 200 msec after the step change in clamp potential. Closed circles: current-voltage curve when test node was exposed to normal Ringer solution (only slow current was present). Open circles: current-voltage curve during exposure of the test node to Ca^{++}-free Ringer solution containing TTX. Holding potential was −100 mv. Exp. F072469.

FIGURE 11. Effect of removal of sodium or calcium ions on action potentials from fibers with slow inward sodium currents. Top half of figure shows action potentials from a fiber where removal of sodium abolished both the fast rate of rise and peak and plateau of the action potential (Exp. C070269). The bottom half of the figure shows action potentials from a fiber where removal of calcium and application of TTX did not markedly affect the action potential (Exp. F072469). Each action potential tracing begins at the termination of the stimulus.

by comparison of the maximum value of slow inward current before and during exposure of the test node to TTX (see Table II, Group 1). In preparations exhibiting only slow inward current when exposed to normal Ringer solution, the effect of TTX was evaluated in a manner similar to that of the

above preparations (Table II, Group 2). In some preparations, the fast inward current was first abolished by removal of Na^+ and the test node was then exposed to Na^+-free Ringer solution containing TTX (Table II, Group 3). The data from these three groups of experiments are given in Table II.

TABLE II
EFFECT OF TETRODOTOXIN ON MAXIMUM VALUE OF SLOW INWARD CURRENT

Exp.	Control	TTX
Group 1		
C031369	−0.30	−0.10
B061769	−0.40	−0.23
B051669	−0.27	−0.32
B062569	−0.90	−1.60
C073069	−0.55	−0.36
A042470	−0.44	−0.32
	(−0.48)	(−0.49)
Group 2		
C051569	−0.30	−0.30
C051669	−0.56	−0.69
D051669	−0.23	−0.37
B010770	−0.30	−0.30
A042270	−0.90	−0.40
	(−0.46)	(−0.41)
Group 3		
A070269	−0.29	−0.38
B070269	−0.58	−0.52
C070369	−0.18	−0.17
C070769	−0.59	−0.67
D070769	−0.26	−0.16
C070969	−0.17	−0.18
E070969	−1.00	−0.70
	(−0.44)	(−0.40)

Values are in μa; average values are given in parentheses.
Group 1, distinct fast and slow inward currents when fiber was bathed in normal Ringer solution (control).
Group 2, only slow inward current when fiber was bathed in normal Ringer solution (control).
Group 3, control is slow inward current when fiber was bathed in low Na^+ solution.

It is apparent that there was a great deal of variability in the results. In some cases TTX markedly reduced the magnitude of the slow inward current (e.g., Group 2, Exp. A042270). In some cases the magnitude was greatly increased (e.g., Group 1, Exp. B062569). But in the majority of cases, TTX had no marked effect on the magnitude of the slow inward current; this was

especially true of Groups 2 and 3 where the slow inward current was not contaminated with residual fast inward current.

The effect of TTX on the current-voltage relationship of the slow inward current recorded from one of the Group 3 preparations is shown in Fig. 12. There is a slight suppression in the peak magnitude of the inward current which is probably not significant.

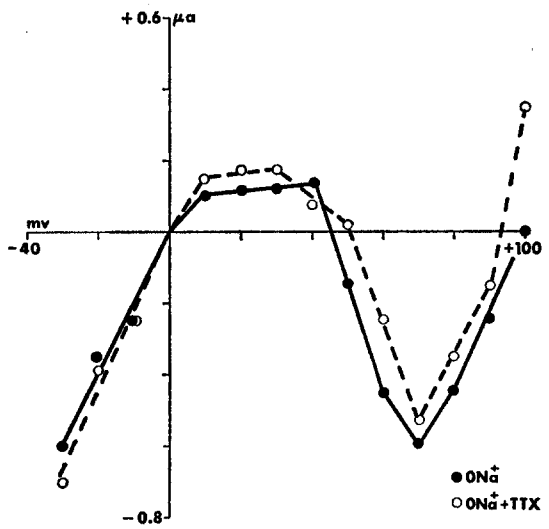

FIGURE 12. Effect of TTX on the current-voltage relationship of the slow inward calcium current. Current-voltage curves were constructed as follows. Currents associated with depolarizations are either maximum values of inward current or minimum values of outward current; currents associated with hyperpolarizations are currents 60 msec after the step change in clamp potential. Closed circles: current-voltage curve when test node was exposed to Na^+-free Ringer solution. Open circles: current-voltage relationship during exposure of the test node to Na^+-free Ringer solution containing TTX. Holding potential was −100 mv. Exp. B070269.

Selectivity of Manganese

Exposure of the test node to Ringer solution containing 1–3 mM $MnCl_2$ markedly suppressed the slow inward current, and slightly suppressed the fast inward current (see Fig. 13 and Table III). At a larger concentration (10 mM), both the fast and slow inward currents were abolished (see Fig. 14 and Table III.

DISCUSSION

The hypothesis that two distinct and different ionic mechanisms are responsible for the upstroke and peak of the cardiac action potential and a variety of evidence to support this hypothesis have often appeared in recent literature.

The action potentials of amphibian and mammalian cardiac fibers often display two distinct phases of depolarization, each phase responding differently to drugs and variations in the extracellular ionic environment (Wright and

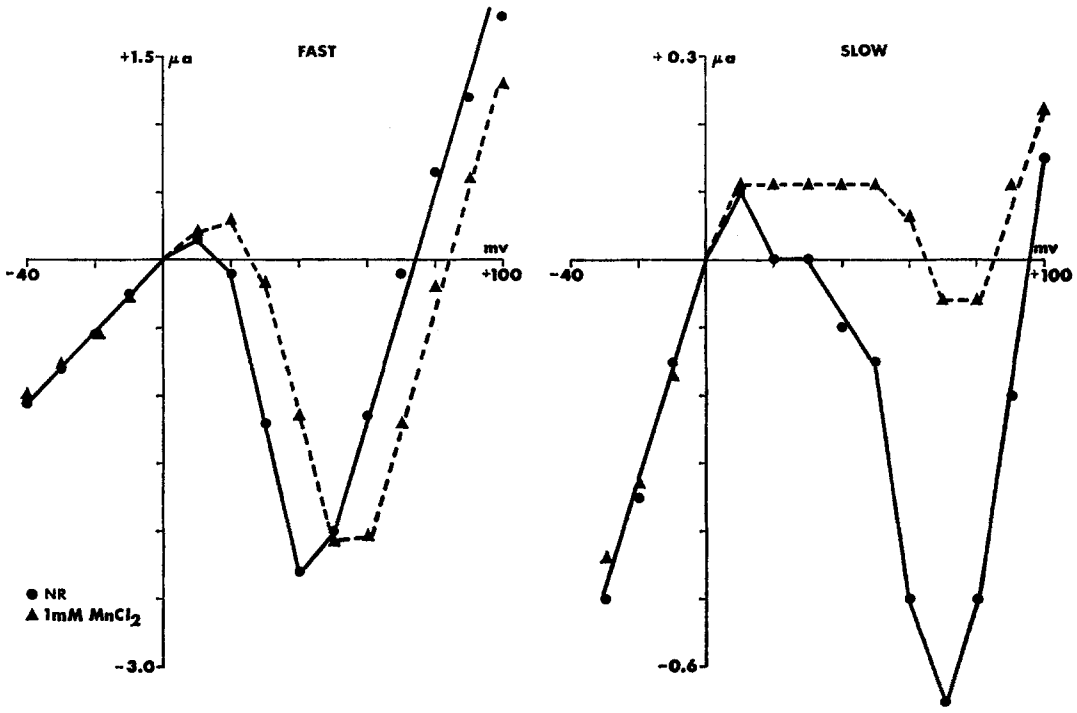

FIGURE 13. Effect of $MnCl_2$ (1 mM) on the current-voltage relationships of the fast and slow inward currents. Fast inward current-voltage curve is shown in the left half of the figure; slow inward current-voltage curve in right half of figure. Closed circles: current-voltage curve when test node was exposed to normal Ringer solution. Closed triangles: current-voltage curve when test node was exposed to normal Ringer solution containing 1 mM $MnCl_2$. Fast inward current-voltage curves were constructed as follows. Outward currents associated with depolarizations and inward currents associated with hyperpolarizations are currents 5 msec after the step change in clamp potential; inward currents associated with depolarizations are maximum values of fast inward current. Slow inward current-voltage curves were constructed as follows. Outward currents associated with depolarizations and inward currents associated with hyperpolarizations are currents 50 msec after step change in clamp potential; inward currents associated with depolarizations are maximum values of slow inward current. Holding potential was −80 mv. Exp. C031369.

Ogata, 1961; Antoni and Delius, 1965; Paes de Carvalho et al., 1969). The initial rapid phase of depolarization appears to be related to an increased membrane conductance to sodium, since it is dependent on extracellular sodium concentration (Draper and Weidmann, 1951; Brady and Woodbury,

1960) and is suppressed by tetrodotoxin (Hagiwara and Nakajima, 1966; Coraboeuf and Vassort, 1968). The slower phase appears to be related to an increased membrane conductance to calcium ions, since it is dependent on the extracellular calcium concentration (Hoffman and Suckling, 1956; Orkand and Niedergerke, 1964; Mascher, 1970; Carmeliet and Vereecke, 1969).

In agreement with the action potential data are the data from voltage-clamp experiments on a variety of cardiac tissue which appear to support the

TABLE III

EFFECT OF $MnCl_2$ ON MAXIMUM VALUES OF FAST AND SLOW INWARD CURRENT

	Fast inward		Slow inward	
Exp.	Control	$MnCl_2$	Control	$MnCl_2$
1 mM $MnCl_2$				
C031369	−2.30	−2.10	−0.65	−0.06
B032769	−0.52	−0.30	−0.15	+
E032669	−1.30	−1.80	—	—
A031969	−5.80	−4.00	—	—
B040369	—	—	−0.55	−0.15
3 mM $MnCl_2$				
A040369	−1.20	−0.90	−0.25	+
B040269	—	—	−0.50	−0.15
C080769	−1.45	−0·73	—	—
A081269	−1.00	−0.50	—	—
10 mM $MnCl_2$				
B030769	−1.70	+	−0.60	+
A031169	−1.00	+	−0.68	+
B032769	−0.52	+	−0.15	+

Values are in μa; a + sign indicates that the current was outward.

two-component hypothesis. These experiments have demonstrated that membrane depolarization elicits a fast inward sodium current which appears to have properties similar to the sodium current responsible for nerve excitation (Rougier et al., 1968; Mascher and Peper, 1969; Beeler and Reuter, 1970 *a*, *b*; Besseau and Gargouil, 1969; Ochi, 1970). The existence of a second component of inward current became apparent after suppression of the fast inward sodium current by various experimental maneuvers. This second inward current had much slower kinetics than the fast inward sodium current and it appeared to be at least partially due to an inward movement of calcium ions (Rougier et al., 1969; Besseau and Gargouil, 1969; Ochi, 1970; Mascher and Peper, 1969; Beeler and Reuter, 1970 *b*). But it should be pointed out that, except for the preliminary data of Besseau and Gargouil

(1969), the properties of the slow inward current have been investigated only after suppression of the fast inward current, since a distinct slow component of inward current was not generally clearly discernible in the presence of the fast inward current. These investigators assumed that suppression of the fast

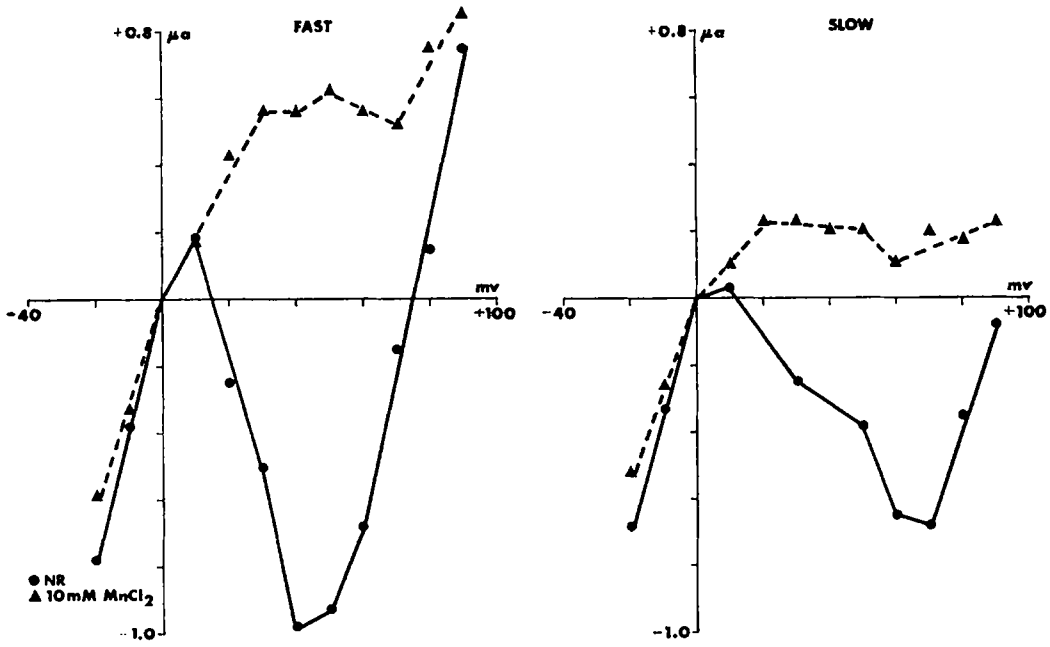

FIGURE 14. Effect of $MnCl_2$ (10 mM) on the current-voltage relationship of the fast and slow inward currents. Fast inward current-voltage curve is shown in the left half of the figure: slow inward current-voltage curve in right half of figure. Closed circles: current-voltage curve when test node was exposed to normal Ringer solution. Closed triangles: current-voltage curve when test node was exposed to normal Ringer solution containing 10 mM $MnCl_2$. Fast inward current-voltage curves were constructed as follows. Outward currents associated with depolarizations and inward currents associated with hyperpolarizations are currents 10 msec after the step change in clamp potential; inward currents associated with depolarizations are maximum values of fast inward current. Slow inward current-voltage curves were constructed as follows. Outward currents associated with depolarizations and inward currents associated with hyperpolarizations are currents 100 msec after the step change in clamp potential; inward currents associated with depolarizations are maximum values of slow inward current. Holding potential was −80 mv. Exp. A031169.

inward sodium current did not affect the properties of the slow inward current.

The data presented in this paper clearly demonstrate the existence of a distinct slow component of inward current in the presence of the fast inward current. Suppression of the fast inward current by exposure of the fiber to

Na^+-free solution or tetrodotoxin (TTX) did not significantly affect the slow inward current (Figs. 3 and 4). In the majority of the preparations, the slow inward current appeared to be primarily carried by calcium ions, since it was rapidly abolished on exposure of the fiber to Ca^{++}-free solution but persisted on exposure of the fiber to Na^+-free solution. However, in a few preparations, the slow inward current appeared to be primarily a TTX-insensitive Na^+ current. In addition, the data presented in this paper support the conclusion that TTX is a selective inhibitor of the fast inward sodium current, but demonstrate that manganese is not a selective inhibitor of the slow inward current. The finding that manganese is not a selective inhibitor of the slow inward current is not suprising, since many metal cations suppress the action potential in nerve (Blaustein and Goldman, 1968; and Hafemann, 1969).

Rougier et al. (1969) found in similar experiments on frog atrial tissue that the slow inward current persisted when the fiber was exposed to either Na^+-free solution or Ca^{++}-free solution containing ethylenediaminetetraacetate (EDTA). These investigators concluded that both Na^+ and Ca^{++} contribute to the slow inward current. The data presented in the present paper do not definitively rule out the possibility that Na^+ also contributed to those slow inward currents classified as calcium currents. Removal of Na^+ in many cases slightly suppressed the slow inward current, but whether or not this suppression was due to removal of a slow sodium current or the result of an accumulation of internal calcium ions in the absence of external sodium (Reuter and Seitz, 1968) cannot be decided from these data. The data certainly do not support the hypothesis of Rougier et al. (1969) that the duration of the action potential is dependent on a slow inward sodium current, since removal of Na^+ did not significantly decrease the duration of the action potential (see Fig. 8) in preparations with slow calcium currents.

The crucial point to be decided is with which of the following hypotheses the voltage-clamp data are most compatible: (*a*) two distinct inward currents contribute to the generation of the cardiac action potential or (*b*) the slow inward current results from an inhomogeneous voltage clamp (Johnson and Lieberman, 1971).

I presented no data to prove that inhomogeneity of voltage control did not occur. Such proof would require the difficult, if not impossible, experiment of monitoring the potentials of a large number of cells in the test node with intracellular microelectrodes. I suspect that if such an experiment were carried out, one would indeed find cells whose potentials were under poor voltage-clamp control and that these cells would most likely be found deep within the preparation. But the meaning of such a finding would be obscure, since one would not be able to determine the contribution of the membrane current of that cell to the total membrane current recorded from the entire test node. The question becomes one of degree. That is, is the inhomogeneity

of voltage control so severe as to preclude the acquisition of meaningful voltage-clamp data? Again, this may be a difficult question to answer definitively.

Data which would be useful in formulating a reasonable conclusion would be the degree of correlation between the membrane action potential recorded under nonvoltage-clamp conditions and the membrane currents recorded under voltage-clamp conditions. For a nonpropagated action potential, as was recorded in the present experiments, the total membrane current is zero (i.e., after cessation of the stimulating current) and the ionic current equals the capacitive current (i.e., $I_i = -C_m dV_m/dt$). In other words, some conclusions as to the wave form of the ionic currents which should be recorded under voltage-clamp conditions can be inferred from the wave form of the action potential.

The action potential of frog atrial tissue often shows two distinct components in the upstroke phase: an initial rapid phase of depolarization followed by a slower phase of depolarization reaching the peak of the action potential (Fig. 7, upper half). From this characteristic action potential wave form one would infer that the following should be observed under voltage-clamp conditions. (*a*) A rapidly activated inward current should be recorded in the potential region of the initial rapid phase of depolarization; (*b*) a more slowly activated inward current should be recorded in the potential region of the slower phase of depolarization. The voltage-clamp data demonstrate that this was indeed the case: at small depolarizations a fast inward current was prominent; at large depolarizations, a slow inward current was prominent (see Fig. 1).

If the rapid and slow phases of depolarization during the upstroke phase of the action potential were due to the same excitatory mechanism, it should not be possible to selectively abolish one phase of depolarization without affecting the other phase. In contrast, if different excitatory mechanisms were involved, selective abolition should be possible and there should be a good agreement between the action potential data and voltage-clamp data. For example, abolition of the fast phase of depolarization should result from abolition of the fast inward current. Such an effect was observed upon exposure of the test node to TTX or Na^+-free solution (see Figs. 2, 3, 7, and 8). In contrast, abolition of the slow phase of depolarization should result from abolition of the slow inward current. Such an effect was observed upon exposure of the test node to Ca^{++}-free solution (see Figs. 4 and 7). The plateau-type responses which were sometimes observed upon exposure to Ca^{++}-free solution (see action potentials in right half of Fig. 7) are compatible with the voltage-clamp data, since dV_m/dt is negative and the ionic current in this potential region should be outward.

As has previously been stated, Johnson and Lieberman (1971) have

suggested an alternative explanation to the voltage-clamp data purported to demonstrate the existence of a slow inward current. They suggest that membranes of the peripheral cells were responsible for the fast inward current, and that membranes of the deeper cells were responsible for the slow inward current. To explain the agreement between the action potential data and voltage-clamp data presented in this paper and to agree with the suggestion of Johnson and Lieberman (1971), one would have to conclude that the membrane action potentials recorded in the present experiments are not representative of the action potentials of individual cells. Although no intracellular recordings were done in these experiments, there is an abundance of data to make the above conclusion untenable. Intracellular microelectrode recordings from frog myocardium give action potentials similar to those shown in this paper (Wright and Ogata, 1961; Tarr and Sperelakis, 1964; Antoni and Delius, 1965; Hagiwara and Nakajima, 1966). In particular, the rapid and slow phases of depolarization are clearly apparent in the intracellular recordings (Wright and Ogata, 1961; Antoni and Delius, 1965; Hagiwara and Nakajima, 1966).

It is my opinion that the voltage-clamp and action potential data presented in this paper support the hypothesis that two distinct inward currents contribute to the upstroke phase of the action potential in frog atrial tissue: a fast inward sodium current which is responsible for the initial rapid phase of depolarization, and a slow inward current (generally a Ca^{++} current) which is responsible for the slow phase of depolarization and peak of the action potential.

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