SUMMARY

In the perfused rabbit heart, the upstroke of the transmembrane action potential of fibers of the atrioventricular (AV) node presents two distinct components. The first depends strongly on extracellular sodium concentration, but the degree to which it is activated is influenced by extracellular calcium, as indicated by the correlation between its $V_{Na}$ and $[Ca^{2+}]_{o}$. The second component depends on calcium and sodium concentrations and is blocked by Mg ions. An analysis comparing action potentials from atrial (A), atrioventricular (AN), and nodal (N) fibers shows that the second component of the upstroke of the action potential contributes 12%, 27%, and 34% to the total depolarization. The results suggest that the upstroke of the nodal action potential results from the activation of two inward currents, as in ordinary cardiac fibers. We postulate that (1) the degree of steady state inactivation of $g_{Na}$ is larger in N fibers than in A fibers because of the low resting potential of the former, and (2) the contribution of the second channel to the upstroke depends on the time course of the previous depolarization and the potential level at which this component is activated.

IT IS CURRENTLY accepted that excitation of cardiac muscle results from the activation of two inward currents. 

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These currents may give rise to two distinguishable components in the upstroke of the transmembrane action potential. Moreover, the current flowing through the second channel not only contributes to the summit of the action potential, but determines the potential level of the plateau components in the upstroke of the transmembrane action potential. This information is not available for cells of the atrioventricular (AV) node. Because of its size and geometrical features, this structure is not suitable for study by voltage clamp, and direct measurement of ionic currents in nodal cells is not possible with presently available techniques. Therefore, indirect methods must be used to study these currents. In addition, experimental evidence is contradictory regarding the effect of changes in ionic environment on the electrical activity of this region. De Mello reported that the nodal cells are only slightly sensitive to changes in the external sodium concentration, [Na⁺], but others reported that lowering [Na⁺] slows AV conduction and decreases the action potential amplitude. Paes de Carvalho et al. suggested that only the slow component of depolarization is responsible for the upstroke of the nodal potential, but this view is opposed by our report on the existence of two components of the depolarization phase in low sodium medium.

The experiments reported here were performed to investigate the ionic dependence of those two components in an attempt to infer whether they arise from the activation of one or two ionic channels. The configuration of the action potential upstroke, as well as the amplitude and maximum rate of depolarization of its components in media of different ionic composition, were the basis of our analysis. A similar approach has been used to study the action potential of frog atria, frog ventricle, and dog ventricular muscle. Despite the limitations arising from this indirect method and the inherent sources of error, the results permit the following hypothesis to be advanced: The mechanism for excitation of nodal cells is qualitatively the same as that of other cardiac fibers; the main difference is the degree of activation of the rapid phase which is attributed to the fast sodium system.

Methods

Twenty-eight successful experiments were performed. Rabbits weighing between 1.5 and 2 kg were stunned by a blow on the head. The heart was rapidly removed, placed in oxygenated Krebs’ solution at 4°C, trimmed of pericardium and connective tissue, and then perfused through the aorta at 33°C and with constant coronary flow. The hearts were driven at a frequency of 2–3/sec by bipolar electrodes placed in the region of the sinoatrial node. The experimental procedure and recording technique have been described elsewhere. Surface electrograms were displayed synchronously with the transmembrane potential. To measure the maximum rate of rise (Vmax), the action potentials were differentiated with an operational amplifier circuit (designed by Dr. O. Schanne and built in the departmental workshop). The time constant of the amplifier was set to 25 or 6.5 μsec, depending on the fiber type. The relationship between the Vmax and the output of the amplifier was approximately linear.

Solutions

The control solution was a modified Krebs-Henseleit solution with the following composition (mM): Na, 145; K, 5; Ca, 1.25; Mg, 1.2; Cl, 126; SO₄, 1.2; H₂PO₄, 1.2; HCO₃, 25; and glucose, 5.5. Table 1 shows the composition of the experimental perfusion media. Components (e.g., Mg) that were never varied do not appear in the table. Although chelating agents were not used, solutions 4 and 8 are referred to as Ca-free. Tris(hydroxymethyl)aminomethane buffer was used instead of HCO₃⁻ in solutions 9 and 10 to avoid precipitation of Mn, and the pH was adjusted with 0.2 N HCl. This accounts for the increase in chloride concentration. Solutions 1 to 8 were gassed with a mixture of 95% O₂-5% CO₂ whereas 100% O₂ was used for solutions containing Mn. Under these conditions, the pH was 7.35–7.40.

Experimental Protocol

Control records were taken during perfusion with Krebs’ solution. Then, the perfusion was switched to the desired test solution. The dead space of the system was so small that the effect of a test solution could be detected within 5 seconds (see Results, Fig. 6). The time course of the effect of a test solution was observed until a steady state was reached. Only the values measured during the steady state were used for the quantitative analysis presented in Figures 4 and 5 and Tables 3 and 4. The hearts then were perfused again with the control solution. Several experiments could be performed on each heart, either with the same or different test solutions, depending on the viability of the preparation. This was judged by the characteristics of the electrogram, the contractile activity, and ability to maintain coronary flow.

Method of Evaluation

The transmembrane potentials recorded from cells of atrial muscle and the upper and middle layers of the AV junction were measured from enlargements projected by a microfilm reader. The nomenclature of Paes de Carvalho and Almeida was adopted. The classification of a given fiber type was made according to the following criteria: (1) anatomical location determined by direct microscopy during the experiment, (2) temporal correlation with the electrocardiogram, the contractile activity, and ability to maintain coronary flow.

Table 1  Composition (mM) of Perfusion Media Used

<table>
<thead>
<tr>
<th>Medium</th>
<th>Na</th>
<th>Ca</th>
<th>Cl</th>
<th>CO₃H</th>
<th>Mn</th>
<th>Tris</th>
<th>Sucrose</th>
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<tbody>
<tr>
<td>1</td>
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<td>0</td>
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<td>0</td>
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</table>
gram, configuration of the action potential, and (4) activation time, measured as the delay between the time of the stimulus and the onset of the action potential. Although the latter, for a given cell type, varied from one heart to another, it was quite constant for the same heart throughout all the control periods, and during the steady state of an experimental challenge. Mapping the preparation during perfusion with test solutions during the steady state allowed us to classify the cell type according to criteria 1, 2, and 4. To reduce inaccuracies derived from multiple impalements, we selected data for quantitative analysis to include: (1) only the hearts for which all the concentrations of a given ion were tested, and (2) only those measurements for which the cell type could be identified unequivocally. All the transitional fibers were rejected. This procedure reduced the number of data from a total of 360 measurements during test periods to the 265 measurements included in Figures 4 and 5 and Table 3. Including the controls, the results of 1,020 impalements are reported. A less strict application of the criteria outlined above did not modify significantly the relations shown below.

Figure 1 shows a control record from an atrionodal (AN) fiber to illustrate the method applied to measure the different variables studied. As previously described, the upstroke consists of two components with different depolarization rates. These components, here called phase I and phase II, are more evident in records taken at high sweep speed. The inflection point on the upstroke was used to determine the amplitude of each phase. Phase II of the upstroke has been called by others "secondary depolarization" and "slow potential change after the rapid upstroke". The differentiated record of the upstroke shows a distinctive peak followed, for most AN and nodal (N) fibers, by a slow wave which represents the second component of depolarization. This becomes evident when both traces are displayed synchronously at a high sweep speed. Similar records from frog ventricle were reported by Antoni and Delius. Even though recordings of dV/dt of phase II usually are obtained the accuracy of the measurement of Vmax depends on the fiber type. In fibers with a rather high depolarization rate [atrial (A) and some AN cells], Vmax of phase II is small compared to Vmax of phase I. Therefore, its determination is less accurate because of the low resolution of the calibration curve in that range. Consequently, the values of Vmax of phase II for A and AN fibers reported in Tables 2 and 3 should be considered as an approximation. In contrast, Vmax of both components of the upstroke for N fibers could be measured more precisely because they are of the same order of magnitude. The results were tested for statistical significance with Student's t-test.

Results

Table 2 shows the control values of membrane potential parameters of atrial (A), atrionodal (AN), and nodal (N) fibers for all hearts. The values of resting and action potential and Vmax, of phase I agree with previous reports. The amplitude of the two components of depolarization and Vmax for phase II have not been previously reported for these fibers. Niedergerke and Orkand and Matsubara and Matsuda carried out similar experiments on frog and dog ventricular muscle, and although they distinguished selective effects on different portions of the action potential, the variations of Vmax observed in different ionic environments were expressed as a percentage change from the control.

If one compares the number of measurements of action potential amplitude and of phases I and II of the upstroke, it is evident that these components could not be identified in all the records obtained. In 57% of A fibers, the upstroke consisted only of a very fast depolarization so that a slow phase was not discernible (see control record of Fig. 2). However, the resting potential, the action potential amplitude, and the maximum rate of rise of these two groups did not differ significantly and therefore they appear together in the table. On the other hand, 25% and 20% of action potentials from AN and N fibers had upstrokes with such a smooth transition between phases that it was difficult to determine the inflection point. Antoni and Delius reported that 14% of the action potentials recorded from frog ventricle presented a smooth upstroke without a distinguish-
TWO INWARD CURRENTS IN AV JUNCTION/Ruiz-Ceretti and Ponce Zumino

### Table 2

Membrane Potential Parameters of Different Cell Types under Control Conditions

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Resting potential (mV)</th>
<th>Action potential amplitude (mV)</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mV</td>
<td>V/sec</td>
</tr>
<tr>
<td>Atrial</td>
<td>-80.4 ± 0.9 (47)</td>
<td>94.4 ± 0.7 (151)</td>
<td>83.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>Atnonodal</td>
<td>-68.8 ± 0.9 (44)</td>
<td>75.4 ± 0.5 (136)</td>
<td>53.5</td>
<td>±0.9</td>
</tr>
<tr>
<td>Nodal</td>
<td>-59.6 ± 1.3 (29)</td>
<td>61.6 ± 0.5 (90)</td>
<td>38.1</td>
<td>±1.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Number of measurements are given in parentheses.

able breaking point. The mean contribution of phase II to the total amplitude of the upstroke amounts to 12%, 27%, and 34% for A, AN, and N cells, respectively. Regardless of the electrical equivalent applicable to these structures, the maximum depolarization rate is an approximate measure of the net ionic current. Thus, one can estimate that the net current during phase II depolarization represents approximately 11%, 30%, and 49% of the current underlying the fast depolarization.

### EFFECTS OF CALCIUM AND SODIUM CONCENTRATIONS

Figure 2 shows typical changes in action potential configuration observed during perfusion with calcium free solution. Removal of extracellular calcium had two different effects on the shape of the action potential. The first type of response (middle column in Fig. 2) was characterized by a decrease in the amplitude of phase I; therefore the inflection appeared at more negative potentials. There was an evident delay between the two phases, and the time course of phase II was slowed. The summit of the action potential was rounded, and the total duration increased considerably. The second type of effect (third column of records) resembled the long duration response described by Tarr and the one appearing during exposure to Mn⁺⁺ (see Fig. 6). The amplitude of phase I of the action potentials from AN and N fibers decreased. Phase II merged into a long, rounded plateau. The transition from phase I to phase II may be abrupt, as in the AN fiber, or may occur with appreciable delay. For all fiber types, removal of calcium produced a slowing of phase I, regardless of the type of change in configuration. This decrease is summarized in Table 3.

Figure 3 shows the responses of N fibers to changes in extracellular sodium and calcium concentrations. The numbers in brackets correspond to the solutions in Table 1. Lowering [Na⁺]₀ (middle column) resulted in a decrease of the amplitude of phase I. This is in agreement with results we have reported previously. Moreover, a delay appeared

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![Figure 2](image-url)  
*Effects of calcium removal (solution 4 of table 1) on action potential configuration. C = control of atrial (A), atrionodal (AN), and nodal (N) fibers. Notice different action potential configuration in middle and right column. Vertical calibration = 20 mV; time calibration = 50 msec. (Redrawn from original records)*
between phase I and the beginning of phase II. Increasing \([Ca^{2+}]_0\) to 5 mM produced an increase in action potential amplitude, and the slow component no longer was discernible because the inflection point was less marked. A low sodium concentration and omission of Ca ions from the solution (medium 8) produced a marked decrease in the amplitude of phase I and the appearance of the long duration response shown in Figure 2.

### ANALYSIS OF THE SODIUM-DEPENDENCE OF ELECTRICAL ACTIVITY

If the electrical transient recorded from nodal cells results from a regenerative response of the membrane, the amplitude of the upstroke and its components should vary as a function of the logarithm of the extracellular concentration of the current-carrying ions, provided the mean ionic concentration of the cytoplasm does not change during the experimental period.** This slope of this relation may indicate whether only one or more than one ionic species are involved. In the first case, the slope approaches the value predicted by the Nernst equation. In addition, there should be a correlation between \(V_{\text{max}}\) and the extracellular concentration. Although we did not check the value of \(V\) at which \(V_{\text{max}}\) occurred, this does not invalidate our approach because changes in the equilibrium potential do not alter the voltage-dependence of the ionic current.** Our results were tested for their relationship with the log of \([Ca^{2+}]_0\) and \([Na^+]_0\).

Figure 4 shows the relationship between \([Na^+]_0\) and the amplitude of the action potential, the overshoot, and phases I and II for A, AN, and N cells. The statistical significance of the difference between the control values and the measurements performed during exposure to test solutions is indicated by asterisks. For A fibers, the action potential amplitude, the overshoot, and the amplitude of phase I show a clear linear dependence on sodium concentration. The slope of the line relating action potential amplitude to log \([Na^+]_0\), 59 mV for a 10-fold change, agrees with the value predicted by the Nernst equation (60.7 mV for 33°C). The amplitude of phase II decreases as the sodium concentration increases. In AN fibers, the slope of the relation for action potential amplitude is 45 mV per decade. The curves for the overshoot and the amplitude of phase I show a nonlinear increase and level off at concentrations between 115 and 145 mM. The amplitude of phase II did not change significantly with sodium concentration. In N fibers the amplitude of phase II also is insensitive to changes in \([Na^+]_0\), but, in contrast to AN fibers, there is a linear relationship, with a slope of 25 mV per decade, between phase I and log \([Na^+]_0\). The total amplitude of the action potential and the overshoot depend less strongly on \([Na^+]_0\) at concentrations higher than 85 mM. The resting potential did not change significantly in solutions containing low concentrations of sodium. Table 3 shows the values of \(V_{\text{max}}\) for both phases for all fiber types as a function of sodium and calcium concentration. Low sodium solutions (1, 2, and 3) drastically decreased \(V_{\text{max}}\) of phase I for A fibers. A similar but less pronounced effect was observed for AN and N fibers. The mean decrease in 72.5 mM sodium was between 40% and 46% for the latter, whereas a 73% reduction was found for A fibers. The \(V_{\text{max}}\) of phase II did not change significantly in A or AN cells, but it was reduced in N cells when exposed to solutions containing 85 and 72.5 mM sodium (solutions 2 and 3).

### ANALYSIS OF THE CALCIUM-DEPENDENCE OF ELECTRICAL ACTIVITY

The plots of the relationship between action potential parameters and \([Ca^{2+}]_0\) for A fibers are not presented because only \(V_{\text{max}}\) of phase I showed a significant relationship to \([Ca^{2+}]_0\) throughout the range of calcium concentrations explored (see Table 3, solutions C, 4, 6, and 7). As for the other variables, the action potential amplitude and the amplitude of phase I decreased significantly only in Ca-free medium (75 ± 3 mM and 51 ± 3 mM, respectively; \(P < 0.001\), compared to control values). In addition, phase II became undetectable at \(Ca^{2+}\) concentrations higher than 1.25 mM.

In Figure 5, which shows the calcium-dependence of AN and N cell action potentials, the values corresponding to...
FIGURE 3 Effects of low sodium, high calcium, and a combination of low sodium plus calcium-free solutions on action potential shape in nodal (N) fibers. C = control recordings; the numbers in brackets correspond to the solutions as in Table I: vertical calibration = 20 mV; time calibration = 50 msec in upper row and 20 msec in lower row. (Redrawn from original records.)

Ca-free medium have been plotted to represent a concentration of 0.1 mM, because chelating agents were not added to the medium. In AN fibers the action potential amplitude and the overshoot increased slightly in relation to log $[\text{Ca}^{2+}]_o$ between 0.1 and 1.25 mM and reached a plateau beyond the higher value. The slope of the curve relating action potential amplitude to $[\text{Ca}^{2+}]_o$ is 12 mV per decade (for $[\text{Ca}^{2+}]_o < 1.25$); this is less than half the value predicted by the Nernst equation (30.4 mV at 33°C). The figure shows that the relative contribution of the two phases to the total amplitude differs at low and high calcium concentrations.

In N fibers the slope of the relations between action potential amplitude and overshoot and log $[\text{Ca}^{2+}]_o$ is 6 mV per decade. The amplitude of phase I was depressed at

FIGURE 4 a (upper panels) plots of action potential amplitude (Δ) and amplitude of phase I (●) and II (○) vs. log $[\text{Na}^+]_o$. b (lower panels): overshoot vs. log $[\text{Na}^+]_o$. Each point represents the mean ± SEM of 8–16 measurements for atrial (A) fibers, 10–16 for atrionodal (AN) fibers, and 10–15 for nodal (N) fibers from four hearts. *P < 0.01; **P < 0.005; ***P < 0.001. Curves are drawn as best fits by hand.
concentrations below normal, whereas the amplitude of phase II increased between 0.1 mM and 1.25 mM [Ca\(^{2+}\)]_o and then decreased at 2.5 mM. No data are available for the amplitude of the two phases of the upstroke for [Ca\(^{2+}\)]_o = 5 mM because they could not be measured at this concentration because of the change in action potential configuration (see Fig. 3). As for the influence of calcium ions on the maximum depolarization rate, Table 3 shows that concentrations below normal decreased \(\bar{V}_{\text{m}}\) of both phases in AN and N fiber action potentials. The values leveled off for concentrations higher than 1.25 mM. It should be mentioned that the resting potential did not change significantly as a function of calcium concentration. In Ca-free solution the mean resting potential was 66 ± 1.3 mV and 59.8 ± 1.4 mV for AN and N cells, respectively.

Removal of calcium chloride from a low sodium solution (solution 8 of Table 1) resulted in a further decrease in amplitude of phase I from 49 ± 2.6 mV to 36 ± 2.7 mV in AN cells (\(P < 0.02\)), and from 32 ± 2.6 to 27 ± 3 mV in N cells (\(P < 0.05\)). The amplitude of phase II increased from 18 ± 2.3 to 28 ± 3 mV in AN fibers (\(P < 0.02\)), and from 19 ± 2.1 to 27 ± 2.3 mV in N fibers (\(P < 0.001\)). These changes correspond to the action potential configuration shown in Figure 3. The total action potential amplitude did not change significantly. The effects on \(\bar{V}_{\text{m}}\) for both fiber types are shown in Table 3, where the statistical significance corresponds to the difference between the mean values in solutions 2 and 8.

**EFFECT OF MANGANESE IONS**

Manganese was used as an inhibitor of the current transported through the slow channel. The effects of Mn\(^{2+}\) on the action potential of A, AN, and N fibers are shown in Figure 6. Each of the rows in this figure was obtained during long-lasting impalements so that the time course of the effect of Mn\(^{2+}\) could be followed for the same cell until a steady state was reached. The records were photographed every 5 seconds and the full effect occurred...
within 1–1 ½ minutes. The time interval between the records shown in Figure 6 is 10–15 seconds. For three fiber types Mn⁺⁺ produced a decrease in action potential amplitude and a progressive abolition of the second phase of the upstroke, which was then replaced by a long-lasting plateau. This type of response persisted in A fibers during the steady state. In contrast, a complete abolition of the second phase in records from AN cells led to a final stage during which the fast phase of the upstroke was followed by a rapid repolarization so that the action potential acquired a triangular shape. The long-lasting plateau also was observed in records from N fibers during exposure to Mn⁺⁺. However, the disappearance of this type of response did not lead to the triangular action potential found for AN fibers, but rather to a small and very slow depolarizing response. In some experiments potential transients (difficult to identify as all-or-none responses or electrotonic potentials) occurred in the N layer at a stage when the triangular action potentials were recorded from AN fibers (Fig. 6, last record in the N row). The amplitude and $V_{\text{mH}}$ of the first phase consistently decreased during exposure to Mn⁺⁺. Recovery always was complete in a few minutes.

Table 4 shows the effects of two different concentrations of Mn⁺⁺: Two groups of data are given. Those identified as single penetration (SP) correspond to seven long-lasting impalements during which the time course of the effect of Mn⁺⁺ could be followed for the same cell. The degree of depression of phase II was a function of the concentration of Mn⁺⁺. The decrease in $V_{\text{mH}}$ of phase I during exposure to Mn⁺⁺ may be due to the reported inhibitory effect of the Tris buffer on the sodium current.

**Discussion**

Discussion of these results in terms of the excitation mechanism or ionic currents underlying a given phase of the action potential requires an evaluation of the validity of our approach to analyze the ionic dependence of the two phases of the upstroke, on the basis of their dissociation under different experimental conditions. Our method is by no means original; it has been widely used by others, and most frequently prior to the extensive application of voltage clamp techniques to cardiac muscle. As reviewed by Reuter, several reports on the effects of ionic changes or inhibitors of membrane conductance on action potential configuration indicated that the fast-rising phase of the action potential depended on a mechanism different from that determining the overshoot and the plateau. Wright and Ogata suggested that the frog atrial potential was comprised of a rapid and a slow prolonged depolarization. This conclusion was based on the different sensitivities of the spike and the plateau to acetylcholine and anoxia. Antoni and Delius separated two phases in the upstroke of the frog action potential (ventricular trabecula), and showed that these responded differently to changes in $[\text{Na}^+]_o$, $[\text{K}^+]_o$ and exposure to epinephrine. Niedergerke and Orkand and Matsubara and Matsu showed the existence of a calcium current in frog and dog myocardium from analysis of the action potential configuration and dependence of the overshoot and $V_{\text{mH}}$ on $[\text{Ca}^{2+}]_o$ and $[\text{Na}^+]_o$. The two components of the upstroke became distinctively separated in low sodium solution, as reported by us for AV nodal cells. Moreover, Langer quoted the report of Niedergerke and Orkand as strong evidence in favor of the existence of an inward calcium current. Tarr found a good correlation between changes in configuration of the upstroke and the ionic currents underlying it. Benitez et al. estimated the relative contribution of both inward currents to the His bundle action potential from an analysis of changes in shape observed during exposure to tetrodotoxin (TTX), MnCl₂, and high $[\text{K}^+]_o$. This evidence suggests that the approach used here is valid to draw qualitative conclusions about the ionic currents determining the action potential.

The two phases of the upstroke were identified in terms of the inflection point that often became a notch under experimental conditions. It has been suggested that these notches arise from electrotonic spread in neighboring

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**Table 4** Percent Changes Caused by Mn⁺⁺

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$[\text{Mn}^{2+}]_o$ (mM)</th>
<th>Group</th>
<th>Resting potential (mV)</th>
<th>Action potential amplitude</th>
<th>Phase I</th>
<th>Phase II</th>
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<td></td>
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<td>AMP</td>
<td>$V_{\text{mH}}$</td>
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</table>

AMP = amplitude; SP = single penetration.

Mean values are from 11 test periods performed on four hearts. All single penetrations were in the same heart, except for one, denoted by an asterisk.
regions and that part of, or the whole, electrical transient recorded from AV cells might be of electrotonic nature. However, the following arguments oppose this view:

1. During ischemia or at high driving rates, stimulation of an AV cell before full repolarization or recovery resulted in low and slow responses which failed to propagate. This suggests that time-dependent processes of activation and inactivation of ionic currents are involved.

2. Changes in sodium and calcium concentrations profoundly affect the upstroke after the foot of the action potential. If the potential transient were electrotonic in nature, this would indicate that the passive electrical properties of the membrane are strongly dependent on sodium and calcium concentrations. This disagrees with Reuter's finding of no change in membrane resistance and capacity in Na-free Tyrode's solution at calcium concentrations of 0, 1.8, and 7.2 mM.

3. The changes in the nodal action potential configuration observed during exposure to solutions with differing ionic content or containing Mn2+ are highly comparable to the recordings obtained by others.4,10 Under current clamp conditions, when electrotonic spread is considered to be negligible.

4. Paes de Carvalho et al.13 remarked that the nodal action potential propagates with a finite velocity while keeping an appreciable overshoot. Their arguments and our linear relationship between overshoot and log [Ca2+]o are difficult to reconcile with pure electrotonic interaction. Moreover, the recordings of Paes de Carvalho and Zipes and Mendez show that the existence of an inflection point in the upstroke does not depend on the mode of activation, antegrade or retrograde.

5. If a blocker of an active current, such as Mn2+, produces a given change in the upstroke, it is unlikely that this part of the potential transient is electrotonic in nature.

From all the above, we conclude that the electrical transient recorded from AV cells represents a regenerative membrane response and that the notches observed in the upstroke are not electrotonic in nature, even though there always is a certain degree of electrotonic interaction in cardiac muscle. Therefore, it is justified to analyze our data according to the electrophysiological criteria usually applied to active membrane responses. We will discuss our results in terms of the model for excitation of cardiac muscle,1,17 and therefore we postulate that the two components of the upstroke of the nodal potential represent the two inward currents described for several types of cardiac tissues.15,16 The relative contribution of each current component to the upstroke will vary according to the amount of current activated. This, in turn, is a complex function of potential, time, and ionic gradients. This should be kept in mind when discussing the plots shown in Figures 4 and 5, where only one of the variables determining the current is considered. Moreover, the strict separation of both currents is difficult because during an action potential there is a voltage range within which the currents overlap: the slow inward current is activated and contributes to depolarization before the fast inward current has subsided completely. When the latter is depressed, separation becomes more distinct, as shown by the delay between phases I and II (Figs. 2 and 3).14,18 The opposite occurs when the fast component is predominant and may obscure the contribution of the slow current to the upstroke, as observed in 57% of control A fiber action potentials and in N fiber action potentials exposed to solutions containing 5 mM calcium (Fig. 3).

The plots of Figure 4a indicate that a sodium current underlies the depolarization during phase I of the upstroke in the three fiber types. However, the low slopes of the curves relating the action potential amplitude to [Na+]o for AN and N cell suggests that another ionic current contributes to the total action potential amplitude. This interpretation is further supported by the fact that the overshoot does not change significantly for concentrations between 85 and 145 mM. It is known that the size of the overshoot or the slope of the relation between action potential and log [Na+]o does not adequately reflect the sodium-dependence of the upstroke for cases in which the sodium mechanism becomes inactivated before the summit of the action potential is reached, as in guinea pig myocardium.19 Departure from linearity of some of the plots shown in Figures 4 and 5 most likely results from the fact that not only the ionic gradients but the kinetics of activation and inactivation play a role in determining the amount and rate of depolarization produced by a given current.

Although phase I of the upstroke in atrioventricular cells depends on [Na+]o, its Vmax is also influenced by the extracellular calcium concentration (Table 3). Because removal of calcium did not produce depolarization, this finding may be explained by the known influence of [Ca2+]o on the h∞ curve. This mechanism also explains the calcium dependence of Vmax of atrial action potentials (Table 3). Similar effects were reported for the squid axon17 and for Purkinje fibers.18 Beeler and Reuter4 found a shift of the potential for half inactivation of the Na system from -55.7 mV to -64 mV when the [Ca2+]o was lowered to 0.2 mM. The Vmax of phase II of AN and N action potentials varied with [Ca2+]o in the same range as reported for dog ventricular muscle.19 Only for N fiber action potentials does the Vmax of phase II vary with both sodium and calcium, and the slopes of the curves relating the action potential amplitude and the overshoot to [Ca2+]o are much lower than expected from the Nernst equation. These observations may imply that changes in current kinetics result in the size of the overshoot being less than expected for a calcium electrode or that both ions contribute to the current during phase II of the upstroke. The existence of a mixed ionic current for this phase is plausible because the slow inward channel is not selective for Ca ions.16 Moreover, a competition between Na+ and Ca2+ also could explain the observed results. The decrease in the amplitude of phase II with increasing [Ca2+]o (Fig. 5), may result from an increased Ca uptake with the subsequent decrease of calcium equilibrium potential (ECa).20 This seems likely because the overshoot and the total amplitude are not depressed in solutions containing a high calcium concentration, and the figure shows that the overshoot is a linear function of the external calcium concentration. Other possibilities are (1) that the reduction of phase II is due to a lower degree of steady state inactivation of the sodium system,16 and (2)
that the lack of inflexion point in 5 mm Ca\textsuperscript{2+} results from a greater degree of activation of phase II than of phase I current, but these hypotheses were not verified experimentally.

Thus interpreted, our results support the hypothesis that the excitation mechanism currently accepted for cardiac muscle is applicable to cells of the AV node. This interpretation contradicts the mechanism suggested by others\textsuperscript{14,15} who favored the view that the nodal potential results from the activation of only the slow channel. It is unlikely that this discrepancy is due to a mistake in our classification of the fiber types because of the agreement between our control values and those reported by others. Furthermore, Freeman and Turner\textsuperscript{16} observed notching in 75\% of the phase plane trajectories recorded from AV cells of the rabbit heart. These corresponded to inflection points in the upstroke of the action potential and were interpreted as an indication of two distinct current-carrying mechanisms. This agrees with our finding of two distinct phases in 80\% of N fiber action potentials. The existence of the inflexion point on the upstroke does not prove, per se, the two channel hypothesis, but the different sensitivity of the resulting phases to experimental challenges strongly supports it. On the other hand, the proposal that the nodal potential results solely from the activation of the slow channel cannot be sustained in view of the recent results of Shigeto and Irisawa,\textsuperscript{4,17} who showed that the voltage-dependence of V\textsubscript{max} of AV potentials corresponds to that of the fast sodium system.

Moreover, our unpublished observations on the effect of TTX (10^{-7} g/ml) on the nodal potential showed that this inhibitor selectively depresses the amplitude and the V\textsubscript{max} of phase I of the action potential. This is accompanied by an increase in the amplitude of phase II which is triggered at more negative levels than under control conditions. The V\textsubscript{max} of this phase is not altered by TTX. This confirms the view that the relative contribution of each current to the upstroke is a complex function of time and the potential at which it is activated. It is difficult to reconcile these results with the finding of persistence of automatic activity in the AV junction in the presence of TTX.\textsuperscript{4,17} However, this was observed in cells of the lower node (NH) and Wit and Cranefield,\textsuperscript{18} reported that these cells were less affected by blockers of the ionic conductances.

The apparent abolition of regenerative activity in some N fibers during exposure to Mn\textsuperscript{2+} (Fig. 6) seems to support the hypothesis of a single channel. However, because, under our experimental conditions, effective stimulation of nodal cells depends on the arrival of an activating wavefront, failure of excitation in this region may be secondary to action potential changes in cells other than the N fibers. During exposure to Mn\textsuperscript{2+} the action potential of AN fibers is reduced to a small depolarization whose V\textsubscript{max} has fallen to about 40\% of its original value (Table 4). It is known that the margin of safety for transmission in the AV junction is very low,\textsuperscript{20} due to the characteristics of the action potential, and to structural features.\textsuperscript{21} Therefore, electrotone spread must be weak and the effectiveness for propagation of an action potential, as that shown for AN fibers exposed to Mn\textsuperscript{2+}, is very low. Moreover, failure of conduction from AN to His bundle as reported by Zipes and Mendez\textsuperscript{21} (in their Figure 1C) might not result necessarily from a lack of response in the N layer, but from a very low and slow potential (as shown in our Figure 6, 4th and 5th N record) which can be ineffective to excite other cells below in the pathway. This argument was rejected by Zipes and Mendez on the basis of gross depression of the overshoot of N potentials before block occurred. However, this finding agrees with our results that the overshoot depends mainly on the slow current. Moreover, due to the short length constant of this region, the subthreshold responses shown in AN fibers during retrograde block, during exposure to Mn\textsuperscript{2+},\textsuperscript{22} are unlikely to appear unless some degree of activity remains in the N layer.

From the above discussion the following is proposed to explain the differences in the action potentials of A, AN, and N fibers in the rabbit heart: (1) These differences are mainly quantitative inasmuch as the degree of activation of the two inward currents is concerned. (2) The fast current, sodium-dependent, is responsible for only 66\% of the total depolarization in nodal cells. Several transitional degrees exist. (3) The slow membrane potential of nodal cells may explain the inactivation of a larger fraction of maximum \(g_{NH}\) at the resting level as suggested by the voltage-dependence of \(V_{max}\) in cells from the AV node\textsuperscript{4,17} and upper His bundle.\textsuperscript{22} (4) The two ionic channels seem to be independent, but the contribution of the slow channel to the upstroke depends on the time course of the previous depolarization and the potential level at which it is activated.

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