Intracellular Potassium Activity in Rabbit Sinoatrial Node
Evaluation during Spontaneous Activity and Arrest

Augustus O. Grant and Harold C. Strauss
From the Departments of Medicine and Pharmacology, Duke University Medical Center Durham, North Carolina

SUMMARY. Measurement of intracellular potassium activity ($a_K$) is important in furthering our understanding of the basis of the low resting and maximum diastolic potentials and the membrane currents underlying pacemaker activity in the sinoatrial node. $K^+$-selective microelectrodes have been successfully applied to measure $a_K$ in Purkinje fibers, ventricular muscle, and atrium. Similar measurements in the sinoatrial node are difficult, however, because of the cellular heterogeneity, small cell size, and spontaneous activity. We measured $a_K$ in shortened, electrically homogeneous strips of rabbit sinoatrial node. Transmembrane potential was recorded with microelectrodes of 20–30 MΩ resistance. Ion-sensitive signal was measured with similar microelectrodes filled with a $K^+$-selective liquid ion exchanger. All experiments were performed with simultaneous impalements with ion-sensitive and conventional microelectrodes. During spontaneous activity, cycle length was 341 ± 12 msec, maximum diastolic potential (MDP) was —62.0 ± 1.4 mV, and ion-sensitive signal ($V_K$) was 27.0 ± 1.2 mV. Apparent $a_K$ calculated from the maximum diastolic potentials and $V_K$ was 151 ± 6 mM. Replacement of chloride ion by thiocyanate ion led to rapid reversible arrest with a maximum diastolic potential of —49 ± 3.3 mV and $V_K$ 25.0 ± 2.7 mV. Calculated $a_K$ was 85 ± 3 mM ($n = 12$, $[K^+]_o = 4$ mM). $a_K$ measured after sequential arrest by thiocyanate ion and 1 mg/liter verapamil agreed within 2 mM. The close agreement between values obtained in preparations arrested by thiocyanate ion and verapamil suggests that, over the period of time required for arrest, the agency of arrest does not change $a_K$. We also measured a value of 80 ± 3.3 mM for $a_K$ in quiescent right atrial strips. We conclude that (1) $a_K$ is similar in the sinoatrial node and right atrium, (2) the slow frequency response of the ion sensitive microelectrode may lead to a considerable overestimate of $a_K$, (3) the low MDP in SA nodal cells compared with the atrium is not the result of a low $a_K$, and (4) the net driving force on potassium ions is outward throughout the range of pacemaker potentials at normal $[K^+]_o$. (Circ Res 51: 271–279, 1982)

MEASUREMENTS of the transmembrane distribution of $K^+$ with ion-sensitive microelectrodes have confirmed that the distribution of $K^+$ is largely responsible for the maximum diastolic and resting potentials in Purkinje fibers and working myocardium (Walker and Ladle, 1973; Lee and Fozzard, 1975; Fozzard and Lee, 1976; Miura et al. 1977; Fozzard and Sheu, 1980). Maximum diastolic and resting potentials of sinoatrial (SA) and atrioventricular (AV) nodal cells are much lower (Irisawa, 1978; Sperelakis, 1979). A relatively high permeability to other ions compared with potassium or a low level of intracellular potassium ($K^+_i$), may account for the low potentials in nodal tissues. Early experiments have yielded conflicting results. Davies et al. (1952) reported much lower total tissue potassium content in the right atrium, SA, and AV nodes than in ventricular myocardium of the ox. A similar distribution of total tissue potassium in frog and turtle atrium and sinus venosus compared with ventricular myocardium was reported by Mazel and Holland (1958). On the other hand, Danielson (1964) reported that $[K^+]_o$ was the same in all regions of the frog heart. DeMello and Hoffman (1960) reported similar $[K^+]_i$ in the rabbit atrium and SA nodes. Recently, considerable controversy has arisen as to the ionic current(s) responsible for pacemaker activity in the sinus node (Yanagihara and Irisawa, 1980; Brown and DiFrancesco, 1980; DiFrancesco and Ojeda, 1980; Maylie et al., 1981). Previous results suggested that pacemaker activity resulted from a decline in $K^+$ conductance (Irisawa, 1978). However, Bonke (1973) and Maylie et al. (1981) failed to show a consistent change in membrane conductance between early and late diastole. Recent measurements of extracellular $K^+$ activity and currents under voltage clamp condition suggested that pacemaker activity resulted from an increasing inward time-dependent current (Maylie et al., 1981). Further, failure to define a reversal potential of this current around the expected $K^+$ reversal potential was consistent with a minor role, if any, of changes in $K^+$ permeability. Actual measurement of $a_K$ and calculation of the true $K^+$ reversal potential would more clearly define the role of $K^+$ in the pacemaker mechanism. Therefore, a knowledge of the transmembrane distribution of $K^+$ would be important in an understanding, not only of the comparative electrophysiology of various regions of the heart, but also of the current mechanisms underlying pacemaker activity in the SA node.
Activities rather than the concentrations determine the contribution of ions to membrane potential and the magnitude and direction of membrane currents during activity. Using K+-selective liquid ion exchanger microelectrodes, Walker and Ladle (1973) showed that $a_k$ differed in each region of the frog heart, being lowest in the sinus venosus. In contrast, $a_k$ has been reported to be similar in rabbit atrium and ventricle (Lee and Fozzard, 1975; Skinner and Kunze, 1976). Most electrophysiological experiments on nodal tissues are performed in rabbit SA and AV nodes. We therefore decided to measure $a_k$ in rabbit sinus node and atrium with K+-selective IS microelectrodes.

Measurement of $a_k$ in rabbit sinus node presents major problems. It requires potential determinations with the K+-selective microelectrode and an independent measure of membrane potential with a conventional microelectrode. The small cell size places a major limitation on the use of double-barreled microelectrodes. Because of the spontaneous activity of nodal cells, the slow frequency response of the K+-selective microelectrodes may introduce an artifact. In an attempt to circumvent these problems, we measured $a_k$ in small electrically homogeneous preparations of rabbit sinus node using single barreled conventional and K+-selective microelectrodes during both spontaneous activity and arrest induced by SCN− or verapamil. Our results show that $a_k$ is the same in rabbit SA node and atrium and that the slow frequency response of the K+-selective microelectrode may introduce a considerable measurement artifact. Low potentials in the node are not the result of low $a_k$.

Methods

Preparation and Solutions

Rabbits weighing 1.5–2 kg were heparinized with 1500 U of sodium heparin (Upjohn), iv. Each rabbit was killed by a blow to the neck and the heart rapidly excised. That region of the right atrium bounded by the superior and inferior vena cavae, the crista terminalis, and the interatrial septum was dissected free. Under an Olympus dissection microscope, cuts 300–400 μm apart were made at right angles to the crista terminalis starting at its rostral end (Fig. 1). After each cut, the preparation was exposed briefly to a high-calcium solution and allowed 20 minutes to recover in Tyrode’s solution. The procedure was repeated until 4–5 parallel strips were obtained. Each strip was ligated 1–1.5 mm medial to the crista terminalis with 8-0 silk suture or monofilament nylon. After a 20-minute recovery period, one or two additional ligatures 300–400 μm apart were applied medially. This yielded small preparations approximately 300–400 μm square (Noma and Irisawa, 1976; Seyama, 1979). The shortened preparations were pinned to the waxed base of the same chamber.

The modified Tyrode’s solution had the following composition (mM): Na+, 149.8; K+, 4; Ca++, 2.7; Mg++, 0.5; Cl−, 140.4; HCO₃⁻, 18; H₂PO₄⁻, 1.84; dextrose, 5.5. The high calcium solution was of similar composition but, with the [Ca]++, was 8.1 mM. Thiocyanate (SCN⁻) Tyrode’s solution had the same concentration of Na+, K+, Ca++, and Mg++. The NaCl and KCl were replaced by their corresponding thiocyanate salts ([SCN⁻] = 135 mM). This solution also contained (mM) Cl−, 6.5; HCO₃⁻, 18; H₂PO₄⁻, 1.84. Verapamil hydrochloride (Knoll Pharmaceutical) was prepared as a stock solution and aliquots were added to the Tyrode’s solution to give a final concentration of 1 mg/liter (2 × 10⁻⁶ M).

Principles of Intracellular Potassium Activity Measurement

We determined $a_k$ according to method 2 of Walker and Brown (1977). This method required the following: (1) fabrication, and calibration of ISME in known solutions; (2) electrical measurement of potential with both ion-sensitive and conventional electrodes, the ISME potential being referenced to the external solution; and (3) data analysis for actual calculation of $a_k$ using results of the ISME calibration and the potential measurements. A description of (1) and (2) is followed by the protocol that we used during our typical experiment. We finally describe the technique of data analysis.

Microelectrode Fabrication and Calibration

Micropipettes were pulled from filament-borosilicate glass (1 mm o.d.), and filled with 3 m KCl buffered to a pH of 8.5 with glycylcyline (Thomas, 1978). Microelectrodes of resistance 20–30 MΩ were selected for transmembrane potential measurements.

The consistent fabrication of similarly pulled K+-sensitive
microelectrodes was difficult. Initially, we used modifications of Walker’s "tri-n-butylchlorosilane method" (Walker, 1971; Skinner and Kunze, 1976; Thomas, 1978). Despite variation of the silane concentration from 1 to 5%, only 10–20% of the electrodes were calibrated successfully (see below). The majority had blocked tips. We abandoned this silanization technique in favor of a vapor method which yielded 40–80% calibrated electrodes. Micropipettes were exposed to a dry vapor of trimethylchlorosilane (Pfaltz and Bauer) at room temperature for 1–2 minutes, then baked at 200°C for 1 hour. The micropipettes were back-filled with the liquid ion exchanger resin (Corning 477317) and stored overnight. Prior to use, the microelectrode barrel was back-filled with 0.5 mM KCl. The slope of the IS microelectrode was determined in pure 0.01, 0.1, and 1 mM KCl solutions. Selectivity was determined using Tyrode’s solution as the single mixed solution, according to the method described by Walker and Brown (1977).

A plot of the ISME potential against the logarithm of the activity of the pure potassium-calibrating solutions gave a straight line. The ISME potential in the mixed solution then was projected onto the line and the corresponding activity $a_K$ mixed read from the graph. The selectivity coefficient $K_{KNa}$ then was calculated according to the following equation:

$$K_{KNa} = \frac{a_{K_{mixed}} - a_K}{a_{Na}}$$

where $a_K$ = calculated potassium activity of a pure K solution ($[K^+] = 40$ mM),

$a_{K_{mixed}}$ = Potassium activity in mixed solution determined by interpolation as described above.

$a_{Na}$ = Calculated sodium activity of the mixed solution.

IS microelectrodes selected for use had slopes greater than 55 mV/decade (range 58–62) and a selectivity coefficient of <0.03 (range 0.009–0.025). Although preparations were exposed to SCN$^-$ Tyrode’s or verapamil-containing solutions only when the IS microelectrode was intracellular, we also performed calibration in SCN$^-$ Tyrode’s solution and verapamil-containing solutions. Figure 2 illustrates that calibration curves in Tyrode’s and SCN$^-$ Tyrode’s solution were virtually the same. Similar results were obtained in Tyrode’s solution containing 1 mg/liter verapamil.

Calibration curves were repeated at the end of the experimental protocol. In some experiments, we saw a parallel shift in the calibration curve without a change of slope and with selectivity coefficients agreeing to within 0.003 of the preimpalement value. We discarded four completed experiments because changes in selectivity exceeded this range.

The electrical response time of the ISME was assessed by a replica of the source signal. The bath reference electrode consisted of an Ag/AgCl pellet (In Vivo Metric Systems) embedded in a 3-mm tube containing 3 mM KCl in 3% agar.

The output of both electrometers was displayed on an oscilloscope (Tektronix RM 565). Data were collected on a Gould 2400 recorder.

In our initial experiments the output from the conventional microelectrode was filtered with a variable active filter (cut-off frequency 1 Hz — 100 kHz), the output of the IS microelectrode was subtracted electronically and the difference signal was also registered on the recorder. When it became clear that we could not compensate for the differences in frequency response of the ISME and conventional microelectrodes by filtering, we recorded the difference signal without prior filtering (discussed in details under Results).

**Experimental Protocol**

Following the complete dissection, the preparations were allowed 30 minute to equilibrate in the tissue chamber before any impalements were made. Approximately 60% of the sinus node preparations were spontaneously active; we report data on these preparations only. Impalements were made in the strips to determine the action potential characteristics.

We selected for study those strips with action potentials that had the following characteristics: (1) maximum diastolic potential less than or equal to -70 mV, (2) a smooth transition between phases 4 and 0 of the action potential, and (3) absence of a discrete plateau. Previous studies from this laboratory have shown that these are features of true pacemaker cells of the sinus node (see Discussion). If no strip had action potentials with these characteristics, further strips were prepared as previously described. Those experiments in which action potentials with characteristics of true pacemaker cells could not be found were abandoned.

In a suitable strip, a stable impalement was obtained with the IS microelectrode. The impalement was considered stable if the maximum diastolic potential and action potential amplitude remained unchanged for at least 2 minute. A second impalement was obtained with a conventional microelectrode 50–150 μm from the ion-sensitive electrode. If both impalements were stable, the Tyrode’s solution was changed to SCN$^-$ Tyrode’s solution. This led to rapid arrest of the preparation. After arrest, the superfusate was changed back to the Tyrode’s solution. Both microelectrodes were withdrawn from the cells. In some experiments, both
microelectrodes were maintained in the strips until spontaneous activity resumed. In two experiments, the preparation as arrested with 1 mg/liter verapamil after recovery from arrest by SCN⁻ Tyrode's solution. In one experiment, the preparation was arrested with verapamil without prior exposure to SCN⁻ Tyrode’s solution. Experiments were accepted for analysis provided that the difference signal was stable immediately (within 50 msec) after arrest.

To compare intracellular potassium activity in the sinus node and atrium, we performed experiments on five atrial strips removed from the right atrial appendages of five hearts. Following an equilibration period of 30 minutes, a minimum of three impalements that were stable for at least 1 minute were obtained with a conventional microelectrode. As it was anticipated that the transmembrane potential recorded with the IS microelectrode would be close to 0 mV, the preparation was stimulated at 0.5-1 Hz while impalements with the IS microelectrode were obtained (Lee, 1981). Recording of stable but attenuated action potentials indicated that the IS microelectrode was intracellular. Stimulation was discontinued and the IS microelectrode was withdrawn after it had recorded a stable potential for at least 1 minute.

Data Analysis

Cycle length, maximum diastolic potential of the action potentials recorded with the IS and conventional microelectrodes, and resting potentials immediately after SCN⁻ or verapamil arrest were measured from the Gould recorder output. Intradcellular potassium activity, \(a_K\), was calculated according to method 2 of Walker and Brown (1977), using their Equation 5.

\[
a_K = (a_K + K_{KNa}a_{Na}) \exp\left(\frac{2.303}{5}\right) \left[V_K - E_m\right] - K_{KNa} a_{Na}
\]

where \(a_K\) = calculated potassium activity of the superfusate
\(a_{Na}\) = calculated sodium activity of the superfusate
\(K_{KNa}\) = ISME selectivity coefficient
\(S\) = slope of the IS microelectrode
\(V_K\) = ISME potential referenced to the external solution; i.e., the ISME potential in the external solution is taken as zero
\(E_m\) = transmembrane potential measured with the conventional microelectrode

Activities were calculated using an extended form of the Debye-Hückel equation. The slope and selectivity coefficients were determined from the calibration curves as described above. \(E_m\) and \(V_K\) were the potentials measured with the conventional and ISME, respectively.

The last term in Equation 1 has been neglected. If one assumes a value of \(a_{Na}\) of 20 mM [twice that of other estimates in cardiac tissues (Lee, 1981)], the error introduced was an overestimate of \(a_K\) of less than 1 mM. For each sinus node experiment, there were two estimates of \(a_K\); one based on maximum diastolic potential recorded during spontaneous activity, the other on resting potentials recorded following SCN⁻ or verapamil arrest. Values quoted in the text are means ± SEM. \(a_K\) values in the sinus node and atrium were compared by unpaired t-test (Dixon and Massey 1969).

Results

Although strips from the primary and latent pacemaker regions of the intercaval area retain spontaneous activity, it was possible to identify these regions by criteria based on action potential characteristics. The action potentials in panels A and B of Figure 3 were recorded from neighboring strips in the sino-atrial node region. The maximum diastolic potentials (MDP) in panels A and B were −62 and −56 mV, respectively.

The transition between phases 0 and 4 of the action potential was similar to that in panel B. However, the maximum diastolic potential was −80 mV and a plateau was evident. Only strips with action potentials similar to those shown in panels A and B were used for \(a_K\) determinations.

In our initial experiments, we explored the degree of electrical homogeneity in the strips by recording simultaneous action potentials with two conventional microelectrodes and multiple impalements with a single microelectrode. Panel A of Figure 4 shows action potentials recorded simultaneously in a strip from the primary pacemaker region. MDP was −57 and −55 mV in the upper and lower traces, respectively. There was a difference of less than 5 msec in the time required for the peak positive potential. The remaining time courses of the action potentials were superimposable. Multiple impalements (4 or more) obtained in three experiments yielded a range of MDP of −63.5 to −66, −60 to −63, and −57 to −60 mV. We appreciated that this variability in MDP would introduce a random error when the conventional and ISME impaled separate cells. However, the alternative of using double-barreled microelectrodes would produce cell damage. Using Figure 1 of Walker and Brown (1977), this variability in \(V_m\) could lead to a 10% error in the estimate of \(a_K\).

Action potentials recorded simultaneously with ion-sensitive and conventional microelectrodes in the same strip used for panel A are shown in panel B of Figure 4. The action potentials in the upper trace...
Simultaneous impalements with conventional and IS microelectrodes. Panel A shows simultaneous impalements with two conventional microelectrodes in a shortened strip. The action potentials have a similar time course. Panel B shows simultaneous impalements with an IS and conventional microelectrodes in the same strip (upper and lower traces, respectively). Note the differences in amplitude and time course of the action potentials. The voltage calibration in the upper right applies to both traces in panel A, and the lower trace in panel B, all of which were recorded with the same conventional microelectrode. The other voltage calibration refers to the upper trace in panel B recorded with the IS microelectrode. A time calibration is shown in the lower right. The broken lines on the left were drawn through the peak of the action potential in the lower trace of each panel.

recorded with the IS microelectrodes are markedly attenuated with an amplitude of 11 mV compared with the 62-mV amplitude of the action potentials recorded with the conventional microelectrode. Further, there was a 60-msec delay in the time-to-peak of the action potential. This suggested that the slow frequency response of the ISME precluded an accurate measure of MDP in spontaneously active preparations with cycle length in the range of 250-350 msec.

We measured the electrical time constant of five representative IS microelectrodes. Electrical time constant was 230 ± 48 msec. These were clearly long time constants. However, they were similar to the following values reported for K+-selective IS microelectrodes for intracellular use in various tissues: (1) Skinner and Kunze (1970) 75-200 msec, rabbit atrium; (2) Walker JL (1980) 100 msec, Purkinje fibers; (3) Khuri (1973) 85 msec, rise time 160 msec, renal tubules; (4) Browning and Strauss (1981) <200 msec, Purkinje fiber and ventricular muscle.

The somewhat longer electrical time constants that we assessed are most likely the results of the smaller tip diameters of our electrodes for impaling the small SA node cells. It is clear that microelectrodes with time constants of 230 msec would place a severe limitation of the accuracy of potential measurements of a signal of cycle length 250-350 msec.

To investigate the possibility that the slow frequency response of the IS microelectrode precludes an accurate measure of MDP, we arrested the preparation in SCN⁻ Tyrode's solution while impalements were maintained with both microelectrodes. The results of such an experiment are illustrated in Figure 5. The MDP and action potential amplitudes were stable initially. After exposure to SCN⁻ Tyrode's solution, the signal from the IS microelectrode (VK) showed marked hyperpolarization at a time when there was little change in the MDP recorded with the conventional microelectrode. The latter eventually showed some depolarization prior to and following arrest of spontaneous activity. The potential changes shown in E_m and VK records (panels A and B) need to be discussed further. E_m undergoes slight hyperpolarization followed by progressive depolarization during the solution changes. Possible causes of these potential changes include: (1) change in the liquid junction potential of the reference electrode, (2) influx of SCN⁻ causing hyperpolarization, and (3) enflux of intracellular chloride causing depolarization.

Changes in liquid junction potential for the different [KCl] are of the order of 1-2 mV (Bates, 1973). The correspondence of the calibration curves in regular and SCN⁻ Tyrode's solution indicates a minor effect, if any, of changes in liquid junction potential. Further, a change in the liquid junction potential does not alter the calculation of a_K by method 2 of Walker and Brown (1977) which we used in this study. Both E_m and VK have the same reference electrode, and VK-E_m is used to measure a_K. The cell membrane is freely permeable to Cl⁻ and 5CN⁻.

VK rapidly declines during spontaneous activity, and some of the possible causes for the rapid decline of VK after the change from normal to SCN⁻ Tyrode's solution and before any appreciable change in E_m...
include the following: (1) rapid decline in $a_K$ as $Cl^-$ was replaced by SCN$^-$, (2) change in the slope and selectivity of the ISME caused by SCN$^-$ diffusing into the intracellular space, (3) decline in the solution level in the bath following the change in solution level, which would lead to reduced capacitative coupling of the ISME to ground and an improved frequency response, and (4) slowing of the preparation prior to arrest, which would lead to a more faithful recording of MDP.

The first possibility seems unlikely. $Cl^-$ replacement is known to decrease $K^+$ permeability in the sinus node (Irisawa, 1978). The electronic difference signal shown in panel C of Figure 5 was stable following arrest. If thiocyanate substitution led to a decline in $a_K$, the difference signal should decrease even after arrest. Location of the IS microelectrode in a rapidly exchanging $K^+$ compartment cannot be excluded but is considered unlikely. The second possibility is unlikely, as the calibration curve shown in Figure 2 demonstrated no change in IS microelectrode characteristics in SCN$^-$ Tyrode’s solution. This third possibility is also unlikely in that the flow was regulated between the bath and the manifold used for solution changes. No change in measured flow was observed once switching was complete. The results of another experiment shown in Figure 6 support the fourth possibility. The chart speed was increased so that cycle length and MDPs from both microelectrodes could be measured for the cycles preceding arrest. The ($V_K - E_m$) difference is plotted against cycle length. ($V_K - E_m$) is the experimentally derived parameter used to calculate $a_K$ in Equation 1. ($V_K - E_m$) declined progressively as the cycle length increased. The extent of the decline was variable. However, as shown in Table 1, ($V_K - E_m$) always declined with arrest of the preparation.

Estimates of $a_K$ based on potential measurements during and after arrest and spontaneous activity are shown in Table 1. The estimates are much higher during spontaneous activity. Although ($V_K - E_m$) is only an average of 14 mV less during arrest than during spontaneous activity ($V_K - E_m$) appears in the exponential term of Equation 1. We believe the very high estimates of $a_K$ in spontaneously active preparations are the result of an artifact introduced by the slow frequency response of the IS microelectrode.

In an effort to substantiate this conclusion further, we compared $a_K$ following SCN$^-$ and verapamil-induced arrest. The tracings in panels A and B of Figure 7 are from the same two cells shown in Figure 5. The preparation had been returned to normal Tyrode’s solution with complete recovery of action potential characteristics. Exposure to 1 mg/liter verapamil took

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**Table 1**

Comparison of Estimates of $a_K$ in Spontaneously Active and Arrested Sinus Node Preparations

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>CL msec</th>
<th>$E_m$ mV</th>
<th>$V_K$ mV</th>
<th>$a_K$ mm</th>
<th>$E_m$ mV</th>
<th>$V_K$ mV</th>
<th>$a_K$ mm</th>
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<td>-67</td>
<td>27.0</td>
<td>163</td>
<td>-60</td>
<td>19.5</td>
<td>90</td>
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<td>2</td>
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<td>30.0</td>
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<td>390</td>
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<td>85</td>
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<td>12</td>
<td>295</td>
<td>-55</td>
<td>34.0</td>
<td>174</td>
<td>-56</td>
<td>17.0</td>
<td>91</td>
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<tr>
<td>Mean ± se</td>
<td>341 ± 12</td>
<td>-61.9 ± 1.4</td>
<td>27.1 ± 1.2</td>
<td>151.1 ± 5.9</td>
<td>-49 ± 3.3</td>
<td>25 ± 2.7</td>
<td>85 ± 2.8</td>
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In each experiment, $E_m$ and $V_K$ were measured with the same two impalements during spontaneous activity and arrest.
potential measurement with conventional and IS microelectrodes during spontaneous activity and arrest by 1 mg/liter verapamil. The upper trace was obtained with the conventional microelectrode, the lower trace with the IS microelectrode. Because arrest required more than 15 minutes, the traces have been interrupted to show the potentials during the onset and arrest. The impalements were from the same cells shown in Figure 5. Voltage and time calibrations are shown on the right.

longer than 15 minutes to arrest the preparation completely, $\alpha_k$ determined during thiocyanate arrest was 78.5 mM; it was 77 mM following verapamil arrest. It was not possible to get return of spontaneous activity even after washing for 1 hour with drug-free solutions. Further, even brief exposure of the preparation to verapamil caused slowing and a decline in MDP that was not readily reversible. Because of this, we were unable to do many sequential experiments of the type shown in Figures 5 and 7. In our other successful experiment, $\alpha_k$ was 96 and 98 mM following arrest by thiocyanate and verapamil, respectively. An $\alpha_k$ value of 75 mM was obtained in one further experiment with verapamil arrest but without prior exposure to thiocyanate.

We made $\alpha_k$ determinations in five quiescent atrial strips. Records from one experiment are shown in Figure 8. Strips were initially impaled with a conven-

**Table 2.** Intracellular Potassium Activity in Atrial Preparations

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>$E_m$ mV</th>
<th>$V_K$ mV</th>
<th>$\alpha_k$ mM</th>
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</thead>
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<td>-11</td>
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<tr>
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<td>-11</td>
<td>78</td>
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</tbody>
</table>

Mean ± se = -83.4 ± 1

Discussion

We have used K$^+$-selective liquid ion exchanger microelectrodes to measure intracellular K$^+$ activity in rabbit sinoatrial node and atrium during spontaneous activity and arrest with SCN$^-\,$ or verapamil. The determination required a potential measurement with the IS microelectrode and another independent measurement of transmembrane potential with a conventional microelectrode. Ideally, both microelectrodes should impale the same cell. Our previous experience with double-barreled microelectrodes suggested that they could cause significant cell damage. In the course of the present studies we frequently observed progressive decline in the MDP and action potential amplitude recorded with the single-barreled IS microelectrodes. This suggests that even a single-barreled electrode may cause significant damage in the small SA nodal cells. For this reason, it was very important to secure stable impalements before proceeding with the experimental protocol.

In an attempt to minimize the changes in action potential characteristics that may occur over short distances in the SA node, we used the shortened preparation as previously described (Noma and Irisawa, 1976; Seyama, 1979; Kerr et al., 1980). As reported in those studies, the true pacemaker cells retained their usual characteristics in the shortened preparation. We appreciated that the characteristics we used to define pacemaker cells in the isolated strips are somewhat arbitrary. However, the action potentials are similar to those that we and others have identified in the intact preparation where the primary pacemaker was defined (e.g., Lu, 1970; Strauss and Bigger, 1972).

The slow frequency response of the IS microelectrode posed a serious potential for error in the determination of $\alpha_k$. The resistance of the IS microelectrode was of the order of $10^8$-$10^{10}$ ohms. When this
resistance is coupled with a distributed capacitance of 1 pf/mm of immersed microelectrode tip (Cornwall and Thomas, 1981), together with the amplifier input and stray capacitances, a meantime constant of the IS microelectrode system of 230 msec was observed. The cycle length of the sinus node preparations varied from 280 to 410 msec in this study. This suggested that the frequency response of the IS microelectrode would not be fast enough to faithfully record the MDP.

Our results substantiate this prediction. Action potentials recorded with the IS microelectrode were always of attenuated amplitude and showed large phase shifts when compared with action potentials recorded with conventional microelectrodes. Simultaneous impalements with two conventional microelectrodes suggests that the attenuated action potential amplitude and phase shift were not the result of potential heterogeneity in the shortened strips. Arrest of the preparation with SCN− or verapamil confirmed that the slow frequency response of the IS microelectrode did introduce a significant error in the αk determination.

Mean αk estimated from the MDP measurements during spontaneous activity was 151 mM compared with 85 mM during thiocyanate arrest. Kerr et al. (1980) reported preliminary results on αk determinations in spontaneously active SA nodal preparations from this laboratory. Their estimates of αk were considerably less than those which we report in the present study. The source(s) of these differences remain speculative. The criteria for accepting an experiment, e.g., stability of impalement with IS microelectrode, was more stringent in this study. As the time required for thiocyanate arrest was very brief, we believe that 85 mM is valid estimate of αk. The similarity in the αk estimates obtained after thiocyanate and verapamil arrest suggest that the agency of arrest did not markedly change αk. In the course of these studies, we explored other means of slowing of the preparation, e.g., acetylcholine exposure. Acetylcholine produced substantial changes in electrode slope and selectivity and was not used in these experiments. The estimate of αk in the sinus node is similar to the 80 mM that we observed in right atrial strips in this study, and the 86 mM and 82.6 mM reported for rabbit atrium and ventricular muscle, respectively (Skinner and Kunze, 1976; Lee and Fozzard, 1975). Where regional differences in αk have been reported (Skinner and Kunze, 1976; Lee and Fozzard, 1975), the low values of αk observed in the smaller cells. This suggests the need for caution to exclude significant cell damage as a cause for low values of αk. Using the simplifying assumption that αk at the cell membrane is the same as that in the bulk solution, the range of potassium equilibrium potentials in the sinus atrial node is calculated to be −83 to −92 mV. The normal range of pacemaker potential of the sinus node is −50 to −70 mV. During spontaneous activity, the MDP is achieved when the net membrane current is zero. The net membrane current will be zero if inward and outward current components balance or if the cell has reached the reversal potential of a single ion which dominates the membrane permeability. The fact that the MDP is so positive to the potassium equilibrium potential (EK) at physiologic [K+]i, suggests that, at the MDP membrane, permeability to other ions is significant. Similarly, the resting membrane potential in quiescent SA nodal cells is positive to EK and suggests a significant permeability to other ions in resting cells (Irisawa, 1978).

Noma and Irisawa estimated by extrapolation a reversal potential for the time-dependent outward current in SA Node of −85 mV at a [K+]o of 5.4 mM. We calculate a mean K+ equilibrium potential of −89 mV at [K+]o of 4 mM based on the αk determinations in this study. No reversal potential of the inward pacemaker current was found over ranges of potential that bracketed the presumed K+ equilibrium potential (Maylie et al., 1981). The range of EK which we report in this study is further evidence that the inward pacemaker current is not likely to be carried by potassium ions.

Using the chemical determination of 114 mM for [K+]i ([K+]o, 2.7 mM) in rabbit sinus node reported by DeMello and Hoffman (1960), the intracellular K+ activity coefficient is calculated to be 0.69. This value is somewhat less than that of K+ in free solution and suggests binding and/or compartmentalization of K+ in sinus node cells. Lee and Fozzard (1975) reached a similar conclusion about the state of K+ in ventricular muscle.

The introduction of ion-selective electrodes holds great potential for the furthering of our understanding of ion transport and conduction in heart muscle. However, their uniformly high resistance will continue to pose a serious problem in spontaneously active preparations such as the sinus node and in the ability to follow rapid changes in ion activity.

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Address for reprints: Dr. A.O. Grant, Box 3504, Duke University Medical Center, Durham, North Carolina 27710.

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A O Grant and H C Strauss

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