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Comparison of Steady-State Electrophysiological Properties of Isolated Cells from Bullfrog Atrium and Sinus Venosus

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Summary. Single electrode whole cell voltage-clamp experiments and frequency domain analyses have been used to study and compare the K^+ currents in enzymatically dispersed single cells from the atrium and the sinus venosus (pacemaker region) of the bullfrog heart. Admittance measurements made near the 'resting' or zero-current potential yield data from which the equivalent circuit of each cell type may be obtained. Data from both atrial and pacemaker cells are well-fitted by a model consisting only of parallel resistance-capacitative elements, as predicted from their micro-anatomy. Neither of these amphibian cardiac cells contain a transverse tubule system (TT) and both have very little sarcoplasmic reticulum (SR). These results complement and extend two earlier investigations: (i) Moore, Schmid and Isenberg (*J. Membrane Biol.* **81**:29–40, 1984) have reported that in guinea pig ventricle cells (which *do* contain an internal membrane system consisting of transverse tubules and a substantial SR) the SR may be electrically coupled to the sarcolemma; (ii) Shibata and Giles (*Biophys. J.* **45**:136a, 1984) have shown that although bullfrog atrial cells have an inwardly rectifying background K^+ current, I_{K1}, pacemaker cells from the immediately adjacent sinus venosus do not. Data from admittance measurements also provide evidence that a TTX-insensitive inward Ca^{2+} current is activated in the pacemaker range of potentials.

Key Words atrium · sinus venosus · pacemaker · inward rectifier · impedance · isolated cardiac cells · linear analysis

Introduction

Within the past five years enzymatically dispersed single cardiac cells have become a quite commonly used experimental preparation (*cf.* Dow et al., 1981; Isenberg & Klöckner, 1982; Farmer et al., 1983). The existing data strongly indicate that such preparations have the following significant advantages for quantitative electrophysiological studies: (i) the problem of extracellular K^+ accumulation-depletion is minimized; (ii) possible effects of transmitter release from endogenous nerve varicosities are removed; (iii) under voltage-clamp conditions single cells behave as a short cable. In general, the space constant of the single cell measured near the resting potential is approximately 5 to 10 times the length of the cell indicating that 'space-clamp' conditions can be obtained.

In order to interpret the transmembrane ionic currents which are measured in voltage-clamp experiments and to quantitatively simulate or reconstruct the action potential or the pacemaker potential it is essential to know the equivalent circuit of each type of single cell which is being studied. Surprisingly, relatively little experimental data of this kind is available (but *see* Powell et al., 1980; Brown et al., 1981; Hume & Giles, 1981). Recently, Moore et al. (1984) have used frequency domain analysis to study in detail the equivalent circuit of isolated myocytes from guinea-pig atrium and ventricle. These results suggested that an *intracellular* membrane system is electrically coupled to the sarcolemma. The intracellular organelle most likely to give rise to effects observed in this study is the sarcoplasmic reticulum (SR). This electrical coupling must be considered in equivalent circuit analysis, and in the interpretation of physiological and pharmacological phenomena.

Interestingly, in the frog heart the cells from the sinus venosus, the atrium, and the ventricle have no transverse tubule system and also are virtually devoid of a sarcoplasmic reticulum (Page & Niedergerke, 1972; Niedergerke & Page, 1981). We have therefore carried out a frequency domain admittance analysis of both sinus venosus and atrial cells from the bullfrog heart. Our aims were: (i) to determine the equivalent circuit of the atrial and sinus venosus cells, (ii) to explore further the question of whether the effects observed by Moore et al. (1984) were likely to be due to the SR, and (iii) to document and extend a very important but unexpected observation from conventional voltage-clamp studies—that cells derived from the sinus

venosus (i.e. cardiac pacemaker cells) lack an inwardly rectifying background K^+ current.

Some of these results have been presented previously as abstracts (Giles et al., 1984; Shibata & Giles, 1984; Moore et al., 1985).

Materials and Methods

Cell Isolation and Maintenance

The procedure for isolating single atrial cells was very similar to that used previously (Hume & Giles, 1981). The heart was removed from an adult bullfrog (*Rana catesbeiana*) and, after the atrium was separated from the sinus venosus, it was placed in a small flask containing 5 ml of Ca^{2+}-free Ringer's solution, 0.13% crude bacterial collagenase (type I, *Clostridium histolyticum,* 200 U/mg; Sigma Chemical Co., St. Louis, Mo.) and 0.08% bovine pancreatic trypsin (type III, dialyzed, lyophilized, and salt-free, 10,100 U/mg protein; Sigma Chemical Co.). This solution was stirred continuously for 45 min at room temperature and then replaced with 5 ml of Ca^{2+}-free Ringer's containing 0.1% bovine albumin (essentially fatty acid free and prepared from fraction V; Sigma Chemical Co.) and stirred for another 5 min. Next, the atrium was incubated in fresh collagenase (0.07% in 5 ml of Ca^{2+}-free Ringer's solution) for 30 min. After three or four such 30-min cycles, the solution contained a high density of single cells. Aliquots were removed using a Pasteur pipette, and placed in the recording chamber for electrophysiological experiments.

The enzymatic dispersion procedure developed for isolating pacemaker cells from the bullfrog sinus venosus is, in principle, very similar to that for the atrium (Giles & Shibata, 1985). However, after the initial collagenase/trypsin treatment, elastase (type I, Sigma) is applied in Ca^{2+}-free Ringer's for 45 min. The elastase-containing solution is then pipetted off and the tissue is reincubated in collagenase-Ringer's (100 units/ml) for 20 min. This procedure is repeated after which the tissue is gently triturated (5 times) using a fire-polished Pasteur pipette. Finally, the tissue segments are treated for a third 20-min period with collagenase-Ringer's. The resulting suspension of cells is then transferred to the recording chamber filled with normal Ringer's.

Approximately 20 min were allowed for the cells to adhere loosely to the lid of a 35 × 10 mm tissue culture dish. They were then superfused with normal Ringer's solution for approximately 30 min and, thereafter, with HEPES-buffered Ringer's solution bubbled with 100% O_2. The cells were viewed with an inverted microscope at a magnification of 600. All experiments were performed at room temperature (22 to 23°C).

Solutions and Drugs

The standard Ringer's solution contained (mM): 90.6 NaCl, 20 $NaHCO_3$, 2.5 KCl, 2.5 $CaCl_2$, 5.0 $MgCl_2$ and 10 glucose and was equilibrated with 95% O_2/5% CO_2 (pH 7.4). However, to avoid precipitation, all Ba^{2+} experiments were performed in Ringer's solution containing (mM): 90.6 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 5.0 $MgCl_2$, 10 glucose, 20 sucrose, and 5 HEPES buffer. The pH of this solution was titrated to 7.4 with NaOH and it was equilibrated with 100% O_2. $BaCl_2$ was dissolved in distilled water to make 0.01 or 1.0 mM stock solutions. Tetrodotoxin (TTX) was purchased from Sigma Chemical Co.; $BaCl_2$ and $CdCl_2$ were purchased from Aldrich Chemical Co. Inc., Milwaukee, Wis.; HEPES was obtained from Research Organics Inc., Cleveland, Ohio.

Electrophysiology

Microelectrodes were pulled on a conventional horizontal puller (Industrial Science Associates, Inc., Ridgewood, N.Y.) using 1 mm o.d. square bore glass (Glass Company of America, Bargaintown, N.J.) and were back-filled with a 1-M potassium gluconate solution containing 60-mM KCl (Giles & Shibata, 1985). Acceptable tip resistances ranged from 3 to 6 MΩ. The 60-mM KCl presence in the electrode solution significantly reduced the junction potentials which were in all cases nulled before impalement. The maintenance of negative pressure in the electrode during the experiment probably minimized the leakage of K^+ ions into the cell. Additional control experiments with a 140-mM potassium gluconate electrode solution showed identical results. Details of the suction impalement technique for single atrial cells have been described previously (Hume & Giles, 1983) and are similar to those originally reported by Hamill et al. (1981). In normal Ringer's solution healthy quiescent atrial cells have measured total input resistances (including the parallel resistance of the electrode seal) (R_{IN}) of 500 megohm to 1 gigohm. Cells from the sinus venosus consistently have a higher R_{IN}—2 to 10 gigohms. The experimental setup used for the conventional voltage-clamp experiments was similar to that described in a previous paper (Hume & Giles, 1983). Series resistance compensation was *not* used in these experiments, but the capacitance of the electrodes was reduced by feedback compensation.

Admittance Measurements

All data collection and analysis procedures were very similar to those described in Moore et al. (1984). In brief, voltage-clamp experiments were done on single cells, where the command potential consisted of the sum of a step pulse and a low level (1 to 2 mV rms) deterministic noise source. The admittance method used was a broad-band white noise approach previously described for the squid axon (Poussart et al., 1977). Linearity was assured by doubling the noise perturbation and observing the same transfer function. The technique was implemented on a laboratory computer following the procedures recently described for similar studies on skeletal muscle (Moore & Tsai, 1983).

The basic principle of this method is to stimulate the preparation with a small signal that contains all the frequencies of interest. The response to such a linear stimulus provides magnitude and phase functions of frequency that can be used to find the parameters of the minimal equivalent circuit that describes the data. For the 200-Hz band used in these experiments a simple isopotential compartment consisting of a parallel resistance and capacitance with a series electrode resistance was the simplest circuit that adequately fit the data. In addition, a *cable* model including elements corresponding to the internal membrane structures (Falk & Fatt, 1964; Schneider, 1970; Validosera et al., 1984) similar to that used for skeletal muscle (Moore & Tsai, 1983) was tested. These fits showed that the cable models were not significantly better than the above simple model which was therefore used for the analysis.

The data analysis followed the procedures of Moore and

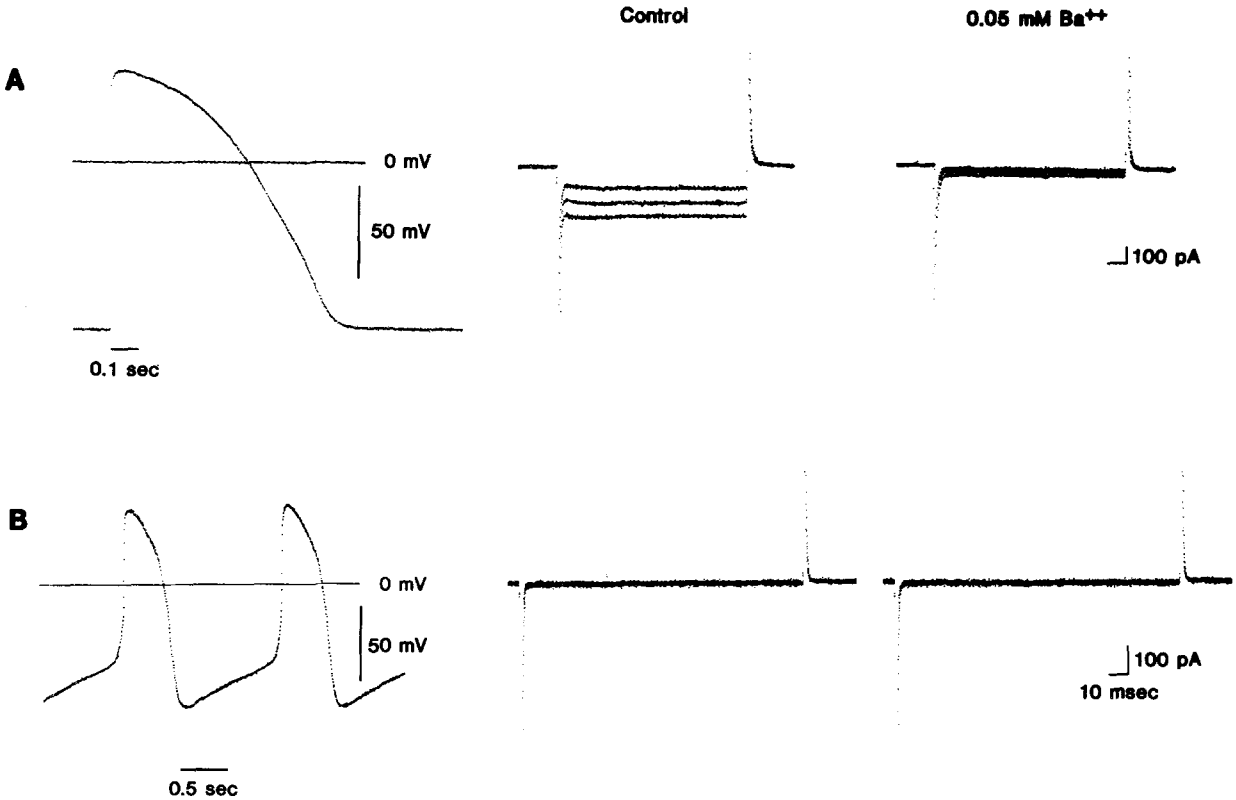

Fig. 1. Action potentials and voltage-clamp records from individual cells of bullfrog atrium (Row *A*) and bullfrog sinus venosus (Row *B*). Action potentials shown in the extreme left-hand columns are from atrium (top) and sinus venosus (bottom). The voltage-clamp data shown in the middle and right-hand columns were obtained from experiments designed to measure the currents present near the resting potentials or at more hyperpolarized potentials. The data from the atrial cells show a substantial inwardly rectifying current when the cell is hyperpolarized from the holding potential −90 mV to −110, −120 and −130 mV. Corresponding data obtained from the same cell after the addition of 50 μM $BaCl_2$ are shown in the right-hand column. $BaCl_2$ selectively and almost completely blocks the I_{K_1} current. In contrast, the data obtained in sinus cells reveals that there is no detectable inwardly rectifying background current (middle column) and that (as expected) $BaCl_2$ has no effect

Tsai (1983) in which the 400 real and imaginary complex numbers were curve fitted with a model circuit by minimizing the chi square using two algorithms, the grid and gradient of the chi square search methods (Bevington, 1969). The plotted data for all cells were arbitrarily scaled for a membrane capacitance of 1 μF/cm^2. In the figure legends the calibrated values of all fitted parameters are given in terms of the whole cell and not scaled according to membrane area. The curve fitting was done on a PDP 11/70 computer.

The general model used for the analysis consisted only of a surface membrane element Y in series with an electrode resistance, R_s. Thus, the admittance Y is:

$$Y = G_m + j2\pi f C_m + \frac{G_i}{1 + j2\pi f \tau_i} \quad (1)$$

where G_m is the frequency-independent steady-state conductance, C_m is the membrane capacitance, G_i is the voltage-dependent conductance, τ_i is the time constant associated with G_i, j is $\sqrt{-1}$, and f is frequency in Hertz.

Results

Conventional Electrophysiological Experiments

Panels *A* and *B* in Fig. 1 show typical action potentials recorded from individual enzymatically isolated atrial and sinus venosus cells from the bullfrog heart. In both cases the electrophysiological activity in the isolated cells is very similar to that recorded in multicellular strips or isolated trabeculae (Hume & Giles, 1981; Shibata & Giles, 1984; Giles & Shibata, 1985). When these cells were voltage clamped at the resting potential or the maximum diastolic potential, and then hyperpolarizing clamp steps were applied, a conventional inwardly rectifying K^+ current I_{K_1} was recorded in atrial cells (top row, middle trace). However, in identical voltage-

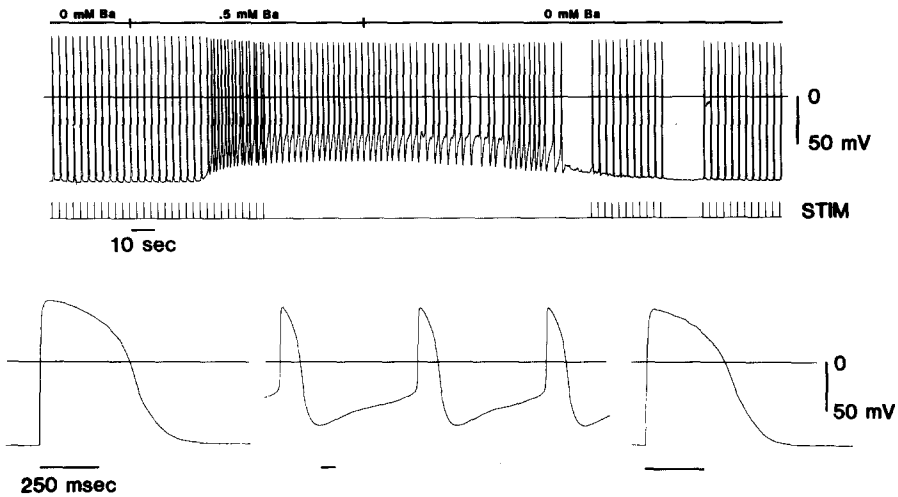

Fig. 2. Induction of pacemaker activity by Ba^{2+}. Trains of action potentials from an isolated atrial cell are shown as a consequence of either electrical stimulation or the addition of Ba^{2+} to the Ringer's solution. The ordinate for the top and bottom panels is membrane potential. The abscissa is time as labeled. The middle panel indicates the time that the stimulus was applied

clamp experiments in cardiac pacemaker cells no detectable inwardly rectifying background K^+ current could be obtained. Moreover (as shown in the extreme right-hand columns of rows *A* and *B*) in atrial cells I_{K_1} could be blocked by small (50 μM) concentrations of $BaCl_2$, but $BaCl_2$ had no effect on the very tiny inward currents recorded in sinus pacemaker cells. These findings strongly suggest that a major difference between cardiac pacemaker cells and immediately adjacent atrial tissue is that the pacemaker cells lack an inwardly rectifying background K^+ current. Perhaps for this reason sinus venosus cells are more depolarized than atrial cells and tiny net inward currents are able to generate the slow diastolic depolarization, or pacemaker potential (Shibata & Giles, 1984; Giles & Shibata, 1985).

The action potential trains of Fig. 2 show that atrial cells can be induced to pace by blocking the I_{K_1} current with barium ions. This effect is probably dependent on both the blockage of the inward rectifier and the concomitant depolarization. In other experiments on ventricular cells blockage of K^+ currents with cesium ions or zero external potassium ions did not lead to pacing although the use of Ba^{2+} did.

Admittance Data Obtained in Sinus Pacemaker Cells

As mentioned previously, bullfrog sinus venosus and atrial cells have no transverse tubule system and only very little sarcoplasmic reticulum. These micro-anatomical features make them particularly interesting for detailed admittance measurements. *A priori* it would be predicted that the equivalent circuit of these cells can be quantitatively modeled by only parallel resistance and capacitance elements, in series with the resistance of the microelectrode. Figure 3 illustrates a fit of such admittance data obtained from an isolated sinus venosus cell. This cell was 'held' at −60 mV, and the noise perturbations were applied during long (4 sec) clamp steps. The resulting admittance function was found to be independent of membrane potential for hyperpolarizations in the range −60 to −90 mV (*data not shown*), consistent with there being no inwardly rectifying K^+ current in these cells.

However, when admittance measurements were made during depolarizing clamp pulses positive to −70 mV, marked changes in the admittance functions were observed. Figure 4 shows admittance functions for a 20-mV hyperpolarization and a 30-mV depolarization from a holding potential of −70 mV. The data obtained in response to the depolarization (top traces) show an increased low frequency magnitude indicative of an enhanced conductance. In this case the phase function approaches 180° at low frequency suggesting an activation of an inward current. The most acceptable fit of these data required that a negative slope conductance having a relaxation time constant of 3.0 msec be used. Relaxation times found at −50, −20 and 0 mV were 3.4, 2.2 and 1.1 msec, respectively. Since all of these measurements were made in the presence of Ringer's solution containing 2×10^{-6} M TTX, the most likely source of the relaxing negative conductance is the gating process of the calcium channels underlying the current I_{Ca}. Giles and Shibata (1985) have previously shown in conventional voltage-clamp studies that a TTX-insensitive current I_{Ca} is activated within the diastolic range of potentials, at approximately −65 mV.

Admittance Measurements in Atrial Cells

Admittance experiments similar to those shown in Figs. 3 and 4 were also carried out in *atrial* cells so that (i) the equivalent circuit in this preparation (*cf.*

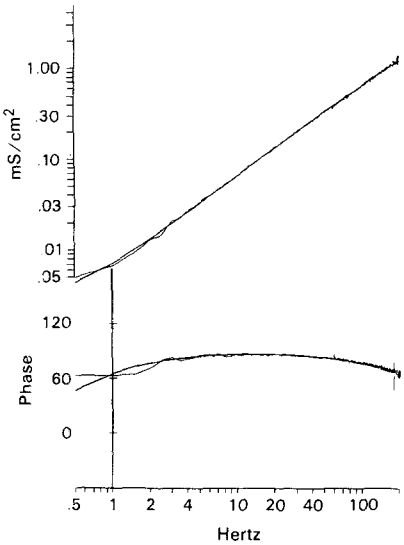

Fig. 3. Magnitude and phase functions obtained from an isolated bullfrog sinus venosus cell. The complex admittance was measured at a holding potential of −60 mV in Ringer's solution containing 3×10^{-6} TTX. The smooth curves are model fits using Eq. (1), that gave parameter estimates of $C_m = 4.3 \times 10^{-11}$ farads, $R_m = 4.7 \times 10^9$ ohms and $R_s = 8.4$ megohms. Note that the plotted data have been rescaled to give $C_m = 1.0 \times 10^{-6}$ farads/cm²

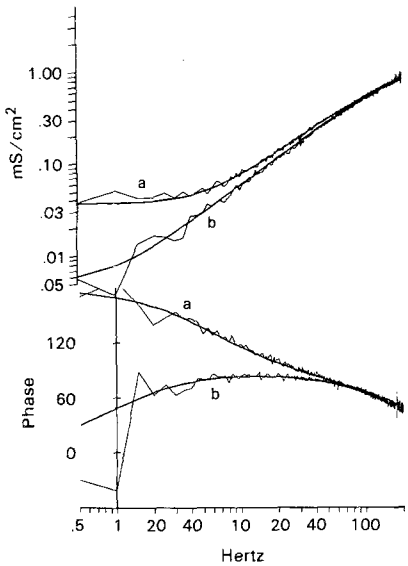

Fig. 4. Effect of changes in the 'resting' potential on the admittance of an isolated sinus venosus cell. Curves *a* and *b* of the superimposed magnitude and phase functions represent the complex admittances for a 30-mV depolarization (*a*) and a 20-mV hyperpolarization (*b*), respectively, from a holding level of −70 mV. The parameter estimates of the smooth curve model fits are $C_m = 5.2 \times 10^{11}$ farads, $R_s = 13.8$ megohms, $G_i = -2.4 \times 10^{-9}$S, at $\tau_1 = 3$ msec, and $R_m = 3.4 \times 10^9$ ohms for the depolarization. The passive parameters, C_m and R_s, remain the same for the hyperpolarization and $R_m = 4.0 \times 10^9$ ohms. Note that the plotted data have been rescaled to give $C_m = 1.0 \times 10^{-6}$ farads/cm²

Hume & Giles, 1981) could be determined; (ii) a comparison of the effect on the admittance function of the presence of an inwardly rectifying potassium current I_{K_1} could be made. The effect of I_{K_1} on the admittance function is illustrated in Fig. 5. When the atrial cell is hyperpolarized progressively from −73 to −113 mV the low frequency magnitude increases dramatically, indicating that the slope conductance continuously increases from less than 0.01 to nearly 0.3 mS/cm². Thus, as has been indicated in Fig. 1 from conventional voltage-clamp data, activation of the inward rectifier produces a substantial conductance increase, in this case about 40-fold over this 40-mV potential range. The phase function shown in the lower part of Fig. 5 also reflects this progressive conductance increase by shifting into higher frequency regions.

Application of small *depolarizing* clamp pulses (e.g., to −63 mV, *not shown here*) led to a phase function approaching 180° and required a negative conductance to accurately fit the data. As expected, when $BaCl_2$ was used to block I_{K_1} in atrial cells, the resulting admittance functions were very similar to those obtained from sinus venosus pacemaker cells in control Ringer's solution.

Figure 6 illustrates admittance data obtained from an atrial cell in the presence of 0.5 mM $CdCl_2$ without TTX. This cell, like the one shown in Fig. 5, exhibited inward rectification, and a negative conductance in the range of potentials −80 to −60 mV. In this preparation $CdCl_2$ very selectively blocks I_{Ca} (Giles, Hume & Shibata 1983); therefore the negative conductance obtained in this relatively negative voltage range is not likely to be due to I_{Ca}. It could be due to the activation of TTX-sensitive Na^+ channels. Perhaps more likely, it could arise from the negative slope conductance in the I_{K_1} *I-V* relation (*see* Vereecke et al., 1980; Cleeman, 1981; Hume & Giles, 1983; Sakmann & Trube, 1984; Giles & Shibata, 1985).

Discussion

The Equivalent Circuit of Single Cells From Bullfrog Atrium and Sinus Venosus

The admittance functions of single cells from both the atrium and the sinus venosus of the bullfrog heart were best-fitted by a very simple equivalent circuit involving only parallel resistance and capacitance elements. This is consistent with the microanatomy of these cells; neither has a transverse tubule system, and the sarcoplasmic reticulum is very sparse in both tissue types (Page & Niedergerke, 1972). Hume and Giles (1981), using conventional DC cable analysis, have previously reported values

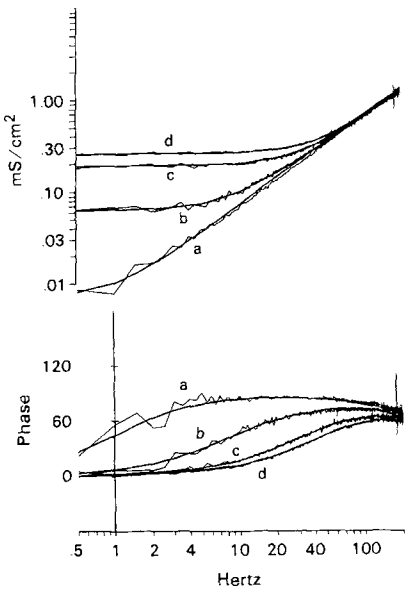

Fig. 5. Effect of changes in membrane potential on the complex admittance of an isolated frog atrial cell. The four superimposed magnitude and phase functions, *a* through *d*, were measured at −113, −103, −93 and −73 mV. Curve fits were done without relaxation terms. The parameter values were: $C_m = 5.4 \times 10^{-11}$ farads, $R_s = 6.1$ megohms, and $R_m = 2.8 \times 10^9$, 2.7×10^8, 9.6×10^7, 7.0×10^7 ohms, at −73, −93, −103 and −113 mV, respectively. All measurements were made in 3×10^{-6} M TTX from a holding potential of −93 mV. Note that the plotted data have been rescaled to give $C_m = 1.0 \times 10^{-6}$ farads/cm²

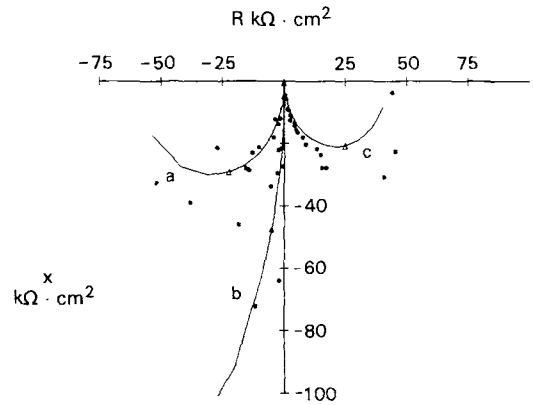

Fig. 6. Effect of changes in membrane potential on the impedance plane of an isolated frog atrial cell. The three superimposed plots from left to right, *a*, *b* and *c*, correspond to −60, −70 and −80 mV, respectively. The measurements were made in Ringer's solution containing 0.5 mM $CdCl_2$ from a holding potential level of −80 mV. The smooth curve fits using Eq. (1) had the following parameter estimates: $C_m = 7.6 \times 10^{-11}$ farads, $R_s = 5.3$ megohms, $G_i = 0$, and $R_m = 9.0 \times 10^9$ ohms at −80 mV; $R_m = 5.0 \times 10^8$ ohms, $G_i = -2.1 \times 10^{-9}$S, and $\tau_1 = 1.4$ msec at −70 mV; $R_m = 1.5 \times 10^9$, $G_i = -1.5 \times 10^{-9}$S, $\tau_1 = 3.4$ msec at −60 mV. Note that the plotted data have been rescaled to give $C_m = 1.0 \times 10^{-6}$ farads/cm²

for the passive cable parameters of bullfrog atrial cells. In those experiments the input resistance R_{IN} of cells with a normal resting potential was 220 ± 77 megohms and the total cell capacitance was calculated to be 50 to 100 pF per cell. Both of these are in qualitative agreement with the values derived from curve-fits of the admittance data reported here.

Contrast Between Atrial Cells and Sinus Venosus Cells

The data reported here confirm the initial report of Shibata and Giles (1984) that bullfrog cardiac pacemaker cells do *not* exhibit any measurable inwardly rectifying background potassium current I_{K_1} (Giles & Shibata, 1985). Thus, hyperpolarizing clamp steps from the maximum diastolic potential did not activate any significant inward current (Fig. 1); and admittance measurements also failed to identify any increase in conductance in response to hyperpolarizing clamp steps (Fig. 4). Very recently, Irisawa and his colleagues (Irisawa, 1984; Noma et al., 1984) have reported that in the mammalian heart the pacemaker cells also lack an inwardly rectifying background potassium current. This observation (in rabbit heart) has been confirmed by Giles and van Ginneken (1985) working with cells from the crista terminalis, a peripheral region of the anatomical S-A node. In summary, it now appears that an interesting and fundamental property of cardiac *pacemaker* cells is that they lack an inwardly rectifying background potassium current. This may be of functional importance. These cells are relatively depolarized and have a very high input resistance; thus very small net inward currents will significantly depolarize these pacemaker cells from their maximum diastolic potential into the voltage range where the time- and voltage-dependent inward current initiates the action potential.

Actions of $BaCl_2$ on Atrial and Sinus Cells

Hermsmeyer and Sperelakis (1970) first reported that $BaCl_2$ depolarized frog ventricular tissue, often resulting in 'induced pacemaker activity' and Hiroaka et al. (1980) suggested a possible explanation for this. Our data (Fig. 1) show clearly that in small concentrations $BaCl_2$ very strongly and selectively inhibits I_{K_1}.

In skeletal muscle fibers (Standen & Stanfield, 1978), and in mammalian ventricular cells (Sakmann & Trube, 1984) $BaCl_2$ has previously been shown to have very similar effects on I_{K_1}. Interestingly this may depolarize the atrial cells and

give rise to sinus-like pacemaker activity. This indicates that a major difference between pacemaker and nonpacemaker cells of the heart may be the presence or absence of an inwardly rectifying background K^+ current.

Identification of Functionally Important Negative Conductances

Our admittance data (Fig. 4) also provide additional evidence that activation of I_{Ca} is an important factor in generating the final one-third of the pacemaker potential in bullfrog sinus venosus cells. Previous conventional voltage-clamp measurements had strongly suggested this (Giles & Shibata, 1985; Fig. 4). Additional admittance studies done under conditions in which heart rate and I_{Ca} are changed systematically (i.e. varying doses of isoproterenol) will be of importance.

The admittance data in *atrial* cells confirm the very large increase in conductance when these cells are hyperpolarized and I_{K_1} is activated. In addition, this frequency domain analysis adds an important piece of information to the conventional studies by suggesting that even under these circumstances the single atrial cell is adequately space clamped. If this was not the case, then a different model as opposed to a simple parallel R/C element would have been needed to fit the admittance data (Fig. 5). Interestingly, the admittance data from the atrial cells identified a region of negative slope conductance at very hyperpolarized potentials, approximately -70 to -80 mV. This negative conductance was shown to be Cd^{2+} insensitive. It therefore could arise from the noninactivating or persistent inward current of the kind identified by Hume and Giles (1983) in this preparation. Alternatively, and perhaps more likely, it could be due to the negative slope conductance in the current-voltage relation of the inward rectifier (*see* Hille & Schwartz, 1978; Vereecke et al., 1980; Cleeman, 1981; Hume & Giles, 1983; Sakmann & Trube, 1984; Giles & Shibata, 1985; Fig. 14).

Relationship to Previous Work

Comparison of the results reported here with those previously published by Moore et al. (1984) yields a striking difference in the admittance functions from bullfrog atrial or sinus venosus cells versus those obtained from mammalian atrial or ventricular cells. The mammalian cells consistently exhibit a second dispersion which Moore et al. (1984) suggested may represent an internal membrane system, the sarcoplasmic reticulum. In contrast, in the amphibian cells the impedance plane plots (Fig. 6) clearly shows *single* semicircles indicative of only the surface membrane. From anatomical data, the amphibian cell types would be expected to exhibit only surface membrane phenomena in admittance measurements. These results further indicate that for the conditions of these experiments the membrane is space clamped.

In summary, the results reported here further document the differences in steady state or resting conductances between atrial and sinus venosus cells from the bullfrog. Secondly, they illustrate the improved sensitivity of identifying negative conductance regions in steady-state current-voltage relations which is obtained using the impedance analysis approach. In addition these data may be used to further substantiate the interpretation of earlier impedance measurements from mammalian cardiac cells which strongly suggested that an internal membrane system was electrically connected to the sarcolemma. The features of the admittance data which suggested this in *mammalian* cardiac cells were not expected to be present in *amphibian* cardiac cells, and their absence was confirmed. The present data therefore provide validation of the single electrode method for admittance studies; that is, they strongly indicate that the second dispersion cannot be due to microelectrode properties but rather is a genuine property of the cell being studied. The data from the amphibian cells presented here are consistent in that no second dispersion is seen under circumstances when very little or no sarcoplasmic reticulum is present.

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