J. Physiol. (1981), **318**, *pp.* 455–477
With 12 *text-figures*
Printed in Great Britain

VOLTAGE CLAMP AND INTERNAL PERFUSION OF SINGLE RAT HEART MUSCLE CELLS

BY A. M. BROWN, K. S. LEE* AND T. POWELL†
From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, 77550, U.S.A. and the †Department of Physics as Applied to Medicine, Middlesex Hospital Medical School, London W1P 6DB

(*Received 3 July 1980*)

SUMMARY

1. Single cells from adult rat ventricle were dispersed using an enzymic dissociation technique. Electrical properties were measured with either suction pipettes or conventional glass micropipettes and the results were compared.

2. Suction pipette and micropipette measurements of resting membrane potentials and action potentials were comparable. Values were similar to those reported previously for both dispersed cardiac myocytes and whole tissue preparations from adult rat ventricle.

3. Voltage clamp with a single suction pipette was used in initial experiments, but the results were not sufficiently accurate. Consequently, voltage clamp of single cells was carried out using two suction pipettes (tip diameters 10–15 μm), one for passing current and the other for recording membrane potential. Dialysis of cell contents was performed by each suction pipette. A roving micropipette (tip diameter < 1 μm) was used occasionally to measure membrane potential at selected sites.

4. Using the two-suction-pipette method, voltage-clamp steps rose with time constants of less than 10 μsec and the capacitative current transient decayed with a single time constant of less than 100 μsec. These values are more optimal than those observed in other voltage-clamped cardiac muscle preparations.

5. Single cardiac myocytes had membrane input resistances of $44{\cdot}5 \pm 4{\cdot}6$ MΩ, membrane time constants of $16{\cdot}2 \pm 0{\cdot}63$ msec and membrane capacitances of $399{\cdot}7 \pm 42{\cdot}2$ pF. (values are means $\pm$ S.D., $n = 10$). The length constant, λ, of a single myocyte at its resting potential and perfused with KH_2PO_4 was about 500 μm. For cells perfused with Cs aspartate solution commonly used in voltage-clamp experiments, the input resistance was approximately quadrupled and λ was 1100 μm. The average length of a myocyte partially aspirated by two suction pipettes was 50 μm. At maximum $\bar{g}_{Na}$ in 50% extracellular Na, λ was calculated to be about four times the actual cell length between voltage-recording and current-passing pipettes.

6. The half-time for the disappearance or recovery of outward Na currents, following removal or restitution of intracellular Na with two pipettes, was of the order 1 min, indicating that intracellular ionic composition of the cell could be readily controlled and modified.

* Present address: Department of Physiology, Yale University, New Haven, Connecticut, U.S.A.

INTRODUCTION

Voltage-clamp analysis of membrane currents in myocardial tissue is made difficult by the complicated geometry of the cardiac syncytium and associated restricted extracellular spaces (Johnson & Lieberman, 1971; Attwell, Eisner & Cohen, 1979). None the less, some understanding of certain pace-maker, repolarization, background and Ca currents has been possible using several tissue preparations (Noble & Tsien, 1969; New & Trautwein, 1972; Trautwein, 1973; Brown, Giles & Noble, 1977; Isenberg, 1977; Reuter & Scholz, 1977; but see diFrancesco, 1980 and diFrancesco & Noble, 1980 for a recent re-examination of the pace-maker current, I_{K_2}). This has not been the case for Na current, however, which has the most rapid kinetics and the largest maximum conductance of any cardiac membrane current. Many attempts to study the initial rapid inward current using voltage-clamp techniques have been fraught with difficulties, due to the fact that conventional methods of voltage clamping are limited when applied to the study of Na current in cardiac muscle (Dudel & Rudel, 1970; Tarr & Trank, 1974). Recently, Colatsky & Tsien (1979) have been more successful using a two-micropipette voltage-clamp method, by selecting short rabbit Purkinje fibres as the most suitable syncytial preparation. However, rapid inward currents were still partially obscured by capacitative transients during the early phases of conductance changes. The two-micropipette technique has also been applied to spherical clusters of chick embryonic heart cells (Ebihara, Shigeto, Lieberman & Johnson, 1980). Again the early phases of Na current were obscured by capacitative transients, and voltage control was limited by a high external resistance (Mathias, Ebihara, Lieberman & Johnson, 1980).

Many of the complexities inherent in the cardiac syncytium can be minimized by dispersing the whole organ into single cells. This procedure also obviates the problems of restricted extracellular spaces. The electrophysiological characteristics of dispersed cells have been studied using micropipette techniques by both Powell, Terrar & Twist (1978*b*, *c*, 1980) and Mehdi & Sachs (1978), and in the case of cells from rat tissue the isolated myocytes have resting and action potentials that resemble recordings made from cells *in situ* (Weidmann, 1956; Isenberg & Klockner, 1980). The rate of rise and overshoot of the action potential appeared normal, indicating that the Na system was probably not greatly affected by the dispersion method. A study of the Na current in these cells would require a voltage-clamp method that could apply voltage steps to the membrane rapidly and, in addition, control internal as well as external ionic composition. The suction-pipette method developed in this laboratory for voltage clamp and internal perfusion of small cells (Lee, Akaike & Brown, 1977, 1978, 1980) seemed particularly well suited for this purpose, due to the fact that the resistance of suction pipettes is much lower than conventional glass micropipettes, thus allowing more rapid application of a voltage step to the cell membrane. The results of initial experiments have demonstrated the feasibility of voltage clamping single myocytes with a single suction pipette (Lee, Weeks, Kao, Akaike & Brown, 1979), but a limitation of this method applied to small cells having large Na currents, which is the case for cardiac myocytes, is the voltage drop across the tip resistance of the pipette. A similar limitation is present in the experiments reported by Undrovinas, Yushmanova, Hering, and Rosenshtraukh (1980). This problem has now

been circumvented and the present paper describes the experimental method, with the attendant advantages and limitations, and also reports measurements and calculations of the electrical constants of individual heart muscle cells. Experimental data on both the kinetics and conductance of Na channels in cardiac muscle are described in the accompanying paper (Brown, Lee & Powell, 1981).

METHODS

Cell dispersion

The method used for tissue dissociation was similar to that reported previously (Powell & Twist, 1976; Powell *et al.* 1980) and the main features are summarized here. Female rats (Timco Breeding Lab., Houston, Texas) approximately twelve weeks old and 250 g in weight were used. Animals were injected (I.P.) with 0·2 ml. heparin solution (hyperine, 1000 u.s.p., Ricker Lab., California) and 10 min later anaesthetized with sodium pentabarbitone (200 mg I.P., Abbott Lab., Des Moines, Iowa). The chest was opened and the heart extracted quickly into a beaker of ice-cold Krebs solution (Table 1), then mounted on a Langendorff perfusion apparatus for dissociation by crude bacterial collagenase (Powell *et al.* 1980). Dispersed cells were washed and harvested by centrifugation at 24 *g* with nominally Ca-free Krebs solution, before final suspension in normal Krebs solution (Table 1). All measurements reported in this and the subsequent paper (Brown *et al.* 1981) were made at temperatures of 20–22 °C.

Glass micropipettes

Conventional glass micropipettes filled with 3 M-KCl had tip resistances in the range 20–50 MΩ and were connected to a standard electrometer system (WPI Instruments, Model 750, New Haven, Connecticut).

Suction pipettes

The suction pipettes used in the present experiments were made according to the method of Lee *et al.* (1977; 1978; 1980) and had tip diameters of 10–15 μm, which are smaller than those used earlier on dispersed heart cells (Lee *et al.* 1979). In isotonic solutions the pipettes had tip resistances of about 500–800 kΩ and this large tip resistance can cause voltage drops across the tip as great as 40 mV during peak Na current flow, thus preventing adequate control of membrane potential, particularly in solutions containing 100% Na. Series resistance compensation of the usual kind (Hodgkin, Huxley & Katz, 1952) could not correct for the error. To avoid this, we used two suction pipettes on the same cell, one for recording membrane potential and the other for passing the feed-back current. Two arrangements were used: either the suction pipettes were placed one at each end of the cell (Fig. 1*A*) or transversely at the centre of the cell (Fig. 1*B*). These arrangements and the recording circuitry are shown in Fig. 1. Series resistance was evaluated using the arrangement of Fig. 1*A*, and a current pulse was delivered to the cell through the pipette on the left-hand side. In one case, the membrane voltage was measured by the same pipette through which the current was delivered (middle trace, Fig. 1*C*). In the other case, by using the switch, the membrane voltage was measured by the suction pipette on the right-hand side (upper-most trace of Fig. 1*C*). The differences in the instantaneous voltages at the on and off of the current pulse (lower trace, Fig. 1*C*), as well as the steady-state voltage difference (shown in Fig. 1*C*), were due almost entirely to the voltage drop across the pipette tip when a single pipette was used to record voltage and pass current (Fig. 1*C*). In the two-pipette case, the series resistance was measured in current clamp as described above or was calculated from the capacitative current transient produced by the on and off of voltage-clamp steps. Series resistance compensation was used to the point of ringing in the two-pipette experiments. Shunt current compensation was used in initial single-pipette experiments but was not used in later experiments.

Evaluation of intracellular perfusion

The extent and rapidity with which intracellular Na concentration could be changed was estimated as follows. Extracellular Na was replaced with Cs and sucrose, while $[Na]_i$ was adjusted to 16 mM (Table 1). The cell was depolarized to a fixed potential of +20 mV from the holding

TABLE 1. Composition* of solutions used for isolation and perfusion of single adult rat cardiac myocytes

	NaCl (mM)	KCl (mM)	KH_2PO_4 (mM)	Mg_2SO_4 (mM)	$NaHCO_3$ (mM)	$CaCl_2$ (mM)	Collagenase % (w/v)
(1) Ca^{2+}-free	118·41	2·55	1·32	1·16	14·52	—	—
(2) Enzyme solution	118·41	2·55	1·32	1·16	14·52	0·02	0·04
(3) Incubation solution	118·41	2·55	1·32	1·16	14·52	0·02	0·04
(4) Washing solution	118·41	2·55	1·32	1·16	14·52	0·02	—
(5) Normal Krebs (100% Na^+_o)	120	4·8	1·2	1·2	25	1·1	—
(6) 50% Na_o Krebs	47·5	4·8	1·2	1·2	25	1·1	—
(7) 50% Na_o Krebs, K-free	47·5	—	—	1·2	25	1·1	—
(8) K^+, Cl^-, Ca^{2+}-free	—	—	—	2·85	—	—	—
(9) Na^+, K^+, Cl^-, Ca^{2+}-free	—	—	—	2·85	—	—	—
(10) 'Normal' K^+	—	—	135	—	16	—	—
(11) K^+, Cl^-, Ca^{2+}-free	—	—	—	—	16	—	—
(12) Na^+, K^+, Cl^- and Ca^{2+}-free	—	—	—	—	—	—	—

	Bovine albumin % (w/v)	Dextrose (mM)	Sucrose (mM)	Na isethionate (mM)	CsOH (mM)	Tris (mM)	Aspartic acid (mM)	pH
(1) Ca^{2+}-free	0·1	11·1	—	—	—	—	—	7·4
(2) Enzyme solution	0·1	11·1	—	—	—	—	—	7·4
(3) Incubation solution	1·0	11·1	—	—	—	—	—	7·4
(4) Washing solution	4·0	11·1	—	—	—	—	—	7·4
(5) Normal Krebs (100% Na^+_o)	—	5·5	—	—	—	—	—	7·4
(6) 50% Na_o Krebs	—	5·5	72·5	—	—	—	—	7·4
(7) 50% Na_o Krebs, K-free	—	5·5	72·5	—	5·4	—	5·4	7·4
(8) K^+, Cl^-, Ca^{2+}-free	—	5·5	—	145·22	5·4	—	5·4	7·4
(9) Na^+, K^+, Cl^-, Ca^{2+}-free	—	5·5	58	—	72·5	—	72·5	7·4
(10) 'Normal' K^+	—	5·5	—	—	—	—	—	7·2
(11) K^+, Cl^-, Ca^{2+}-free	—	5·5	—	—	135	—	135	7·2
(12) Na^+, K^+, Cl^- and Ca^{2+}-free	—	5·5	—	—	151	—	151	7·2

* Cell isolation, (1)–(4)
Extracellular perfusion, (5)–(9)
Intracellular perfusion, (10)–(12)

potential of −80 mV and outward Na current was measured. The internal solution was then changed to one in which Na was replaced by Cs (Table 1). The cell was then depolarized to the same potential (+20 mV) at regular intervals. After the outward current decreased to a small time-invariant value, the solution containing 16 mM-Na^+ was re-introduced internally and the time course of the reappearance of Na current was measured. Although the relation between the change in Na current and $[Na]_i$ is non-linear, this method does provide a qualitative estimation of the ability to control intracellular ionic environment.

Data acquisition

Records of voltage and current were digitized every 2 μsec using a twelve-bit digital oscilloscope with 2 K memory (Nicolet 1170). The digitized records were stored on a digital tape recorder (Kennedy Model 9700). The linear capacitative and leakage components of voltage-clamp currents were removed by adding two currents produced by identical voltage steps of opposite polarity.

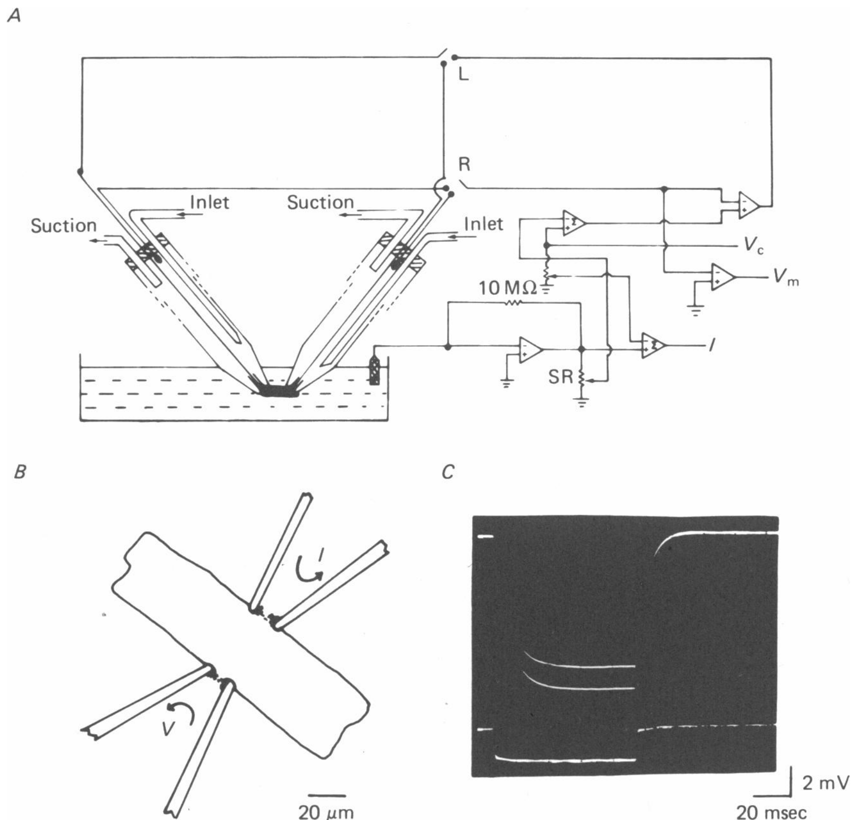

Fig. 1. *A*, arrangement of two suction pipettes on a single ventricular muscle cell (black rod). Using the switches L and R, either of the pipettes can be used to record voltage and/or inject current. Voltage-clamp circuitry shows V_m, membrane voltage, V_c command voltage, *I* current and SR, series resistance compensation (Hodgkin *et al.* 1952). The gain of the clamp amplifier was at least 10^3 and the frequency response was flat to 10 kHz. *B*, two suction pipettes applied centrally across the cell diameter. *I*, current-injecting pipette. *V*, voltage-recording pipette. Arrows indicate flow path of internal solution. *C*, voltage responses to inward current pulses (2 nA) recorded with two-suction-pipette arrangement according to the double method in *A* (smaller steady-state deflexion) and a single suction pipette. The difference in the instantaneous and steady-state voltages is due to the *I–R* drop across the tip of the single pipette. For the two-pipette case the voltage rose exponentially from the base line, although this is not shown in the record in order to illustrate the instantaneous voltage change in the single-pipette case.

RESULTS

Electrical characteristics of single heart muscle cells

Freshly isolated cells in Ca-free Krebs solution had steady resting potentials, measured by either suction pipettes or glass micro-electrodes, which were between −20 and −60 mV. In normal and high $[Ca]_o$ (1·1 and 2·5 mM), the resting potential reached a steady value of −80 ± 10 mV (mean ± S.D.; $n = 10$) (also see Powell *et al.* 1978*c*, 1980). In some cells an inward current of about 50 pA to 0·5 nA was injected

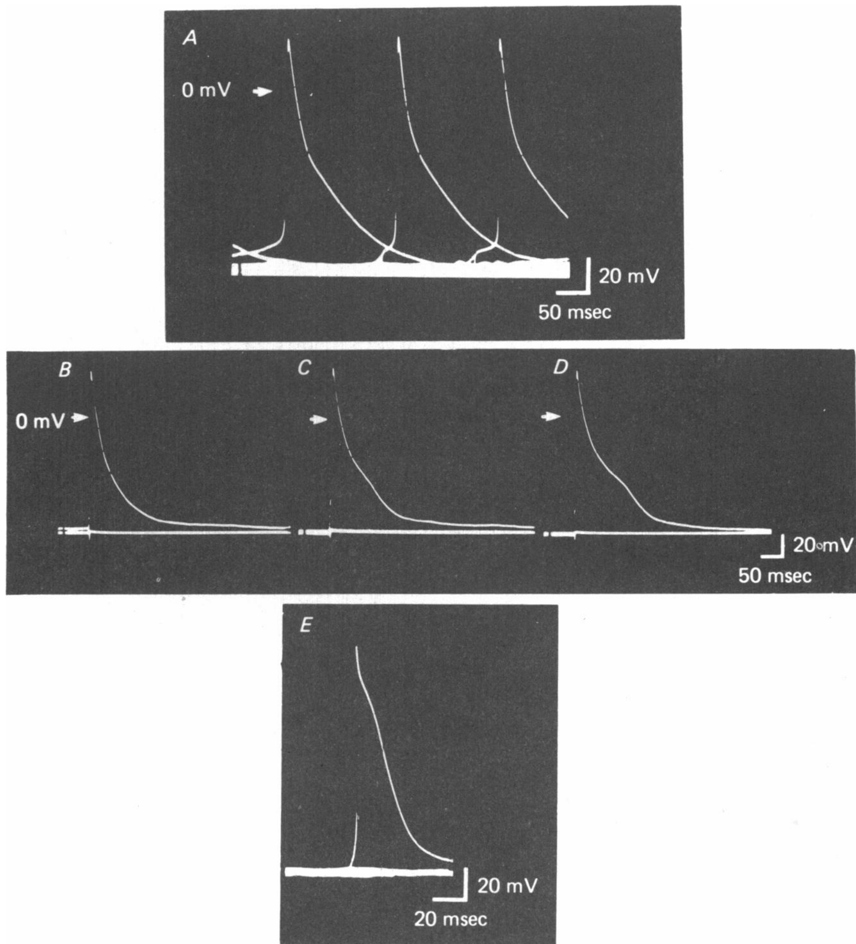

Fig. 2. *A*, spontaneous action potentials at membrane potential of −90 mV. The action potentials are characterized by an initial depolarization followed by the upstroke. The maximum velocity of upstroke averaged 230 V sec^{-1} at room temperature. Total amplitude averaged about 120 mV with 30 mV overshoot. The duration of the action potential is 150–200 msec. *B*, *C* and *D*, action potentials elicited by brief outward currents (2 msec, +3 nA). All the action potentials were taken from same cell, but at membrane resistances of 30, 40 and 60 MΩ, respectively, for *B*, *C* and *D*. The duration and the plateau are more pronounced at higher membrane resistance. Same calibration applies to *B*, *C* and *D*. *E*, another cell with membrane resistance of 10 MΩ. The duration of the action potential is greatly reduced and the upstroke velocity was 150 V sec^{-1}.

to increase the membrane potential to between −90 and −100 mV in normal Krebs solution containing 1·1 mM-Ca. Action potentials recorded in normal Krebs solution from cells with membrane potentials of −90 mV had amplitudes of 120 ± 10 mV, spike durations of 180 ± 20 msec, short plateaus lasting 50 ± 20 msec and maximum rates of rise of about 230 V sec^{-1}. There was often a very prolonged small tail of potential. In some cells action potentials occurred spontaneously (Fig. 2*A*) and in others they were produced by brief depolarizing current pulses (Fig. 2*B–D*). Similar

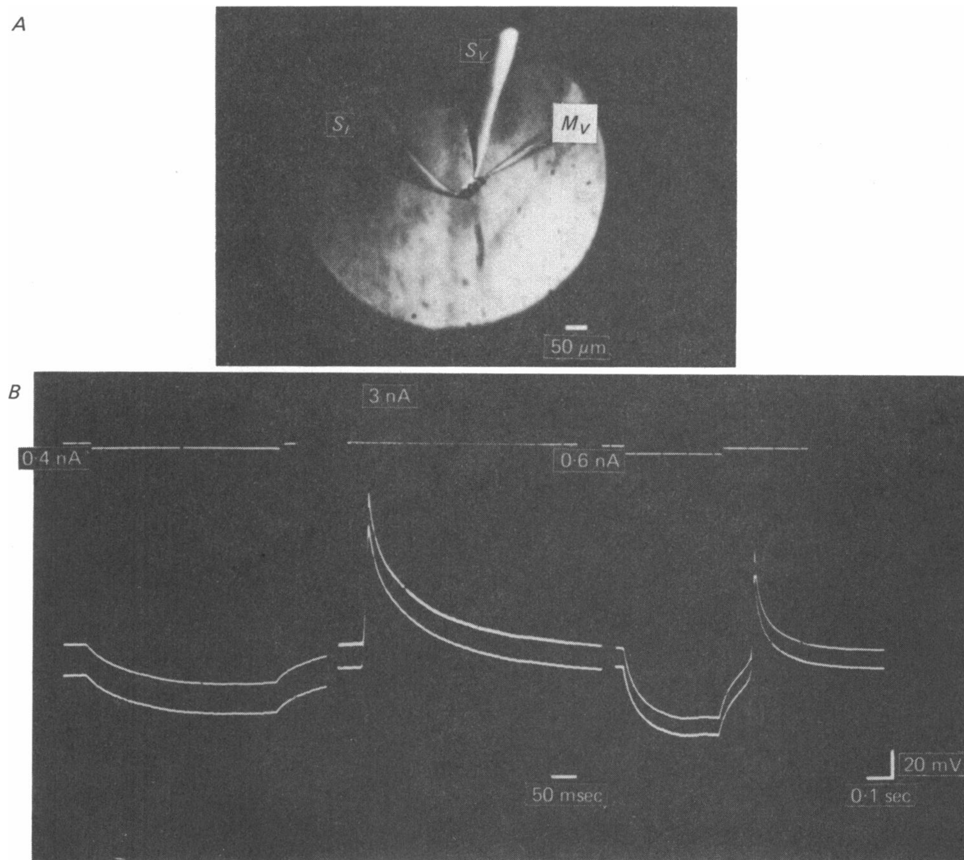

Fig. 3. *A*, photograph of a single myocyte subjected to a triple-pipette experiment. S_i is the current-injecting suction pipette. S_v is the voltage-sensing suction pipette. M_v is the voltage sensing micropipette. During experimental runs, as current was injected through S_i, M_v and S_v measurements were compared. *B*, pair of voltages measured by micropipette (upper trace of pair) and suction pipette (lower trace) applied to the same cell during current clamp. Current records are the very top trace. The potential traces were deliberately separated for recognition. The resting membrane potential measured by both systems was −80 mV in 2·5 mM Ca. Traces in the left hand panel show the membrane response to inward current. Traces in the middle panel show action potentials elicited by a brief outward current pulse. Traces in the right hand panel show membrane response to 0·6 nA inward current step. An anodal break response was observed. Traces at top are the current traces.

action potentials have been recorded from intact rat heart (Weidmann, 1956) and in isolated rat ventricular muscle cells (Isenberg & Klockner, 1980; Powell *et al.* 1980). In the latter case the prolonged tails were also observed. The action potential profile was related to the resting membrane resistance from which activity was triggered. Starting from membrane potentials of −90 mV, lower input resistances of below

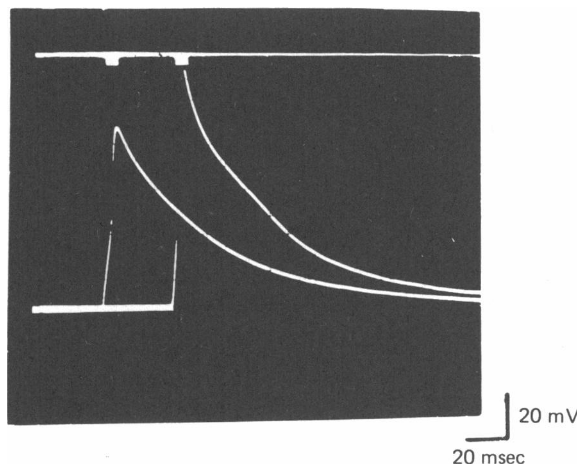

Fig. 4. Comparison of action potentials recorded using one- and two-suction-pipette methods from the same cell at measured holding potentials of −90 mV. Action potential recorded by one suction pipette is shown to the left. The current trace is shown at the very top. The marked difference between the two action potential amplitudes must be due to the fact that the real membrane potential for the single pipette recording was much more positive than in the double-pipette case. Results similar to the data shown were obtained when very large holding currents were required (see text), but in this particular cell the holding current was noted as 0·5 nA. If this is a correct entry, it is difficult to ascribe a mechanism which would produce such a large difference between the two recording methods.

40 MΩ were associated with a reduction in plateau and total durations, although the upstroke was relatively unaffected (Fig. 2*B*–*D*). When the membrane resistance was 10 MΩ, as measured by constant current injection, the duration of the action potential became very short and the upstroke phase was also affected (Fig. 2*E*).

Comparison of membrane properties measured by the suction-pipette or micropipette methods

In these experiments two suction pipettes were used, one to record potential and the other to pass current, and a glass micropipette was also inserted into the same cell between the two suction pipettes (Fig. 3*A*). Measurements of resting potential, action potential, membrane input resistance, and membrane time constant recorded by each method were compared (Fig. 3*B*). The resting potentials obtained with the micropipette or the suction pipettes applied to the same cell were the same. Differences arose only when inward or outward currents were applied. These differences were dependent upon the distance between the voltage-recording pipettes and the current-passing suction pipette and provided a profile of the voltage distribution within the cell. Resting and action potentials recorded by a micropipette

were similar whether or not suction pipettes were applied, indicating that shunt resistance between suction pipette, aspirated myocyte and bath were very high. A minimum value for the shunt resistance, using measurement of membrane resistance with a micropipette as a reference, was about 450 MΩ and in good experiments the shunt resistance remained large. Deterioration of the preparation was associated with a large reduction in shunt resistance.

Comparison of potential measurements made with one and two suction pipettes

When the action potential started from the resting potential, the action potentials measured by both suction pipettes were similar. However, when inward current was required to produce holding potentials of -80 to -90 mV the action potential amplitude measured by two pipettes was larger and an extreme example is shown in Fig. 4. Furthermore, the maximum speed of the upstroke ($\dot{V}_{max}$), the plateau region and the duration of the action potential were enhanced with the two-pipette method. The differences are probably due to the fact that the real holding potential applied to the membrane is less negative in the single-pipette case, resulting in more steady-state inactivation prior to producing an action potential. With holding currents of less than 1 nA, the differences between the two methods were 5–10 %, but data similar to those shown in Fig. 4 were obtained when currents of up to 10 nA were required to maintain a potential of -90 mV, due to cell damage when the second pipette was attached or a marked increase in pipette resistance due to blockage of the tip. However, for the cell shown in Fig. 4 the holding current was simply noted as 0·5 nA and, unless this was an incorrect entry, it is difficult to see how such a large difference between the single- and double-pipette measurements could have arisen.

Comparison of voltage-clamp measurements made with one and two suction pipettes

The advantages gained in using two suction pipettes were strikingly evident in voltage-clamp experiments. An independent measurement of voltage control was achieved either by using a conventional glass micropipette to monitor membrane potential in cells which had two suction pipettes attached for voltage clamp or by recording potential from both suction pipettes. By suitable switching of the electrode connexions (Fig. 1), single or double pipette clamps could be applied to the same cell, with simultaneous voltage recordings taken from the micropipette, the voltage-sensing suction pipette, or current-passing suction pipette. For the latter case, in the absence of series resistance compensation, the voltage differed from the command voltage by the voltage drop due to current flow across the pipette's tip. With two pipettes, membrane potential was measured more accurately and the capacitative current transient was shorter (Fig. 5). In this particular experiment, the decay constant for capacitative current transients was 80 μsec with double-pipette voltage clamp (Fig. 5*A* and *B*) and was about three times faster than the corresponding single-pipette clamp decay constant ($\sim$ 275 μsec, Table 2). This comparison is complicated by the fact that whereas capacitative current decay for double-pipette clamp is monoexponential, a double-exponential fit is required in the case of one suction pipette. The calculated series resistance for the experiment shown in Fig. 5 is 200 kΩ, which is an average value that can be calculated from the data in Tables 2 and 3. Another measure of the series resistance (R_s) can be obtained from the

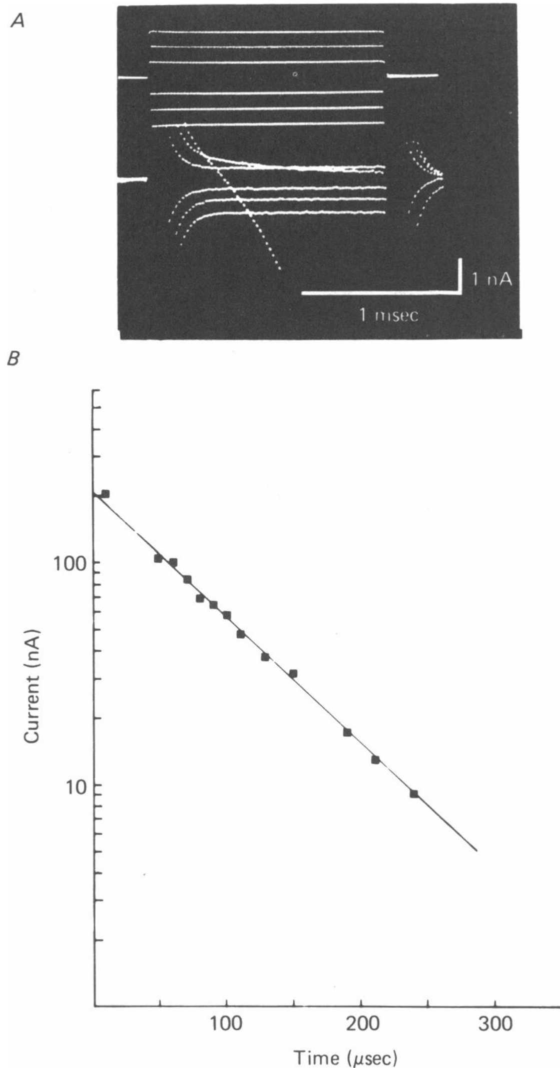

Fig. 5. *A*, voltage (top) and current records using two suction pipettes for voltage clamping. The capacitative current transients and the activation of the inward sodium current can be seen in the current traces. Currents were produced by ± voltage steps of 10, 20 or 30 mV from the holding potential of −80 mV. *B*, time course of the capacitative current transient produced by a voltage step to −102 mV from a holding potential of −80 mV. R_s was 200 kΩ. The current values fell approximately on a straight line giving a time constant of 80 μsec.

instantaneous voltage change produced by a step input of current. In some experiments, such as that shown in Fig. 1*C*, the instantaneous change was not measureable and there was a smooth exponential change in voltage. In other experiments it was possible to reduce the instantaneous voltage change using R_s compensation. It was difficult to resolve instantaneous voltage changes after a step input of current when compensation was applied and we estimate that in a typical

experiment R_s was reduced to about 100 kΩ before ringing occurred. Furthermore, using current records obtained under voltage clamp (Fig. 6), current–voltage relationships for peak I_{Na} (Fig. 7) were markedly different in the two-clamp configurations. In double-pipette clamp, the *I–V* curves were approximately symmetrical in shape with maximum peak I_{Na} at -20 mV membrane potential, whereas

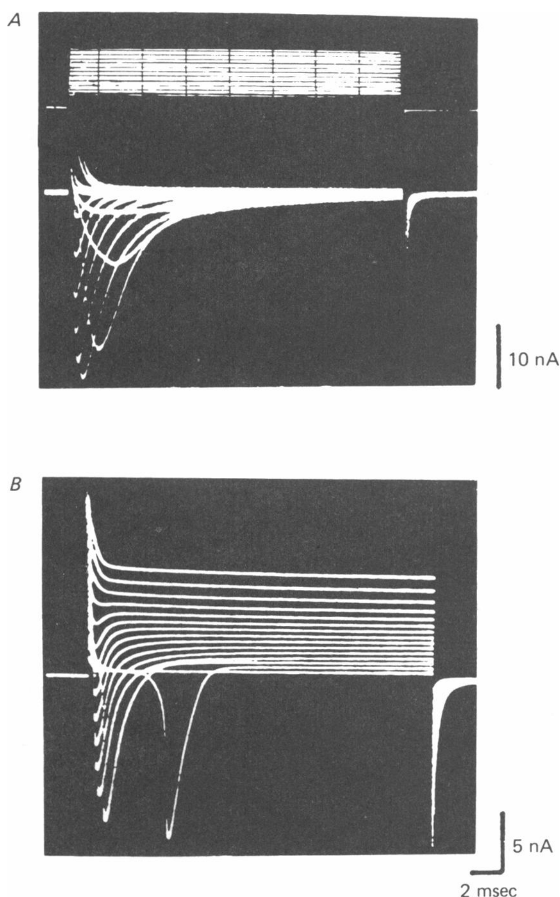

Fig. 6 *A*, Na currents, uncorrected for capacitative and leakage currents, recorded under voltage clamp using two suction pipettes in 50% Na_o solution (solution 7, Table 1). Holding potential was -80 mV and the voltages were applied in 10 mV steps from -60 to $+60$ mV. *B*, Na currents recorded from the same cell using one suction pipette. The current response was not graded but appeared in an all-or-none fashion at a threshold voltage of -60 mV.

single-pipette *I–V* relationships had maxima at more negative potentials (-60 mV) and showed negative slope conductances more than 20 times greater than positive slope conductance (Fig. 7). A similar result for single-pipette clamp was obtained by Undrovinas *et al.* (1980, see their Fig. 1 *B*). Grossly asymmetric *I–V* curves, for inward current having abrupt increases in current on membrane depolarization slightly beyond threshold, are to be expected when voltage control is poor during the early

phase of voltage clamp in cardiac tissue, due to a large series resistance (Jack, Noble & Tsien, 1975).

Maximum peak I_{Na} recorded by two suction pipettes was always at least twice as large as that recorded from the same cell using only a single pipette and in some cases the difference was much greater (Fig. 8). This difference in I_{Na} amplitudes under the two voltage-clamp conditions is consistent with the observation reported above, that

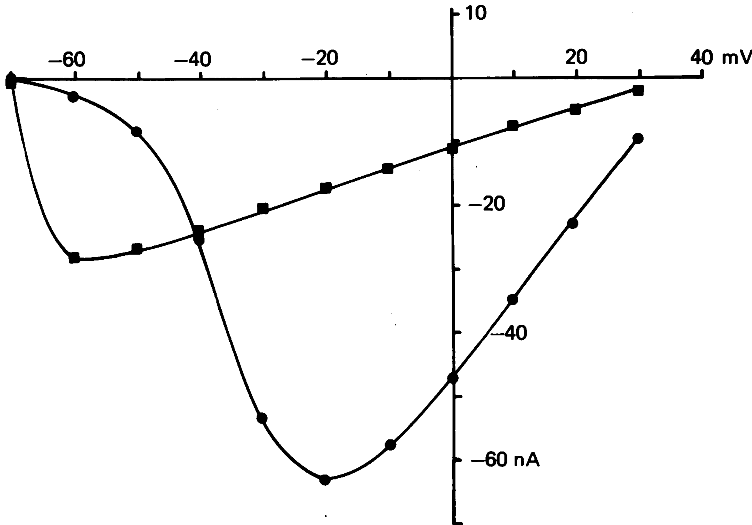

Fig. 7. *I–V* relationships for Na current obtained using single-suction-pipette (left) and double-suction-pipette (right) methods of voltage clamping. Holding potential was −80 mV; same cell.

in current clamp $\dot{V}_{max}$ is larger in the double-pipette clamp system than the single-pipette case. As noted already, the differences are probably due to the lower values of the holding and command potentials actually applied to the membrane in the case of the single pipette clamp. The difference in command potentials was probably about 35 mV for the experiment shown in Fig. 8. Even in the two-pipette case, the true membrane potential differs from the command potential according to the ratio of the series resistance and membrane resistance. The usual value for the positive slope resistance of the *I–V* curve is about 1 MΩ (Fig. 7) so that a maximum difference of 20 % could occur when series resistance compensation was not used.

An additional test for adequate voltage control is to terminate the depolarizing clamp pulse at peak I_{Na} (Colatsky & Tsien, 1979). The resulting tail current should follow a smooth, rapid decay if membrane potential is controlled, otherwise the current may be hooked or show the pattern of an inverted action potential. Fig. 9*A* illustrates a test of this kind, using two pipettes with $[Na]_o$ at 145 mM, and the experimental trace does show a rapid uninterrupted decay following membrane repolarization. It is also possible to reduce $\bar{g}_{Na}$ drastically with tetrodotoxin (TTX) and compare current time traces before and after this reduction. As Fig. 9*B* shows, the time courses of the two currents obtained from the same cell with and without TTX are identical after scaling, indicating that voltage control at high $\bar{g}_{Na}$ without

TTX is adequate. Moreover, the envelope of the tail currents elicited at various times during the course of I_{Na} at a particular voltage is similar to the time course of I_{Na} produced by a prolonged voltage-clamp step of identical amplitude, both decays being described by two exponential components, one having a time constant three to four times as long as the other (see Figs. 7 and 12 and Table 2 of Brown *et al.* 1981). The instantaneous current amplitudes were used to calculate the chord conductance, g_{Na},

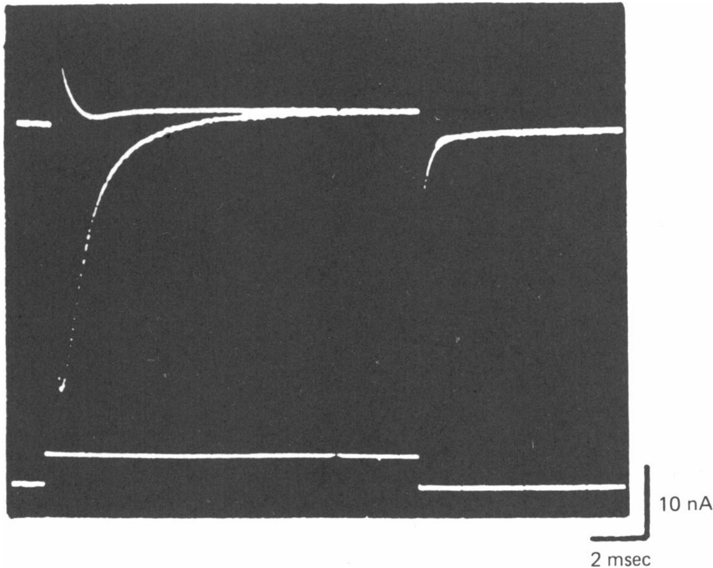

Fig. 8. Comparison of Na currents recorded using one- and two-suction-pipette methods produced by an identical voltage step (bottom trace) to −30 mV from measured holding potentials of −80 mV. Upper trace current was recorded with the single-suction-pipette voltage method.

as described in the next paper (Brown *et al.* 1981). Fig. 10 shows that g_{Na} calculated from tail currents and g_{Na} calculated from the current trace itself have similar time courses.

A comparison of the results obtained with the two- and one-pipette methods of voltage clamping is given in Table 2. Finally, with two suction pipettes perfusing the cell simultaneously, the time course for exchange of internal Na was estimated indirectly, by following changes in I_{Na} produced by modifying the Na content of the internal perfusion solution (see Methods). Efficient substitution of internal Na permits measurement of net outward I_{Na} as internal Na is changed. Fig. 11*A* shows the time course of I_{Na} as internal Na is reduced and then restored to the original level of 16 mM. When peak outward current was plotted at different times after solution changes, the curves shown in Fig. 11*B* were obtained. The half-times for changing I_{Na} are approximately 1 min.

Electrical constants of single cells

Although the experimental data reported here support the contention that double-suction-pipette voltage clamp permits resolution of cardiac Na currents at

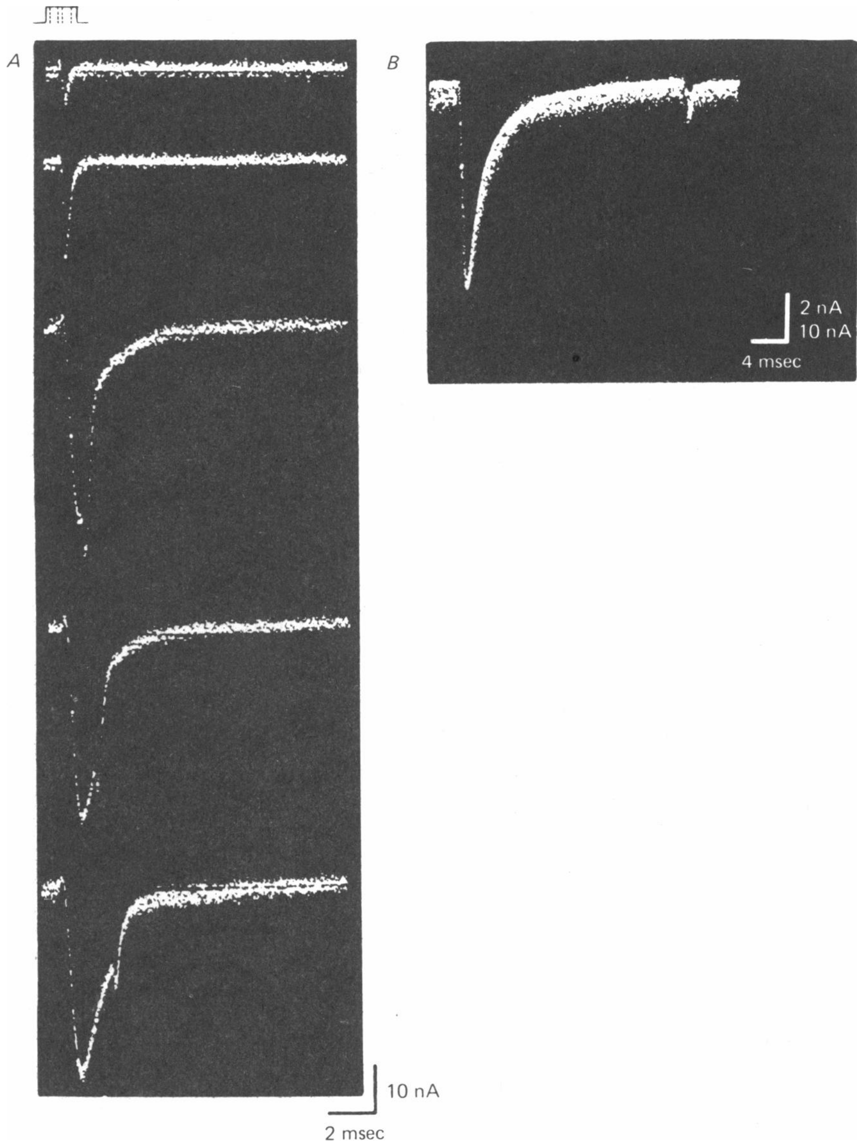

Fig. 9. *A*, Na currents during progressively longer voltage steps. Holding potential, −80 mV; command potential, −50 mV. From above, the voltage steps were increased in duration from 0·2 to 0·8, 1·0, 1·6 and 2 msecs. The envelope of the tail currents would fit the time course of the current produced by this command voltage. Linear components of leakage and capacitative current have been subtracted by adding the current responses to voltage steps opposite in polarity. *B*, net Na currents elicited by identical voltage steps in the presence and absence of 7 μM-TTX. The more dispersed trace is the Na current in 50 % Na_o with 7 μM-TTX. The other trace is Na current recorded from the same cell in the same solution without the TTX at 0·2 X gain. Holding and command potentials as in *A*.

physiological concentrations of extracellular Na, direct measurements of the electrical characteristics of individual muscle cells under the recording conditions employed would enable calculations to be made giving theoretical information on the adequacy of voltage control during the clamp. Central to such estimates is the determination of the space constant (λ) for a cell both at rest and during the peak phase of inward

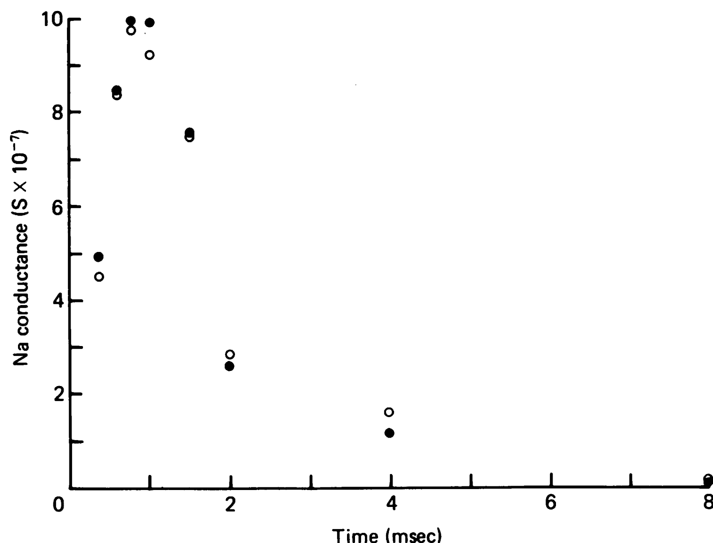

Fig. 10. Conductance–time trajectories. Each point represents chord conductance calculated using tail current (○) measured at times of 0·4, 0·6, 0·8, 1·0, 1·5, 2, 4 and 8 msec after the onset of a voltage step to −30 mV. The values are similar to those obtained from a prolonged step of equal amplitude (●). The time course of tail-current amplitude data used in this Figure could be fitted by time constants of 0·9 and 2·3 msec, which are similar to those found for I_{Na} decay at −30 mV using a prolonged depolarizing step (Table 2 of Brown *et al.* 1981).

I_{Na}. We have attempted to measure λ directly using the three-electrode protocol described above (Fig. 3), with one suction pipette used to inject current and the other pipette, together with a conventional glass micropipette, used to measure membrane potential at two sites in the cell. The current-injection pipette was located at one end of the cell (X_0), the voltage-recording pipette at the other (X_2) and the glass micropipette inserted equidistant (X_1) from the two pipettes. If V_0, V_1 and V_2 are the voltages at X_0, X_1 and X_2 at the end of a 400 msec hyperpolarizing current pulse, then from the theory for a short cable of length L, terminated by open-circuit ends,

$$\frac{V_x}{V_0} = \frac{\cosh\left((L-X)/\lambda\right)}{\cosh\left(L/\lambda\right)}$$

(Weidman, 1952). We can eliminate V_0 knowing V_1 at $X = L/2$ and V_2 at $X = L$, to obtain

$$\frac{V_1}{V_2} = \cosh\left(L/2\lambda\right). \qquad (1)$$

TABLE 2. Comparison of some results obtained with one- and two-pipette method for voltage clamping single adult rat cardiac myocytes

	Time at which peak current appears at −20 mV step potential (msec)		Capacitative current time constant (μ sec)		Peak Na current amplitude (nA)		Voltage at maximum peak current (mV)		Time constants for internal perfusion (min)	
No. of pipettes...	1	2	1	2	1	2	1	2	1	2
	2·5	0·5	250	80	27	65	−50	−30	2·5	0·8
	2·6	0·5	240	65	23	57	−55	−30	4	0·9
	2·5	0·4	210	60	20	50	−60	−25	—	1·2
	2·55	0·5	350	100	30	74	−60	−25	—	0·8
	2·3	0·4	370	120	35	80	−55	−20	—	—
	2·5	0·5	225	70	24	60	—	—	—	—
Mean	2·49	0·47	274	82·5	26·5	64·3	−56·0	−26	3·25	0·925
±S.D.	0·1	0·052	68	23	5·4	11	4·2	4·2	1·06	0·19

Holding potential = −80 mV.

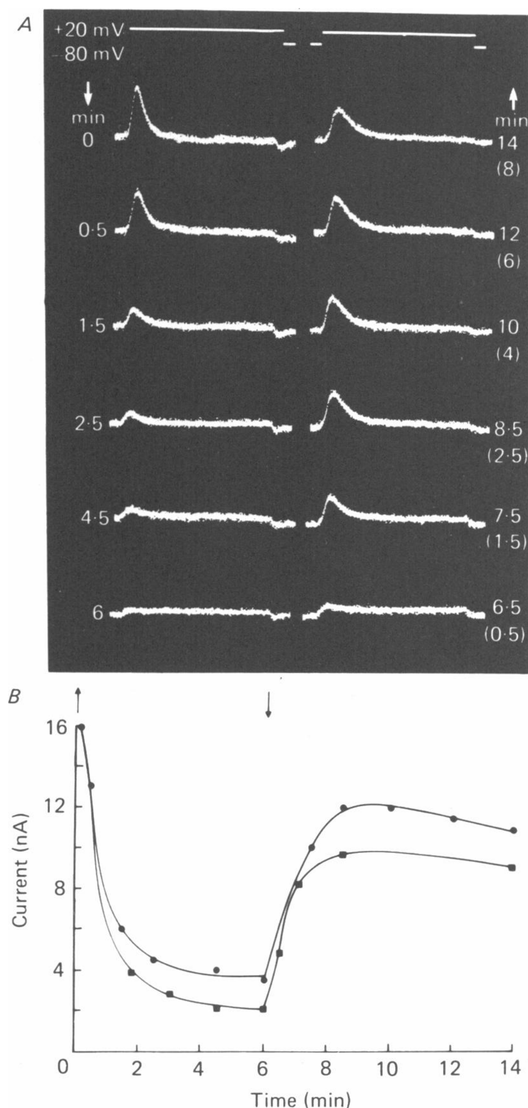

Fig. 11. *A*, outward Na currents in response to change in $[Na]_i$. External solution was Ca-free, Na-free, K-free and Cl-free (solution 9 of Table 1). Internal solution contained 16 mM-Na and 135 mM-Cs. Cell was depolarized to +20 mV for 15 msec from V_H of −80 mV. At 0 min, 16 mM-Na was removed completely. At 6 min, outward transient current disappeared. At this time, 16 mM-Na_i was re-introduced. At 8·5 min, recovery reached steady state. The recovered current was reduced by about 30% from control. Arrows indicate sequence. *B*, peak outward Na currents are plotted against experimental time. Upward arrow indicates removal of Na_i, downward arrow indicates re-introduction of Na_i (16 mM). The half-time for disappearance of Na current was about one min. The half-time for Na current to reach new steady state after re-introduction of Na_i was slightly longer than 1 min. These experiments were done using pipettes with shallow tapers and fast internal flow rates (5 drops/min).

TABLE 3. Electrical constants of single adult rat ventricular myocytes

Stimulating current (nA)	Membrane potential (mV) (V_1 micro)	Membrane potential (mV) (V_2 suction)	Differences in membrane potential (mV)	τ (msec) micro	τ (msec) suction	R_{in} (MΩ)	Membrane input capacitance (pF)	C_m* (μF/cm²)	λ (μm)	r_i ($10^8 \times$ Ω cm⁻¹)	r_m ($10^5 \times$ Ω cm)	R_i (Ω cm)	R_m† ($10^3 \times$ Ω cm²)
0·0	−80	−80	0·00	—	—	—	—	—	—	—	—	—	—
−0·3	−96	−95·43	0·57	17·8	16	53·3	334	4·175	457·7	2·54	5·33	798·0	3·35
−0·4	−100·3	−99·7	0·6	17	16	50·7	335	4·187	456·0	2·44	5·07	766·5	3·19
−0·5	−102·8	−102·5	0·3	18	16	45·6	395	4·975	653·7	1·07	4·56	336·2	2·87
−0·6	−107·72	−107·4	0·32	17	15	46·2	368	4·61	647·9	1·10	4·62	345·6	2·90
−0·7	−108·57	−107·7	0·87	18	17	41	439	5·487	393·6	2·65	4·10	832·5	2·58
−0·8	−113·14	−122·5	0·64	17·8	16	41	434	5·425	469·0	1·86	4·10	584·3	2·58
−0·9	−115·7	−115·2	0·5	18	17	40	450	5·625	536·9	1·39	4·00	436·7	2·51
−1	−124·2	−123·4	0·8	18	17	44	409	5·112	439·3	2·28	4·40	716·3	2·76
−1	−123·7	−122·8	0·9	17·5	16	44	397	4·963	413·2	2·58	4·40	810·5	2·76
−1·5	−138·3	−137·7	0·6	17	16	39	436	5·45	535·8	1·36	3·90	427·3	2·45
Mean				17·6	16·2	44·5	399·7	5·00	500·3	1·93	4·45	605·4	2·79
±S.D.				0·45	0·63	4·6	42·4	0·53	91·5	0·65	0·47	202·6	0·29

* Calculated from input resistance and time constant assuming a cell surface area of 8000 μm² (Page & McCallister, 1973).
† Calculated from length constant measurements.

Although eqn. (1) can be used to calculate λ the measurements are difficult to make, since V_1 and V_2 are nearly equal in magnitude. This is due to the fact that the cells are small, about 100 μm in length (Powell, Steen, Twist & Woolf, 1978*a*; Nash, Tatham, Powell, Twist, Speller & Loverock, 1979), and the voltage decrement for a short terminated cable is much shallower than that for an infinite cable (Jack *et al.* 1975). Table 3 gives the average values of V_1 and V_2 from three cells perfused

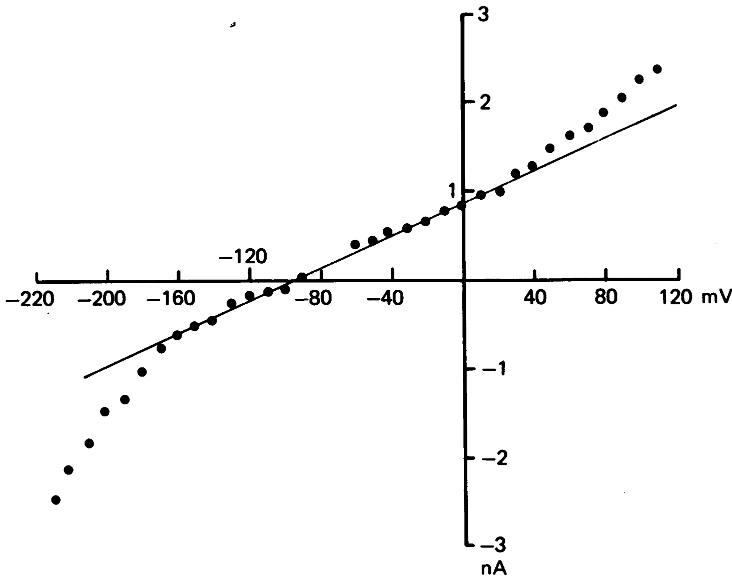

Fig. 12. Leakage current during internal perfusion with Cs-substituted K solutions. The Na current was blocked by 30 μM-TTX and K, Cl, Ca were removed (Table 1, solution 8). Membrane input resistance during internal Cs perfusion was usually 120–150 MΩ. The high input resistance increased the length constant of the cell during voltage clamp (see text). At very negative and very positive potentials the resistance is considerably reduced.

internally with KH_2PO_4, as the injected current amplitude was increased from 0·3 to 1·5 nA. In all cases, the voltage difference between V_1 and V_2 was never greater than 1 mV, giving a steady-state value for λ, calculated from eqn. (1), of $500{\cdot}3 \pm 91{\cdot}5$ μm ($\pm$S.D., $n = 10$). The average membrane time constant measured under these conditions was $16{\cdot}2 \pm 0{\cdot}63$ msec ($\pm$S.D., $n = 10$) and the resting membrane input resistance (R_{in}), determined as the ratio of the steady-state voltage deflexion to the corresponding current pulse amplitude, was $44{\cdot}5 \pm 4{\cdot}6$ MΩ.

Knowing λ and R_{in}, the resistance per unit length of cell interior (r_{i}, Ω cm^{-1}) can be calculated from

$$r_{\text{i}} = \frac{R_{\text{in}} \tanh (L/\lambda)}{\lambda} \tag{2}$$

(Jack *et al.* 1975) and has the value $(1{\cdot}93 \pm 0{\cdot}65) \times 10^8$ Ω cm^{-1} ($\pm$S.D., $n = 10$). Once r_{i} is found, then the resistance × unit length of the cell surface membrane (r_{m}, Ω cm) is calculated simply as $r_{\text{m}} = r_{\text{i}}\lambda^2$. For the data given in Table 3, r_{m} is $(4{\cdot}45 \pm 0{\cdot}47) \times 10^5$ Ω cm ($\pm$S.D., $n = 10$). In fact, if $\lambda \gg L$, then eqn. (2) reduces to

$r_i = (R_{in}L)/\lambda^2$, leading to $r_m = R_{in}L$; since $L \simeq 0{\cdot}01$ cm, then r_m is simply two orders of magnitude numerically smaller than the measured input resistance.

R_i (Ω cm), the specific resistivity of the cell interior, and R_m (Ω cm^2), the specific resistance of the cell surface membrane, can then be calculated directly from r_i and r_m by assuming an average cell radius of 10 μm (Powell *et al.* 1978*a*; Nash *et al.* 1979). We found R_i to be $605{\cdot}4 \pm 202{\cdot}6$ Ω cm and R_m to be $2{\cdot}79 \pm 0{\cdot}29$ k Ω cm^2 for ventricular cells. The specific membrane capacitance was $5{\cdot}00 \pm 0{\cdot}53$ μF cm^{-2}.

The input resistance values used were obtained from cells perfused internally with the KH_2PO_4 solution. Voltage-clamp experiments were done using intracellular solutions (nos. 11 and 12, Table 1) and extracellular solutions (nos. 7 and 8, Table 1) in which Cs was substituted for K to suppress K currents. Under these conditions the input resistance of the resting membrane was 150 ± 20 MΩ (Fig. 12) and the calculated length constant, λ, was 1100 ± 100 μm.

DISCUSSION

Current injection for voltage clamp of myocardial preparations has been achieved with either a micropipette (Weidmann, 1951; Deck & Trautwein, 1964) or a sucrose gap (Morad & Trautwein, 1968; Beeler & Reuter, 1970). Although conventional glass micropipettes can be used with short preparations of Purkinje fibres to record Na current within a few msec of activation (Colatsky & Tsien, 1979) and the single sucrose gap can be modified to measure membrane current and potential as early as 5 msec after the initiation of a voltage-clamp step (Goldman & Morad, 1977), analysis of early Na current has proved elusive. The major conclusion of this paper is that individual muscle cells, used in combination with two suction pipettes, comprise a system which can resolve cardiac Na current throughout a series of voltage-clamp steps, at 20–22 °C, even in solutions containing physiological levels of Na.

Records of resting and action potentials taken from cells with two suction pipettes in place are similar to those reported by Powell *et al.* (1978*b*, *c*, 1980), who used a single glass micropipette in cells prepared by the same dispersal technique as used here and from the same strain of rat. The electrical constants determined with the present system are comparable to those found by Powell *et al.* (1980) and also consistent with those for whole tissue preparations (Weidmann, 1970), with the exception that the membrane time constants for cells having input resistances of the order 40 MΩ at -80 mV membrane potential are about twice as long as those reported previously (Powell *et al.* 1980).

Voltage-clamp records in 100 % extracellular Na show good voltage control, and currents have no 'abominable notches', relaxing smoothly when interrupted at peak. Capacitative current transients have time constants of less than 100 μsec, and Na currents change after modification of the intracellular solution with half-times of about 1 min. *I–V* curves for peak Na current are approximately symmetrical in shape and the voltage for maximum current is 30–40 mV more positive than threshold. These data support our contention that the new technique reported here has great value for quantitative voltage-clamp analysis of rapid cardiac currents.

Our theoretical calculations suggest, however, that in 100 % extracellular Na the voltage control may be more marginal than the records imply. If the resting space

constant is 500–1000 μm and Na conductance increases about 100-fold at peak (Fig. 6 and Weidmann, 1951; Noble, 1975) then during this conductance increase the space constant is of the order 50–100 μm, or approximately between half and the entire length of a cell. However, if two suction pipettes are attached, about 25 μm of cell length is aspirated into each pipette, leaving 50 μm of exposed surface. When two pipettes are placed at the centre of a fibre (see Fig. 1*B*) the effective cell length is reduced by 50 % to about 50 μm. From these calculations, it appears tht when Na conductance is maximal the ratio of cell length to space constant (L/λ) is approximately 0·5, while at all other times it is less than this, declining to a value of 0·05–0·1 at rest. By using non-linear cable equations and functions to reproduce $I-V$ relations during excitation, Jack *et al.* (1975) have estimated that serious distortion of $I-V$ relations in voltage-clamp situations might be expected for values of L/λ of about 0·4. Should this have been the case in our experiments, then we might have expected $I-V$ curves which were often very steep near threshold and showed inward current peaks at more negative potentials, compared to the results obtained for nerve membranes under conditions of more uniform polarization (Johnson & Lieberman, 1971). Although results of inadequate voltage control are seen with single-pipette clamp, they are not evident when two suction pipettes are used. Further evidence of adequate voltage control are the facts that the envelope of the tail currents had the same shape as the decline of the Na current during a single voltage step, and the $\dot{V}_{max}$ calculated from peak I_{Na} and the cell capacitance is about 180 V sec^{-1}, compared to the directly measured value of approximately 230 V sec^{-1}.

There are several reasons which might account for these discrepant observations. Firstly, as described above, the use of two pipettes reduces the series resistance during voltage clamp from 500–800 KΩ to 200 kΩ and when R_s compensation is used this value is reduced further to about 100 kΩ. Secondly, the calculations of resting space constant given above must be approximate due to the small (< 1 mV) voltage difference along the fibre axis, precluding easy measurement. Should resting space constants for cells giving $I-V$ curves with little distortion be nearer the 800–900 μm reported by Weidmann (1970) for trabecular muscle, an assumption consistent with our estimate for λ in cells internally perfused with Cs, then L/λ would be nearer the critical value of 0·4 calculated by Jack *et al.* (1975). Finally, it has been noted by Noble (1975) that Purkinje fibres of length about 0·4 space constants do not have Na $I-V$ relationships that are markedly distorted. We would contend that, at the very least, the double-suction pipette-clamp system on isolated cells represents a marked improvement as a voltage-clamp technique for measurement of cardiac Na currents. There is no doubt that when extracellular Na is half or a third of the normal value, the present technique is perfectly adequate for voltage clamp of myocardial tissue at temperatures around 22 °C.

This is not to say that the two-suction-pipette method has no other disadvantages. The single-pipette method can be used for more than 1 hr, even in solutions containing physiological levels of Ca. Aspirating a cell into two pipettes reduces the chances of a stable preparation from about 50–20 % in Ca-free solutions, while in 1·1 mM-Ca it was successful in only five out of 100 cells. Survival times were also shortened, but it was possible to record from some cells with two suction pipettes for as long as 45 min without changes in amplitudes of currents produced by

sequential voltage-clamp steps. It is not uncommon that in an effort to improve voltage-clamp techniques, such as in the double-sucrose gap (Rougier, Vassort & Stampfli, 1968), experiment duration is limited in favour of improved resolution and voltage control of the preparation.

This work was supported by N.I.H. grants HL-25145, NS-11453 and the British Heart Foundation. It was done as a partial fulfilment of Kai S. Lee's doctoral thesis.

REFERENCES

ATTWELL, D., EISNER, D. & COHEN, I. (1979). Voltage clamp and tracer flux data: effects of a restricted extracellular space. *Q. Rev. Biophys.* **12**, 213–261.

BEELER, G. W., JR. & REUTER, H. (1970). Voltage clamp experiments on ventricular myocardial fibres. *J. Physiol.* **207**, 191–209.

BROWN, H. F., GILES, W. & NOBLE, S. J. (1977). Membrane currents underlying activity in frog sinus venosus. *J. Physiol.* **271**, 783–816.

BROWN, A. M., LEE, K. S. & POWELL, T. (1981). Sodium current in single rat heart muscle cells. *J. Physiol.* **318**, 479–500.

COLATSKY, T. J. & TSIEN, R. W. (1979). Sodium channels in rabbit cardiac purkinje fibres. *Nature* **278**, 265–268.

DECK, K. A. & TRAUTWEIN, W. (1964). Ionic currents in cardiac excitation. *Pflügers Arch.* **280**, 63–80.

DIFRANCESCO, D. (1980). Evidence that 'I_{K_2}' in Purkinje fibres is an inward current activated on hyperpolarization. *J. Physiol.* **305**, 64*P*.

DIFRANCESCO, D. & NOBLE, D. (1980). If 'I_{K_2}' is an inward current, how does it display potassium specificity? *J. Physiol.* **305**, 14*P*.

DUDEL, J. & RÜDEL, R. (1970). Voltage and time dependence of excitatory sodium current in cooled sheep purkinje fibers. *Pflügers Arch.* **315**, 136–158.

EBIHARA, L., SHIGETO, N., LIEBERMAN, M. & JOHNSON, E. A. (1980). The initial inward current in spherical clusters of chick embryonic heart cells. *J. gen. Physiol.* **75**, 437–456.

GOLDMAN, Y. & MORAD, M. (1977). Measurement of transmembrane potential and current in cardiac muscle: a new voltage clamp method. *J. Physiol.* **268**, 613–654.

HODGKIN, A. L., HUXLEY, A. F. & KATZ, B. (1952). Measurement of current–voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 424–448.

ISENBERG, G. (1977). Cardiac purkinje fibers. The slow inward current component under the influence of modified $[Ca^{2+}]_o$. *Pflügers Arch.* **371**, 61–69.

ISENBERG, G. & KLOCKNER, U. (1980). Glycocalyx is not required for slow inward calcium current in isolated rat heart myocytes. *Nature, Lond.* **284**, 358–361.

JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). *Electric Current Flow in Excitable Cells*. Oxford: Clarendon Press.

JOHNSON, E. A. & LIEBERMAN, M. (1971). Heart: excitation and contraction. *A. Rev. Physiol.* **33**, 479–532.

LEE, K. S., AKAIKE, N. & BROWN, A. M. (1977). Trypsin inhibits the action of tetrodotoxin on neurones. *Nature, Lond.* **265**, 751–753.

LEE, K. S., AKAIKE, N. & BROWN, A. M. (1978). Properties of internally perfused, voltage clamped, isolated nerve cell bodies. *J. gen. Physiol.* **71**, 489–508.

LEE, K. S., AKAIKE, N. & BROWN, A. M. (1980). The suction pipette method for internal perfusion and voltage clamp of small excitable cells. *J. neurosci. Methods* **2**, 51–78.

LEE, K. S., WEEKS, T. A., KAO, R. L., AKAIKE, N. & BROWN, A. M. (1979). Sodium current in single heart muscle cells. *Nature, Lond.* **278**, 269–271.

MATHIAS, R. T., EBIHARA, L., LIEBERMAN, M. & JOHNSON, E. A. (1980). Linear electrical properties of heart cell aggregates. *Fedn Proc.* **39**, 2077*A*.

MEHDI, J. & SACHS, F. (1978). Voltage clamp of isolated cardiac purkinje cells. *Biophys. J.* **21**, 165*A*.

MORAD, M. & TRAUTWEIN, W. (1968). The effect of the duration of the action potential on contraction in the mammalian heart muscle. *Pflügers Arch.* **299**, 66–82.

NASH, G. B., TATHAM, P. E. R., POWELL, T., TWIST, V. W., SPELLER, R. D. & LOVEROCK, L. T. (1979). Size measurements on isolated rat heart cells using coulter analysis and light scatter flow cytometry. *Biochim. biophys. Acta* **587**, 99–111.

NEW, W. & TRAUTWEIN, W. (1972). Inward membrane currents in mammalian myocardium. *Pflügers Arch.* **334**, 1–23.

NOBLE, D. (1975). *The Initiation of the Heartbeat.* pp. 48–52. Oxford: Clarendon Press.

NOBLE, D. & TSIEN, R. W. (1969). Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of membrane current. *J. Physiol.* **200**, 233–254.

PAGE, E. & MCALLISTER, L. P. (1973). Studies on the intercalated disc of rat left ventricular myocardial cells. *J. Ultrastruct. Res.* **43**, 388–411.

POWELL, T., STEEN, E. M., TWIST, V. W. & WOOLF, N. (1978*a*). Surface characteristics of cells isolated from adult rat myocardium. *J. Mol. and cell. Cardiol.* **10**, 287–292.

POWELL, T., TERRAR, D. A. & TWIST, V. W. (1978*b*). Membrane potentials in muscle cells isolated from adult rat myocardium. *J. Physiol.* **282**, 23–24*P*.

POWELL, T., TERRAR, D. A. & TWIST, V. W. (1978*c*). Electrical activity in superfused cells isolated from adult rat ventricular myocardium. *J. Physiol.* **284**, 148*P*.

POWELL, T., TERRAR, D. A. & TWIST, V. W. (1980). Electrical properties of individual cells isolated from adult rat ventricular myocardium. *J. Physiol.* **302**, 131–153.

POWELL, T. & TWIST, V. W. (1976). A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. *Biochem. and biophys. Res. Com.* **72**, 327–333.

REUTER, H. & SCHOLZ, H. (1977). A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. *J. Physiol.* **264**, 17–47.

ROUGIER, O., VASSORT, G. & STAMPFLI, R. (1968). Voltage clamp experiments on frog atrial heart muscle fibers with the sucrose gap technique. *Pflügers Arch.* **301**, 91–108.

TARR, M., & TRANK, J. W. (1974). An assessment of double sucrose-gap voltage clamp technique to frog atrial muscle. *Biophys. J.* **14**, 627–643.

TRAUTWEIN, W. (1973). Membrane currents in cardiac muscle fibers. *Physiol. Rev.* **53**, 793–835.

UNDROVINAS, A. I., YUSHMANOVA, A. V., HERING, S. & ROSENSHTRAUKH, L. V. (1980). Voltage clamp method on single cardiac cells from adult rat heart. *Experientia* **36**, 572–574.

WEIDMANN, S. (1951). Effect of current flow on the membrane potential of cardiac muscle. *J. Physiol.* **115**, 227–236.

WEIDMAN, S. (1952). The electrical constants of Purkinje fibres. *J. Physiol.* **118**, 348–360.

WEIDMANN, S. (1956). Comparison of mammalian action potentials. *Ann. N.Y. Acad. Sci.* **65**, 663–678.

WEIDMANN, S. (1970). Electrical constants of trabecular muscle from mammalian heart. *J. Physiol.* **210**, 1041–1054.