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VOLTAGE CLAMP MEASUREMENTS OF SODIUM CHANNEL PROPERTIES IN RABBIT CARDIAC PURKINJE FIBRES

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SUMMARY

1. Voltage clamp studies of the excitatory sodium current, I_{Na}, were carried out in rabbit cardiac Purkinje fibres using the two-micro-electrode technique. Previous work has shown the rabbit Purkinje fibre to have relatively simple morphology (Sommer & Johnson, 1968) and electrical structure (Colatsky & Tsien, 1979*a*) compared to other cardiac preparations.

2. Non-uniformities in membrane potential were kept small by reducing the size of I_{Na} to less than 50 μA/cm^2 of total membrane surface area through prepulse inactivation or removal of external sodium, Na_o. Temporal resolution was improved by cooling to 10–26 °C. These adjustments did not greatly alter the measured properties of the sodium channel.

3. Under these conditions, sodium currents were recorded satisfying a number of criteria for adequate voltage control. Direct measurement of longitudinal non-uniformity using a second voltage electrode showed only small deviations at the time of peak current.

4. The properties of the sodium channel were examined using conventional protocols. Both peak sodium permeability, P_{Na}, and steady-state sodium inactivation, h_∞, showed a sigmoidal dependence on membrane potential. P_{Na} rose steeply with small depolarizations, increasing roughly e-fold per 3·2 mV, and reaching half-maximal activation at -30 ± 2 mV. The h_∞–V curve had a midpoint of $-74{\cdot}9 \pm 2$ mV and a reciprocal slope of $4{\cdot}56 \pm 0{\cdot}13$ mV at temperatures of 10–19·5 °C, and showed a dependence on temperature, shifting to more negative potentials with cooling (~ 3 mV/10 °C). Recovery of I_{Na} from inactivation in double pulse experiments followed a single exponential time course with time constants of 108–200 msec at 19 °C for holding potentials near -80 mV. No attempt was made to describe the activation kinetics because of uncertainties about the early time course of the current.

5. These data predict a maximum duration for I_{Na} of less than 1–2 msec and a maximum peak current density of about 500 μA/cm^2 under physiological conditions, i.e. 37 °C and 150 mM-Na_o. This current magnitude is sufficient to discharge the membrane capacitance at rates comparable to those measured experimentally (311 ± 27 V/sec, Colatsky & Tsien, 1979*a*).

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6. The limitations of the method are discussed. The major problem is the longitudinal cable delay which limits the speed of voltage control. This makes it difficult to separate the activation of I_{Na} from the decay of the capacity transient for potentials positive to -15 mV.

7. It is concluded that the approach described is valid for measurements of sodium currents in the potential range where action potentials are initiated, making it possible to study cardiac sodium channels in an adult mammalian preparation which is free of enzymatic treatment.

INTRODUCTION

It has long been known that the rapid upstroke of the cardiac Purkinje fibre action potential is generated by an inflow of sodium ions (Draper & Weidmann, 1951). However, direct measurements of the excitatory sodium current (I_{Na}) in heart have been difficult because the complex multicellular organization of cardiac muscle limits the degree of spatial and temporal uniformity that can be achieved with the voltage clamp technique (Johnson & Lieberman, 1971; Tarr & Trank, 1974; Connor, Barr & Jakobsson, 1974; Fozzard & Beeler, 1975; Attwell & Cohen, 1977; Beeler & McGuigan, 1978). Longitudinal non-uniformities arise because a point source of current must be used. Furthermore, radial non-uniformities are introduced in many preparations by the presence of narrow intercellular spaces which places a significant external resistance in series with much of the total membrane (Sommer & Johnson, 1968, 1979). Voltage non-uniformities can be tolerated for relatively small membrane currents (Kass, Siegelbaum & Tsien, 1979) but become severe when membrane current flow is intense, as during the excitatory sodium current.

Purkinje fibres from rabbit ventricle have fewer morphological complexities than other naturally occurring cardiac preparations (Johnson & Sommer, 1967; Sommer & Johnson, 1968). They lack transverse tubules, unlike mammalian ventricular muscle, and have fairly wide (1 μm) intercellular clefts, unlike sheep Purkinje fibres and frog myocardium. The electrical properties of the rabbit Purkinje fibre reflect these structural features (Colatsky & Tsien, 1979*a*). Cable analysis showed the rabbit Purkinje fibre to behave as a simple RC cable with an extremely low external series resistance. Furthermore, radial non-uniformities in membrane potential and potassium ion concentration during voltage clamp polarizations were considerably smaller than those found in ungulate Purkinje fibres in similar experiments (cf. Fozzard, 1966; Baumgarten, Isenberg, McDonald & TenEick, 1977).

Earlier studies of I_{Na} in the rabbit Purkinje fibre using the double sucrose gap were complicated by poor viability of the preparations (Harrington & Johnson, 1973) and by the presence of large extracellular shunt currents (Thompson, 1975). Evidence for adequate control of membrane potential could, nevertheless, be obtained (Thompson, 1975), although control often remained insufficient for the analysis of membrane properties (Harrington & Johnson, 1973). The feasibility of reliably measuring sodium currents in the rabbit Purkinje fibre has been recently explored using the two-micro-electrode voltage clamp technique (Colatsky & Tsien, 1979*b*). Voltage non-uniformity was minimized by partially removing the external sodium to reduce the size of the current, and by lowering the temperature to slow the current kinetics. Under these conditions, the recorded currents satisfy a number of criteria for adequate voltage control.

This paper extends the previous analysis of sodium currents in the rabbit Purkinje fibre using the two-micro-electrode voltage clamp method, and provides additional tests for voltage uniformity and for the effects of sodium removal and low temperature on the properties of the sodium channel.

METHODS

Preparations

Adult rabbits weighing 4-5 lb were killed by a sharp blow to the back of the head. The hearts were quickly removed, rinsed free of blood, and placed in warm oxygenated sodium-free potassium glutamate Tyrode solution (solution A, Table 1). The ventricles were then opened and short (0·3–1·0 mm) strands of Purkinje tissue carefully removed. The excised preparations were incubated in potassium glutamate Tyrode solution for 15–30 min to permit healing over before exposure to solutions containing sodium. The exposure to potassium glutamate solution seemed to improve the viability of the preparations (Colatsky & Tsien, 1979*a*).

TABLE 1. Composition of external solutions

Solution	NaCl	KCl	$CaCl_2$	$MgCl_2$	$MnCl_2$	K^+ glutamate	Choline Cl
A	—	—	5·4	0·5	—	150	—
B	150	4	5·4	0·5	—	—	—
C	150	4	1·8	0·5	3·6	—	—
D	15	4	1·8	0·5	3·6	—	135

All solutions contained 5 mM-glucose and were buffered to pH 7·2–7·4 using either 10 mM-Tris base-Tris HCl or 5–10mM-HEPES titrated with NaOH.

Experimental procedure

The experiments were carried out at 10–26 °C, with temperature held constant within ± 0·2 °C in each run by means of a Peltier device (Cambion, Cambridge, Massachusetts) with a feed-back controller. Low temperatures were necessary to improve temporal resolution of I_{Na}. In some experiments, cooling was initiated following the establishment of satisfactory impalements at 37 °C in standard Tyrode solution (solution B) or in Tyrode containing 3·6 mM-manganese–1·8 mM-calcium (solution C). Impalements were usually difficult to maintain during the cooling period, and fibres often showed signs of deterioration with re-impalement. To avoid this problem, later experiments were begun at room temperature (~ 24 °C) and bath temperature gradually lowered to the desired final level. Once a stable condition was obtained, the superfusate was exchanged for one containing low sodium (solution D). Choline chloride (Mallinckrodt) was used as the sodium replacement. TMA (tetramethylammonium) was also tried, but appeared to have deleterious effects on the preparations (cf. Lee, Weeks, Kao, Akaike & Brown, 1979). The presence of manganese in the bathing solution blocked the twitch and greatly reduced the slow inward current (Vitek & Trautwein, 1971) and the delayed outward current (Kass & Tsien, 1975). Thus, the ionic current recorded on depolarization consisted of I_{Na} and a small amount of leak.

Membrane currents were recorded in the majority of experiments using the conventional two-micro-electrode voltage clamp technique of Deck, Kern & Trautwein (1964) with minor modifications (Tsien, 1974). The voltage electrode was positioned about one third of the distance from the current electrode to the end of the fibre. This placement limits the apparent speed of the clamp but provides a more accurate measurement of the average membrane potential in the fibre (DiFrancesco & McNaughton, 1979). The command pulse was lagged with an exponential time constant of 100–400 μsec to reduce the peak capacitance surge and minimize damage to the preparation (Adrian & Marshall, 1977). Voltage and current electrodes were shielded to within 0·5 mm of their tips by a coating of Electrodag 416 (Acheson Colloid, Port Huron, MI) and insulated with nail polish. The voltage electrode shield was driven by the output of the unity gain electrometer, and the shield around the current electrode was grounded. Capacitative

coupling was further reduced by a grounded metal shield positioned between current and voltage electrodes and by keeping the level of solution in the bath low. In later experiments, current electrodes were bevelled to improve ther current-passing capability. Records of membrane potential and membrane current were taken with a chart recorder (Brush 440), a storage oscilloscope (Tektronix 5031 or 5103), and a laboratory computer (DEC, PDP-8/E).

Measurement of I_{Na}

In the present study, longitudinal non-uniformity was reduced to an acceptable level by choosing very short preparations to minimize cable delays and by limiting the maximal sodium conductance using low external sodium or reduced holding potential. Spatial control was considered adequate if the following observations were made: (*a*) no 'abominable notches' in the current record (Cole, 1968), (*b*) graded increases in I_{Na} with small depolarizations beyond threshold (i.e. in the negative slope region of the current–voltage relation, and (*c*) constancy of current time course as current magnitude is varied by prepulse inactivation. Only data from preparations meeting these minimal criteria were accepted.

Measurements of peak inward current were most accurate when a clear separation existed between activation of I_{Na} and the decay of the capacity transient. At 20 °C, this occurred for depolarizations negative to about -15 mV. For stronger depolarizations, it was necessary to adjust the peak current measurement for a capacitative component. This was done by scaling the capacity transient for a small subthreshold depolarization in proportion to the amplitude of the appropriate voltage step and subtracting the resulting waveform from the recorded total membrane current (Colatsky & Tsien, 1979*b*). Steady currents were also subtracted, but these were usually quite small (e.g. Fig. 1). The capacity subtraction removes linear capacitative components only. Since the activation of inward current will assist in depolarizing the membrane during a voltage step, differences in capacitative time course for sub- and supra-threshold depolarizations are expected. The corrected measurements of peak I_{Na} are therefore subject to some error, the magnitude of which would depend on the amount of additional non-uniformity introduced by the presence of inward current (Schoenberg & Fozzard, 1979). For membrane currents of the size recorded in these experiments (≤ 50 μA/cm^2), the voltage distribution should not be greatly altered during I_{Na}. In some cases, the subtraction did not reveal a clear current peak at positive voltages. In these experiments, currents were measured isochronally.

Details of the experimental analyses are presented in the text. Collected data are given as mean ± S.E. of mean. Measurements are referenced to total membrane surface area, as estimated by the total effective capacitance.

RESULTS

After impalement with two micro-electrodes, the rabbit Purkinje fibres used in this study were generally depolarized to between -30 and -10 mV. The depolarization is largely attributed to the use of very short preparations (0·3–1·0 mm), since values near -85 mV are typically recorded in longer fibres (Colatsky & Tsien, 1979*a*). The resting potential could be easily moved to more normal levels by applying a small amount of hyperpolarizing current through the current electrode (~ 1 nA). The size of the current needed was minimized by the presence of marked inward rectification, which gave the I–V characteristic of the membrane a negative or nearly zero slope generally between -70 and -20 mV. In many cases, the fibre remained well polarized when the holding current was removed (cf. Gadsby & Cranefield, 1977). A low input resistance was associated with damaged or derelict preparations, and sodium currents were not studied in these fibres.

Sodium current measurements

In order to improve voltage control during the flow of sodium current, the maximum sodium conductance was reduced, either by lowering the external sodium con-

centration to 10–13% of normal, or, alternatively, by using a reduced holding potential to partially inactivate the sodium channel. Fig. 1 compares sodium currents recorded from the same fibre under both sets of conditions. Panel *A* shows total membrane currents elicited in 155 mM-sodium Tyrode (solution C) by step depolarizations from a holding potential of −71 mV, while panel *B* shows the currents

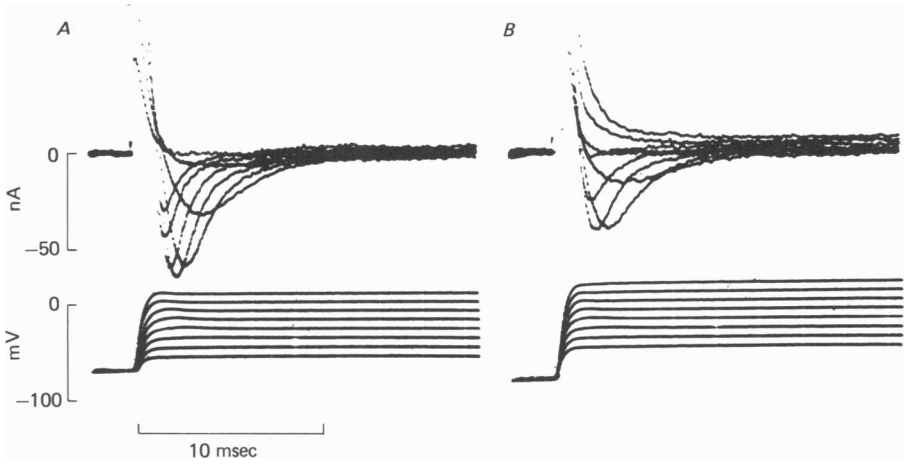

Fig. 1. Early membrane currents in rabbit Purkinje fibres in normal and low-sodium Tyrode solution. Part *A* shows the total membrane currents (ionic + capacitative) elicited by depolarizations of varying magnitude from a holding potential of −71 mV in 155 mM-sodium Tyrode. Part *B* gives a similar family of current records from the same fibre in a later run of 20 mM-sodium Tyrode using a holding potential of −82 mV. The voltage command pulses have been lagged exponentially to blunt the surge of capacitative current. Total effective capacitative, 3·47 nF; apparent cylindrical surface area, 0·00057 cm². 10 kHz filtering was used throughout. Experiment T92-1.

associated with a similar series of voltage steps in low-sodium Tyrode (solution D), using a more negative holding potential of −82 mV. No corrections have been made for leak or capacitative currents. Depolarization produces an initial outward surge of capacitative current which decays with a final time constant of 540 μsec. The relatively slow time course of the capacity transient reflects the longitudinal cable delay inherent in the two-micro-electrode voltage clamp method, rather than the presence of an external series resistance (Colatsky & Tsien, 1979*a*). In the present study, values for the final time constant ranged from 260 to 1300 μsec. These values are consistent with those predicted by cable analysis (Schoenberg, Dominguez & Fozzard, 1975; Colatsky & Tsien, 1979*a*) for rabbit Purkinje fibres at low temperatures (see Discussion).

Small subthreshold depolarizations produce only a brief capacitative surge and a negligible component of steady outward current. When the membrane potential is stepped positive to −55 mV, a transient inward current is elicited. The size of the current increases progressively with further depolarizations to reach a maximum near −18 mV in normal sodium (panel *A*) and −24 mV in low-sodium Tyrode solution (panel *B*). With more positive steps, the peak, current decreases and, in panel *B*, appears to reverse positive to 0 mV. In a total of eight experiments, the

maximum inward current measured was 95 ± 10 nA, giving a mean membrane current density of 19 ± 4 μA/μF referred to the total effective capacitance as estimated by integrating the area under the capacity transient. Individual estimates of total capacitance ranged from 0·57–18 nF, with a mean of $8{\cdot}2 \pm 2{\cdot}5$ nF.

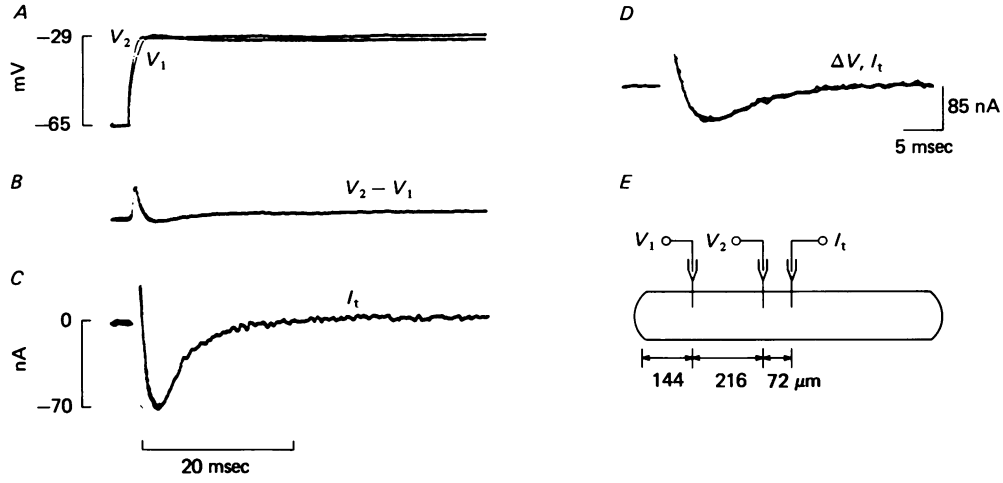

Fig. 2. Three-micro-electrode experiment designed to evaluate longitudinal voltage non-uniformities during the excitatory sodium current. The electrodes were positioned as shown in the lower right panel (*E*). Membrane potential was clamped at V_1. Part *A* superimposes the changes in membrane potential recorded simultaneously at V_1 and V_2 during a step from −65 to −35 mV. The small deviation at the end of the pulses is due to an imperfect seal around the voltage electrode. Part *B* gives the difference signal (V_2–V_1) obtained by computer subtraction of the digitized voltage records. Part *C* shows the total current (I_t) applied by the voltage clamp amplifier during the voltage step. Part *D* compares the ΔV and I_t wave forms after subtracting the outward pedestal and arbitrarily scaling the ΔV trace by 17·15 kΩ to match the downward peaks. 155 mM-external sodium, 18 °C. Total effective capacitance, 7·31 nF; apparent cylindrical surface area, 0·00366 cm². Experiment T01-1.

The kinetics of the inward current were found to depend markedly on membrane potential. Increasing depolarization produced a progressively earlier time-to-peak and a faster rate of inactivation. The decay of I_{Na} followed a single exponential time course. The time constant of inactivation (τ_h) was steeply voltage dependent, ranging from 8·6 msec at −36 mV to 2 msec at −8 mV at 18 °C. Analysis of the activation kinetics was not attempted because of uncertainties about the current time course at early times.

Longitudinal non-uniformity

Previous efforts to measure cardiac sodium currents have been criticized because of doubts about the adequacy of voltage control. In a previous report (Colatsky & Tsien, 1979*b*), experimental evidence was presented which indicated the validity of sodium current measurements in the rabbit Purkinje fibre using the two-microelectrode voltage clamp. These data included the absence of 'abominable notches' during the decay of inward current (Cole, 1968; Connor *et al.* 1975), the absence of an

overly steep negative slope in the peak current–voltage relation (Johnson & Lieberman, 1971), and the rapid shut-off of inward current when the membrane is repolarized at the time of peak sodium permeability (Ramon, Anderson, Joyner & Moore, 1975). Fig. 2 shows the results of an additional test for spatial control using the three-microelectrode technique of Adrian, Chandler & Hodgkin (1970) as adapted for the measurement of regenerative inward currents by Kass *et al.* (1979).

Longitudinal non-uniformity was measured directly using a second voltage electrode. The placement of the electrodes is illustrated in the lower right panel of Fig. 2. A current electrode was placed near the middle, and the two voltage electrodes positioned at distances of $l(V_1)$ and $\frac{5}{2}l(V_2)$ from one end of the fibre. Membrane potential was clamped at V_1. Panel *A* shows the simultaneous measurement of membrane potential at V_1 and V_2 during a voltage step which elicits a sodium current of 70 nA (9·6 μA/μF). A holding potential of -65 mV was used to partially inactivate the sodium channel and reduce the membrane current density. The lower left tracing (panel *C*) is the total current (I_t) supplied by the voltage clamp amplifier during the step. The voltages at V_1 and V_2 deviate from each other during the early part of the clamp. The voltage rises more quickly at V_2 than at V_1, as expected, since V_2 is very near the site of current injection. Panel *B* shows the difference signal (ΔV) obtained by computer subtraction of the V_1 and V_2 traces in panel *A*. The largest non-uniformity occurs at early times, reaching a maximum of about 13·5 mV. At the time of peak I_{Na}, the voltage deviation is only 1·2 mV.

The three-micro-electrode method is susceptible to errors due to imperfect sealing at impalement sites (Adrian *et al.* 1970; Schneider & Chandler, 1976; Kass *et al.* 1979). The difference in steady potential recorded by the V_1 and V_2 electrodes arises from a leaky impalement at V_1 which attenuates the voltage signal at this point. This shows up as a small positive pedestal in the difference trace which cannot be seen on the I_t record (cf. Reuter & Scholz, 1977; Lederer & Tsien, 1976; DiFrancesco & McNaughton, 1979). Such problems were difficult to avoid in these experiments. The amount of distortion introduced by a resistive leak at the V_1 electrode should be relatively small (Adrian & Almers, 1974; Schneider & Chandler, 1976). It therefore seemed reasonable to treat the pedestal as a time-independent leak proportional to the voltage signal at V_1 in the subsequent analysis.

The ΔV measurements not only directly report longitudinal non-uniformity but also provide an independent measure of membrane current density. Kass *et al.* (1979) have shown that close agreement between ΔV and I_t occurs only when both signals report the membrane characteristics that would be obtained with an ideal longitudinal space clamp. Panel *D* shows the superposition of the I_t and the ΔV signals after subtracting the outward pedestal and scaling the ΔV trace by 17·15 kΩ to match the measured peak total current. This conversion factor corresponds to an axial resistance of 352 Ω cm. The time scale has been expanded by a factor of 2 to facilitate comparison of the signal wave forms. It is clear that the agreement is excellent, indicating that adequate spatial control of membrane potential exists during the flow of I_{Na} under these experimental conditions.

Peak current–voltage relations

Fig. 3 presents a more complete analysis of the records in Fig. 1. The experiment provides a useful check on the effects of sodium removal on the properties of the sodium channel. This is important because reductions in external sodium exert

secondary effects on several ionic conductance systems in heart (Deck & Trautwein, 1964; McAllister & Noble, 1966; Dudel, Peper, Rüdel & Trautwein, 1967; cf. Schoenberg & Fozzard, 1979) and can increase intracellular calcium levels via the sodium-calcium exchange mechanism (Reuter & Seitz, 1968). Panel *A* compares the time course of the total membrane current associated with nearly equivalent voltage steps

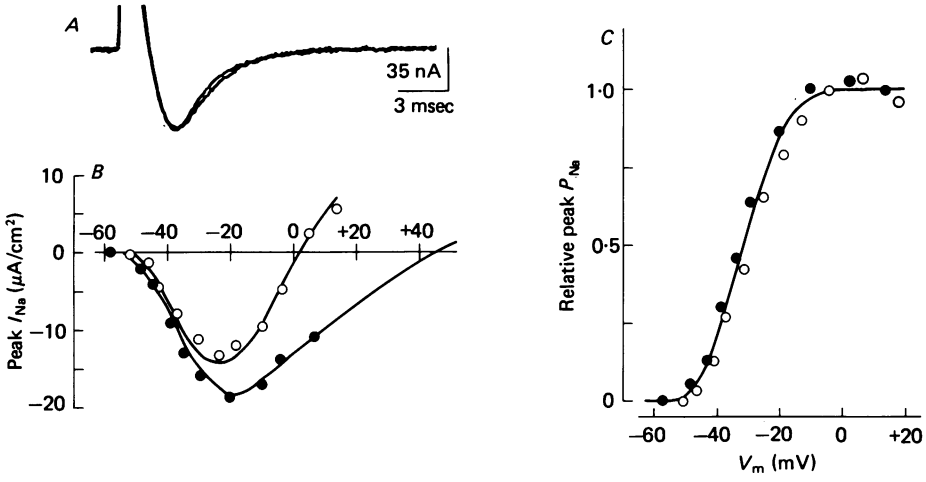

Fig. 3. Effects of lowering external sodium on the measured properties of the sodium channel. Part *A*, comparison of I_{Na} times course in normal and low sodium Tyrode. The traces are total membrane currents recorded at similar voltages in 155 mM (−20 mV) and 20 mM-external sodium (−18 mV). The current in 20 mM-external sodium has been scaled by 1·65 to match the peak current in 155 mM-external sodium. Part *B*, peak current–voltage relations in normal (●) and low sodium Tyrode (○). The continuous curves were calculated from the fit of the permeability data in part *C* assuming independence. Part *C*, voltage dependence of peak sodium permeability. P_{Na} was calculated using the Goldman-Hodgkin-Katz equation and normalized to the maximum value obtained near 0 mV; $1{\cdot}01 \times 10^{-5}$ cm/sec in 20 mM-external sodium, $0{\cdot}11 \times 10^{-5}$ cm/sec in 155 mM-external sodium. The continuous curve was drawn by eye to best fit the data. Same fibre as in Fig. 1. Experiment T92-1.

in normal and low-sodium Tyrode solution. The currents have been scaled to agree in peak magnitude. The current wave forms are largely superimposible, suggesting that the kinetics of the sodium current are not greatly modified by variations in external sodium.

Panel *B* compares the peak current–voltage relations obtained during consecutive runs in 155 mM-sodium Tyrode (filled symbols) and 20 mM-sodium Tyrode (open symbols). The current measurements have been corrected for linear capacitative and steady outward components, as described in Methods. Because the holding potential was increased from −71 to −82 mV in the low sodium run, the *I–V* curves incorporate differences in both driving force and resting inactivation. The voltage dependence of peak I_{Na} is qualitatively similar to that found in other excitable tissues. Beyond a threshold near −55 mV, there is a negative slope region which has a maximum slope conductance per unit effective capacitance of −1·0 mS/μF. The largest inward current occurs at −24 mV in 20 mM-sodium and at −18 mV in

155 mM-sodium Tyrode solution. In a total of eight experiments, current maxima occurred at -26 ± 2 mV in low sodium and -15 ± 1 mV in normal sodium media. The shift of the maxima to more negative potentials in low sodium arises because the reduction in driving force begins to limit the magnitude of the sodium current even though P_{Na} is still increasing (panel *C*).

The ionic current in 20 mM-sodium reverses its direction at $+2{\cdot}5$ mV. Reversal potentials for the sodium current (V_{Na}) could not be measured directly in full sodium because the capacity transient seriously overlaps I_{Na} activation at positive potentials. A rough estimate can be made by linear extrapolation of the positive limb of the $I-V$ curve to zero current. In this experiment, an apparent reversal potential of $+38$ mV was obtained. Although the actual experimental measurement of V_{Na} contains considerable uncertainty, the values are in reasonable agreement with the predictions of the Goldman-Hodgkin-Katz potential equation (Goldman, 1943; Hodgkin & Katz, 1949), using a P_K/P_{Na} ratio of 1/12 (Chandler & Meves, 1965), an internal potassium activity of 130 mM (Miura, Hoffman & Rosen, 1977), and allowing the intracellular sodium activity to vary with external Na as reported by Ellis (1977). In 15 mM-external sodium with internal sodium activity set at 2 mM, a reversal potential of -2 mV is predicted. This compares favourably with the mean of 0 ± 3 mV measured in four experiments at this concentration of external sodium. The predicted reversal potential in normal sodium with an internal sodium activity of 8 mM, is $+48$ mV, which is close to the estimates obtained experimentally ($+44 \pm 2$ mV, $n = 4$).

Voltage dependence of P_{Na}

Peak sodium permeabilities were calculated from measurements of peak inward current using the following equation (Goldman, 1943; Hodgkin & Katz, 1949):

$$P_{Na} = \frac{I_{Na}}{Na_o} \frac{RT}{F^2 V} \frac{\exp(VF/RT) - 1}{\exp((V - V_{Na})F/RT) - 1}, \quad (1)$$

where V is the membrane potential, V_{Na} the sodium current reversal potential, and R, T and F have their usual meanings. The values of P_{Na} obtained were normalized to the maximum permeability calculated for each run and are plotted against membrane potential in panel *C*. P_{Na} shows a sigmoidal dependence on membrane potential varying from almost no activation at -55 mV to nearly full activation at -10 mV. The $P_{Na} - V$ curves obtained in normal sodium (filled symbols) and low sodium (open symbols) show similar voltage dependence. The continuous curve has been drawn by eye to fit the data, and is half maximal at -32 mV. The maximal permeability was $0{\cdot}11 \times 10^{-5}$ cm/sec in 155 mM-sodium, and $1{\cdot}01 \times 10^{-5}$ cm/sec in 20 mM-sodium. The tenfold difference in maximal permeability reflects the different levels of resting inactivation in the two runs. Best estimates of P_{Na} from the fitted curve were then used to predict peak I_{Na} using eqn. (1) after rearranging. The continuous curves in panel *B* are the result of this calculation and fit the data reasonably well, suggesting that the independence principle is obeyed to a first approximation (Hodgkin & Katz, 1949; Hodgkin & Huxley, 1952*a*).

The peak P_{Na} values from eight separate experiments (ten runs) are plotted on a logarithmic scale *versus* potential in Fig. 4. The measurements were made in normal (filled symbols) and low sodium (open symbols) concentrations at temperatures

ranging from 10 to 24 °C. Despite the wide variation in experimental conditions, the voltage dependence of P_{Na} appears relatively constant. P_{Na} rises steeply with small changes in membrane potential, increasing e-fold per 3·2 mV of depolarization, and levels off near −10 mV, reaching half maximal activation at -30 ± 2 mV ($n = 10$). These data are consistent with similar measurements in squid axon (Hodgkin & Huxley, 1952*a*) and frog skeletal muscle (Campbell & Hille, 1976), but differ from previous work in cardiac Purkinje fibres (Dudel, Rüdel, Peper & Trautwein, 1966; Dudel & Rüdel, 1970).

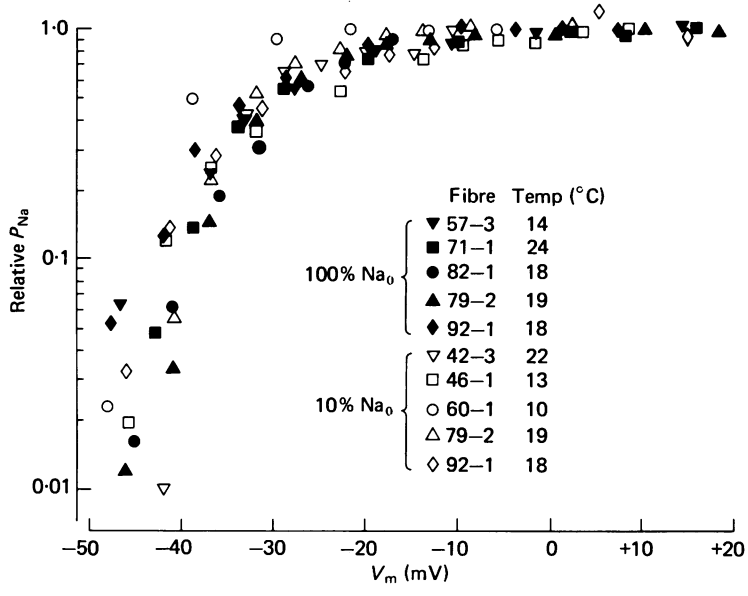

Fig. 4. Voltage dependence of peak sodium permeability in rabbit Purkinje fibres. Measurements of relative P_{Na} collected from ten determinations in eight fibres, plotted semilogarithmically.

Steady-state inactivation

The steady-state voltage dependence of sodium inactivation (h_∞) was studied by measuring the effect of various conditioning prepulses on the peak sodium current elicited at a fixed test potential (Hodgkin & Huxley, 1952*b*). Fig. 5 presents the results from one experiment (15 mM-external sodium, 19 °C) which utilized a 500 msec prepulse and a test pulse to −30 mV. The current records used in the analysis are shown in panel *A* (left) with a small component of holding current omitted. The measured peak currents are plotted as open symbols in panel *B* after normalization to the maximum current elicited with hyperpolarization. The sodium current shows a sigmoidal dependence on prepulse potential which can be fitted by the function (Hodgkin & Huxley, 1952*b*)

$$h_\infty - \frac{1}{1+\exp\left((V-V_h)/k\right)} \tag{2}$$

where $V_h = 69$ mV and $k = 3{\cdot}96$ mV. In a total of eight experiments, $V_h = -74{\cdot}9 \pm 2$ mV and $k = 4.56 \pm 0.13$ mV. Eqn. (2) and similar parameters were used by

TABLE 2. Voltage dependence of sodium inactivation in heart

Preparation	Temp (°C)	$[Ca]_o$ (mM)	V_h. (mV)	k_h. (mV)	Reference
Rabbit Purkinje fibre	10–19·5	1·8 Ca_o+ 3·6 Mn_o	−74·9	4·56	This paper
	19–26	5·4†	−74·4	5·36	Colatsky & Tsien, 1979*a*
	22	4·0	−76·0	8·72*	Thompson, 1975
Sheep Purkinje fibre	8	5·4	−148·4	14·74*	Dudel & Rüdel, 1970
	20	5·4	−101·9	5·52*	Dudel & Rüdel, 1970
Dog ventricle	35	1·8	−55·7	3·0	Beeler & Reuter, 1970
Frog atrium	4·7	1·8	−45 to −62	3·5–5·2	Haas *et al.* 1971

* The parameters were calculated from published data using weighted regression analysis.
† Solutions of 5·4 Ca_o or 5·4 Mn_o + 0·Ca_o or 1·8 Ca_o + 3·6 Mn_o were used in these experiments.

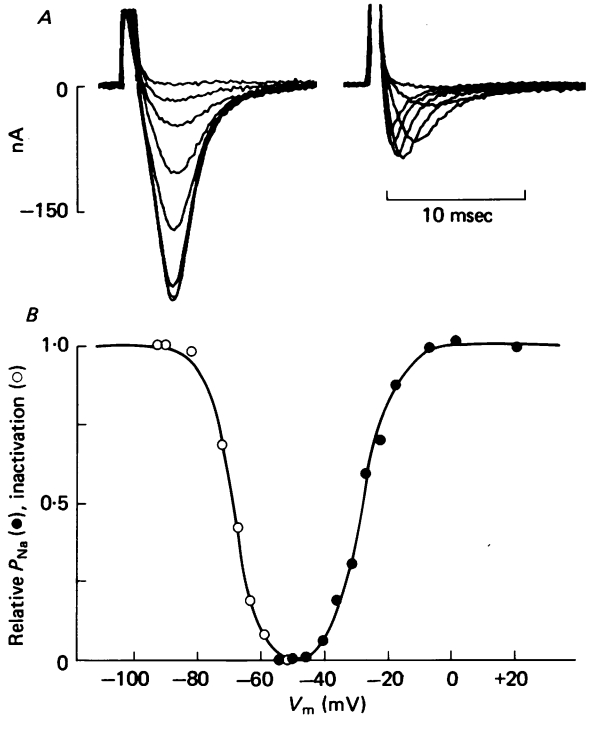

Fig. 5. Relative voltage dependence of sodium inactivation and permeability. Panel *A*, families of total membrane currents obtained using conventional protocols for analysis of sodium inactivation and permeability. The inactivation curve (left) was measured in 15 mM-external sodium using 500 msec prepulses and a constant test pulses to −38 mV. A small component of holding current has been omitted for some of the traces. P_{Na} was measured to 150 mM-external sodium using values of peak I_{Na} recorded at different potentials (right), using a constant holding potential of −58 mV. Panel *B*, voltage dependence of relative sodium inactivation and permeability. The inactivation curve (○) is fitted by $h_\infty = 1/(1+\exp(V-V_h)/k)$, with V_h = 69 mV and k = 3·96 mV. P_{Na} (●) was calculated using the Goldman-Hodgkin-Katz equation, and normalized to a maximum value of $0{\cdot}09-10^{-5}$ cm/sec. Total effective capacitance, 5·66 nF. Experiment T82-1.

Weidmann (1955) in studies using the maximal rate-of-rise of the action potential as an index of P_{Na}. Table 2 compares these results with previous work on sodium inactivation in the rabbit Purkinje fibre and other cardiac preparations. The present data resemble those of Colatsky & Tsien (1979*b*) at slightly warmer temperatures, and of Thompson (1975) for rabbit Purkinje fibres in the double sucrose gap, but differ

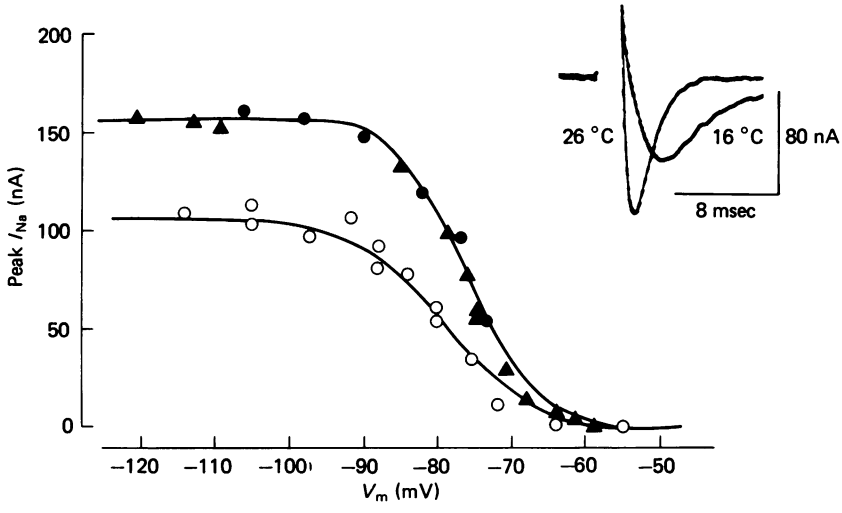

Fig. 6. Temperature dependence of sodium inactivation and peak I_{Na}. Inactivation curves were obtained by varying the voltage of a 500 msec prepulse and measuring peak I_{Na} elicited by a constant test pulse to −28 mV. The temperature was varied from 26 °C (●) to 16 °C (○), then back to 26 °C (▲). Continuous curves were drawn according to $I_{Na} = I'_{Na}/(1+\exp(V-V_h)/k)$, with the following parameters: at 26 °C, $V_h = -76$ mV, $k = 4{\cdot}5$ mV and $I'_{Na} = 157$ nA; at 16 °C, $V_h = 79$ mV, $k = 6{\cdot}0$ mV and $I'_{Na} = 107$ nA. Total effective capacitance, 2·67 nF; 20 mM-external sodium. Inset: total membrane currents elicited with a prepulse to −75 mV. Experiment T98-6.

markedly from the observations of Dudel & Rüdel (1970) in sheep Purkinje fibres. At 20 °C, Dudel & Rüdel found the inactivation curve strongly shifted to more negative potentials, with a midpoint near −100 mV. Differences are also evident with respect to studies in mammalian ventricular (Beeler & Reuter, 1970) and frog atrial trabeculae (Haas, Kern, Einwachter & Tarr, 1971). In these preparations, the inactivation curve is less negative and has a slightly steeper slope than the present results in rabbit Purkinje fibres.

Permeabilities were also measured in this fibre in an earlier run with full sodium and a holding potential of −58 mV which inactivated 96% of the sodium channels. The uncorrected membrane current records are shown in panel *A* (right). Relative sodium permeabilities are plotted in panel *B* as filled symbols, normalized to a maximum P_{Na} of $0{\cdot}09 \times 10^{-5}$ cm/sec. The smooth curve is drawn by eye with half-maximal activation at −28 mV. The separation between the midpoints of the P_{Na} and h_∞ curves is 41 mV, and there appears to be little overlap. In four other experiments where both curves were determined in the same fibre, the average separation was 42 ± 2 mV.

Effects of temperature

Reductions in temperature are known to shift the h_∞–V curve to more negative potentials in frog and rabbit myelinated nerve (Chiu, Mrose & Ritchie, 1979; Mrose & Chiu, 1979), squid axon (Kimura & Meves, 1979) and sheep cardiac Purkinje fibres (Dudel & Rüdel, 1970). A similar dependence of h_∞ on temperature was found in the present study. These results are shown in Fig. 6. Steady-state inactivation was

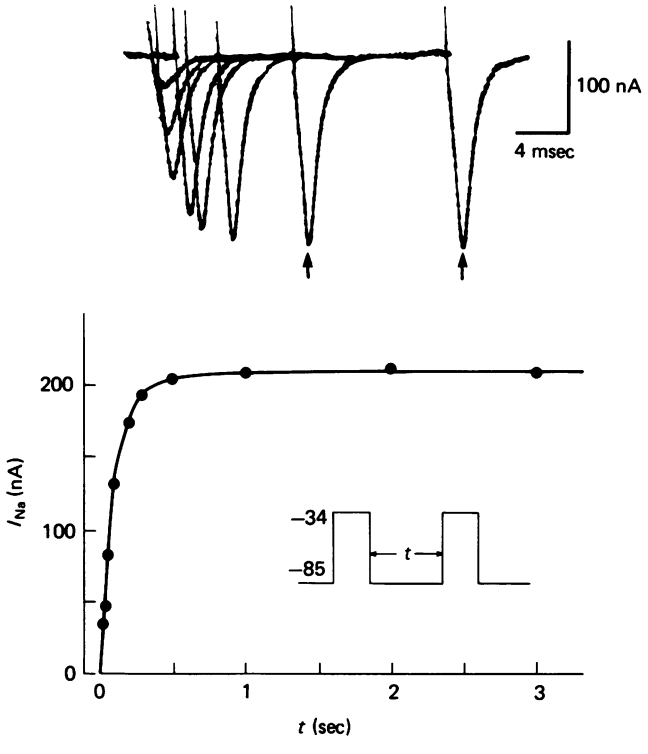

Fig. 7. Recovery of I_{Na} from inactivation. The reactivation time course of I_{Na} was determined using a conventional two pulse protocol, as shown in the inset, consisting of a conditioning pulse and a test pulse, separated by a variable interval, t. Panel A, total membrane currents recorded during the test depolarization for t = 20, 50, 100, 200, 500, 1000 and 2000 msec. The traces are displaced along the time axis according to the appropriate value of t; the distance between the arrows represents 1 sec on this time scale. The calibration bars refer to the individual current records. Panel B, reactivation time course of I_{Na}. The continuous curve is a single exponential with a time constant of 108 msec. 20 mM-external sodium; 19·2 °C. Experiment C02-9.

measured in the conventional way, using 500 msec prepulses and a test pulse to −28 mV, at temperatures of 26 °C (●), 16 °C (○), and again 26 °C (▲). The h_∞ curve shifts to the left with decreasing temperature, and the effect is completely reversible. The points are fitted by eqn. (2) with V_h = −76 mV and k = 4·5 mV at 26 °C, and V_h = −79 mV and k = 6·0 mV at 16 °C. These results are similar to the shifts of 2–8 mV/10 °C found in nerve (Chiu *et al.* 1979; Kimura & Meves, 1979), but are substantially smaller than the shifts previously reported in heart (15–39 mV/

10 °C; see Table 2). Small changes in the voltage dependence of P_{Na} may have occurred (see Fig. 4), but this was not investigated systematically.

Both the rate of inactivation and the peak magnitude of the sodium current were reduced at lower temperature, as shown in the inset in Fig. 6. The time constant for the decay of the inward current at −30 mV increased from 1·36 msec at 26 °C to 3·17 msec at 16 °C (Q_{10} = 2·3). In another experiment, a 10 °C fall in temperature produced a similar 2·6-fold slowing of τ_h. The Q_{10} for peak I_{Na} ranged from 1·23 to 1·46. Comparable values have been obtained in nerve. (Chandler & Meves, 1970; Moore, 1971; Chiu *et al.* 1979) and skeletal muscle (Campbell & Hille, 1976; Adrian & Marshall, 1977).

TABLE 3. Time constants for recovery from inactivation

Fibre	V_m(mV)	τ_h (msec)	Temperature (°C)
T45-1	−69	175	19·0
T78-1	−73	200	19·0
C02·9	−85	108	19·2
T82-1	−85	180	19·0
T94-5	−86	180	17·3

Recovery from inactivation

The time course of removal of inactivation was studied using the double pulse method (Hodgkin & Huxley, 1952*b*). The protocol is illustrated in Fig. 7 (inset). Two 50 msec depolarizing pulses from a holding potential of −85 mV were applied in succession separated by a variable interval, *t*. The upper panel shows the total membrane currents recorded during the second (test) pulse. The traces have been displaced along the time axis to illustrate the time course of reactivation of I_{Na}. The interval between the two largest currents (arrows) represents 1 sec on this time scale. Below, the measured peak currents are plotted as a function of the variable interval. The continuous curve is an exponential having a time constant of 108 msec. Results from this and four other experiments are summarized in Table 3. In each case, a simple exponential reactivation time course was found. In work performed in collaboration with Dr C. J. Cohen, one could, however, observe a small second component of slow inactivation if conditioning depolarizations lasting several seconds were applied. The size of the slowly recovering component did not exceed 10 % of the total current for depolarizations up to 15 sec duration at 20 °C.

DISCUSSION

The present experiments confirm and extend previous results indicating the feasibility of studying the excitatory sodium current in rabbit cardiac Purkinje fibres using the two-micro-electrode voltage clamp (Colatsky & Tsien, 1979*a*, *b*). They also provide useful new information about the properties of the sodium channel in mammalian heart muscle.

Adequacy of voltage control

An important part of this work has been concerned with demonstrating experimentally the adequacy of voltage control during the flow of I_{Na}. The current measurements in each experiment met the following criteria: (i) the absence of 'abominable notches' in the current record (Cole, 1968), (ii) graded increases in peak I_{Na} with small depolarizations in the negative slope region of the $I-V$ curve (Johnson & Lieberman, 1971; Kootsey & Johnson, 1972), and (iii) a constant time-to-peak with increasing current magnitude (Connor *et al.* 1975). Additional tests were performed in other experiments. Repolarisation at the time of peak P_{Na} results in a smooth and rapid shutting-off of the inward current (Colatsky & Tsien, 1979*b*), as expected when voltage is well controlled (Ramon *et al.* 1975). Direct measurements of longitudinal non-uniformity with a second voltage electrode showed voltage deviations of less than 5% along the fibre at the time of peak I_{Na}. This amount of non-uniformity is comparable to that recorded during measurements of slow inward current in cow ventricle (Reuter & Scholz, 1977) and calf Purkinje fibres (Kass *et al.* 1979), and of the delayed rectifier in sheep Purkinje fibres (DiFrancesco & McNaughton, 1979). The agreement between the wave forms of the I_t and ΔV signals provides additional strong evidence that membrane properties are being faithfully recorded (Kass *et al.* 1979).

Intercellular spaces in the rabbit Purkinje fibres are relatively wide (Johnson & Sommer, 1967; Sommer & Johnson, 1968), and do not present a significant external series resistance to transmembrane current flow (Colatsky & Tsien, 1979*a*). Radial non-uniformities are therefore expected to be small in this preparation. The radial length constant (λ_R) can be estimated by $\lambda_R = (w|R'_m|/2R_e)^{\frac{1}{2}}$, where w is the cleft width, R'_m is the cleft membrane resistance, and R_e is the cleft fluid resistivity. Assuming that membrane properties are uniformly distributed throughout the fibre, and that the cleft fluid has the resistivity of normal Tyrode solution (51 Ω cm), a radial length constant of 313 μm is calculated for $R'_m = -1$ kΩ cm^2 (Fig. 3). This estimate of λ_R is very long compared to the total fibre radius (36–90 μm), so little voltage decrement should occur down the clefts. The final time constant for charging the cleft capacitance (τ_0) in the rabbit Purkinje fibre has been estimated to be 10 μsec (Colatsky & Tsien, 1979*a*) using the approach of Schoenberg *et al.* (1975). This is very fast compared to the estimated time course for the longitudinal spread of current (see below). It seems reasonable to assume that spatial and temporal control of the cleft membranes is sufficient for recording I_{Na} faithfully. Non-uniformities would be more serious in a preparation with less favourable geometry, e.g. sheep Purkinje fibres, under the same experimental conditions. For comparable current densities in a preparation with 20 nm clefts, $\lambda_R = 198$ μm and $\tau_0 = 2{\cdot}6$ msec.

Limitations of the method

Accurate measurements of I_{Na} in mammalian cardiac muscle are extremely difficult under normal physiological conditions, i.e. 37 °C and 150 mM-external sodium (Deck & Trautwein, 1964; Dudel *et al.* 1966; Beeler & Reuter, 1970; Harrington & Johnson, 1973; Lee *et al.* 1979). The excitatory sodium current appears to be too large and too fast to resolve with normal voltage clamp techniques. In the present experiments, voltage control and temporal resolution were improved by reducing the

membrane current density below 50 μA/μF and cooling to 10–24 °C. Similar precautions were taken by Adrian & Marshall (1977) in recording I_{Na} in rat skeletal muscle. These adjustments do not greatly alter the properties of the cardiac sodium channel. Decreasing temperature shifts the steady-state sodium inactivation curve in the hyperpolarizing direction 3 mV/10 °C. Shifts of 2–8 mV/10 °C have been reported in squid axon (Kimura & Meves, 1979) and frog and rabbit myelinated nerve under voltage clamp (Chiu *et al.* 1979; Mrose & Chiu, 1979), and in embryonic chick ventricle using the maximal rate-of-rise of the action potential as an index of sodium current (Iijima & Pappano, 1979). Shifts in the P_{Na}-V relationship with temperature have not been studied systematically in the rabbit Purkinje fibre, but they appear to be small (cf. Fig. 4). Such shifts have recently been measured in squid axon (Kimura & Meves, 1979) and myelinated nerve (Mrose & Chiu, 1979). Ionic gradients appear well maintained at 20 °C, as evidenced by estimates of the sodium current reversal potential which are consistent with those predicted for normal values of internal sodium (Ellis, 1977). The fibers appear remarkably stable in low sodium, despite the increase in internal calcium which must occur via the sodium-calcium exchange mechanism under these conditions (Reuter & Seitz, 1968). This may be due to the relatively high threshold for Ca-induced Ca-release in rabbit cardiac muscle (Fabiato & Fabiato, 1977).

The principal limitation of the method is the delay in achieving spatial control. Even at 10–26 °C, the decay of the capacity transient seriously overlaps I_{Na} activation for depolarizations positive to -15 mV. The speed of the clamp is largely determined by the time required for current to spread longitudinally between the current electrode and the ends of the fibre (Kootsey & Johnson, 1972; Colatsky & Tsien, 1979*a*). Extremely short preparations were used to minimize this distance. At 20 °C, a final time constant of 448 μsec is predicted for the longitudinal charging of membrane capacitance (Schoenberg *et al.* 1975) in a typical preparation with a total length of 500 μm, an effective capacitance of 8 nF, and an internal resistivity (R_i) of 450 Ωcm (Colatsky & Tsien, 1979*a*), corrected for the decrease in temperature (Q_{10} = 1·48, Coraboeuf & Weidmann, 1954). The increase in R_i with cooling seriously limits the amount of improvement in temporal resolution that can be expected with further reductions in temperature.

Sodium channel properties

If one assumes, for fixed V, that P_{Na} is independent of the sodium current and the external sodium concentration (i.e. that the independence principle holds) eqn. (1) can be rearranged to predict the intensity of I_{Na} at physiological sodium concentrations. In the experiment shown in Fig. 5, a maximal peak P_{Na} of $1{\cdot}01 \times 10^{-5}$ cm/sec is calculated at 18 °C from a holding potential of -82 mV. At a membrane potential of -15 mV (relative P_{Na} = 0·8), a peak sodium current of 143 μA/μF is calculated for 150 mM-external sodium. This corresponds to a maximal rate-of-rise of 143 V/sec, assuming that $\dot{V}_{max} = I_i/C_m$ and C_m = 1 μF/cm^2 (Hodgkin, Huxley & Katz, 1952; Cole, 1968). Given a Q_{10} of 1·68 for the action potential upstroke velocity (Coraboeuf & Weidman, 1954), a $\dot{V}_{max}$ of 383 V/sec is predicted at 37 °C. This is very close to the value of 311 ± 37 V/sec measured experimentally under normal physiological conditions (Colatsky & Tsien, 1979*a*).

The degree of overlap between sodium inactivation (h_∞) and P_{Na} curves in the rabbit Purkinje fibre appears to be quite small. The existence and extent of crossover between activation and inactivation curves is important because it generates a steady state inward current which can help maintain the plateau and support pacemaker activity (McAllister, Noble & Tsien, 1975). A TTX-sensitive background current believed to be the result of such overlap has been demonstrated in sheep Purkinje fibre under voltage clamp over the potential range -65 to -15 mV (Attwell, Cohen, Eisner, Ohba & Ojeda, 1979). Recently, however, Coraboeuf, Deroubaix & Coulombe (1979) found that TTX shortened the action potential in dog Purkinje fibres at concentrations which did not affect the maximal rate-of-rise, and proposed the existence of a second population of Na channels which lacked the property of inactivation. The action potential duration is also markedly reduced by TTX (16 μM) in rabbit Purkinje fibres (Okada, 1976), suggesting that a significant steady state sodium current is present in this preparation, although it is not clear which type of channels carry the current. The absence of significant overlap in the present experiments may reflect the displacement of the $h_\infty - V$ curve to negative potentials at lower temperature or, alternatively, suggests the presence of a second population of Na channels with modified gating (Coraboeuf *et al.* 1979).

Because of the relative slowness of the voltage clamp, no attempt was made to analyze the activation kinetics of I_{Na} or the instantaneous current–voltage relation of the cardiac sodium channel. However, a rough estimate of the overall current time course can be obtained from the rate of inactivation. At 20 °C and -20 mV, I_{Na} decays exponentially with a time constant (τ_h) of 2–3 msec. Assuming a Q_{10} of 2·3 for the inactivation process (Fig. 6), τ_h at 37 °C for the same depolarization is 485–728 μsec. Thus the inward current transient should last less than 1–2 msec at physiological temperatures. This agrees with estimates of the I_{Na} time course using other voltage clamp techniques (Dudel *et al.* 1966; Dudel & Rüdel, 1970; Ebihara, Shigeto, Lieberman & Johnson, 1979). Following repolarization, I_{Na} recovers exponentially, with time constants of 100–200 msec near 20 °C at resting potentials between -73 and -86 mV. The recovery time constant is decreased at 37 °C to 34–48 msec, using a Q_{10} of 2·3. Gettes & Reuter (1974) found similar time constants of recovery for $\dot{V}_{max}$ in sheep and calf Purkinje fibres at comparable membrane potentials. It was not possible to compare the time constants for inactivation and reactivation at the same potential because of the limited overlap between activation and inactivation curves.

Recently, voltage clamps of cardiac muscle having extremely fast rise times of 10–80 μsec have been reported in single rat myocytes (Lee *et al.* 1979). and in cultured embryonic chick ventricular cells (Ebihara *et al.* 1979). It will be important to determine the amount of non-uniformity in these preparations during measurement of the excitatory sodium current (cf. Nathan & DeHaan, 1979). One advantage of the present approach is that it permits the study of sodium channel properties in an adult mammalian cardiac preparation which is free of enzymatic treatment. Moreover, the morphology and electrical structure of the rabbit Purkinje fibre have been studied in some detail, and evidence for the reliability of I_{Na} measurements under appropriate experimental conditions presented. Further information on the cardiac sodium channel is important, since preliminary data suggest that it may differ in its

pharmacological properties from sodium channels in nerve and skeletal muscle (Baer, Best & Reuter, 1976; Cohen, Colatsky & Tsien, 1979). Such differences will be crucial in understanding the mechanism of therapeutic action of antiarrhythmic drugs.

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REFERENCES

ADRIAN, R. H. & ALMERS, W. (1974). Membrane capacity measurements on frog skeletal muscle in media of low ion content. *J. Physiol.* **237**, 573–605.

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Voltage clamp experiments in striated muscle fibres. *J. Physiol.* **208**, 607–644.

ADRIAN, R. H. & MARSHALL, M. W. (1977). Sodium currents in mammalian muscle. *J. Physiol.* **268**, 223–250.

ATTWELL, D. & COHEN, I. (1977). The voltage clamp of multicellular preparations. *Prog. Biophys. molec. Biol.* **31**, 201–245.

ATTWELL, D., COHEN, I., EISNER, D., OHBA, M. & OJEDA, C. (1979). The steady state TTX-sensitive ('window') sodium current in cardiac Purkinje fibers. *Pflügers Arch.* **379**, 137–142.

BAER, M., BEST, P. M. & REUTER, H. (1976). Voltage-dependent action of tetrodotoxin in mammalian cardiac muscle. *Nature, Lond.* **263**, 344–345.

BAUMGARTEN, C. M., ISENBERG, G., MCDONALD, T. F. & TEN EICK, R. E. (1977). Depletion and accumulation of potassium in the extracellular clefts of cardiac Purkinje fibers during voltage clamp hyperpolarizations and depolarizations. Experiments in Na-free bathing media. *J. gen. Physiol.* **70**, 149–169.

BEELER, G. W. & MCGUIGAN, J. A. S. (1978). Voltage clamping of multicellular myocardial preparations: Capabilities and limitations of existing methods. *Prog. Biophys. molec. Biol.* **34**, 219–254.

BEELER, G. W. & REUTER, H. (1970). Voltage clamp experiments on ventricular myocardial fibres. *J. Physiol.* **207**, 165–190.

CAMPBELL, D. T. & HILLE, B. (1976). Kinetics and pharmacological properties of the sodium channel of frog skeletal muscle. *J. gen. Physiol.* **67**, 309–323.

CHANDLER, W. K. & MEVES, H. (1965). Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* **180**, 788–820.

CHANDLER, W. K. & MEVES, H. (1970). Rate constants associated with changes in sodium conductance in axons perfused with sodium fluoride. *J. Physiol.* **211**, 679–705.

CHIU, S. Y., MROSE, H. E. & RITCHIE, J. M. (1979). Anomalous temperature dependence of the sodium conductance in rabbit nerve compared with frog nerve. *Nature, Lond.* **279**, 327–328.

COHEN, C. J., COLATSKY, T. J. & TSIEN, R. W. (1979). Tetrodotoxin block of cardiac sodium channels during repetitive or steady depolarization in the rabbit. *J. Physiol.* **296**, 70–71*P*.

COLATSKY, T. J. & TSIEN, R. W. (1979*a*). Electrical properties associated with wide intercellular clefts in rabbit Purkinje fibres. *J. Physiol.* **290**, 227–252.

COLATSKY, T. J. & TSIEN, R. W. (1979*b*). Sodium channels in rabbit cardiac Purkinje fibers. *Nature, Lond.* **278**, 265–268.

CONNOR, J., BARR, L. & JAKOBSSON, E. (1975). Electrical characteristics of frog atrial trabeculae in the double sucrose gap. *Biophys. J.* **15**, 1047–1067.

COLE, K. S. (1968). *Membranes, Ions and Impulses*. Berkeley: University of California Press.

CORABOEUF, E. & WEIDMANN, S. (1954). Temperature effects on the electrical activity of Purkinje fibers. *Helv. physiol. pharmac. Acta* **12**, 32–41.

CORABOEUF, E., DEROUBAIX, E. & COULOMBE, A. (1979). Effect of tetrodotoxin on action potentials of the conducting system in dog heart. *Am. J. Physiol.* **236**, H561–H567.

DECK, K. A., KERN, R. & TRAUTWEIN, W. (1964). Voltage clamp technique in mammalian cardiac fibers. *Pflügers Arch ges. Physiol.* **280**, 50–62.

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

DIFRANCESCO, D. & MCNAUGHTON, P. A. (1979). The effects of calcium on outward membrane currents in the cardiac Purkinje fibre. *J. Physiol.* **289**, 347–373.

DRAPER, M. H. & WEIDMANN, S. (1951). Cardiac resting and action potentials recorded with an intracellular electrode. *J. Physiol.* **115**, 74–94.

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

DUDEL, J., PEPER, K., RÜDEL, R. & TRAUTWEIN, W. (1966). Excitatory membrane current in heart muscle (Purkinje fibers). *Pflügers Arch. ges. Physiol.* **292**, 255–273.

DUDEL, J., PEPER, K., RÜDEL, R. & TRAUTWEIN, W. (1967). The effect of tetrodotoxin on the membrane current in cardiac muscle (Purkinje fibers). *Pflügers Arch. ges. Physiol.* **295**. 213–226.

EBIHARA, L., SHIGETO, N., LIEBERMANN, M. & JOHNSON, E. A. (1979). Sodium currents in cultured embryonic heart cells. *Physiologist, Wash.* **22**, 32.

ELLIS, D. (1977). The effects of external cations and ouabain on the intracellular sodium activity of sheep heart Purkinje fibres. *J. Physiol.* **272**, 211–240.

FABIATO, A. & FABIATO, F. (1977). Calcium release from the sarcoplasmic reticulum. *Circulation Res.* **40**, 119–129.

FOZZARD, H. A. (1966). Membrane capacity of the cardiac Purkinje fibre. *J. Physiol.* **182**, 255–267.

FOZZARD, H. A. & BEELER, G. W., Jr. (1975). The voltage clamp and cardiac electrophysiology. *Circulation Res.* **37**, 403–413.

GADSBY, D. C. & CRANEFIELD, P. F. (1977). Two levels of resting potential in cardiac Purkinje fibers. *J. gen. Physiol.* **79**, 725–746.

GETTES, L. S. & REUTER, H. (1974). Slow recovery from inactivation of inward currents in mammalian myocardial fibres. *J. Physiol.* **240**, 703–724.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

HAAS, H. G., KERN, R., EINWACHTER, M. & TARR, M. (1971). Kinetics of Na inactivation in frog atria. *Pflügers Arch.* **323**, 141–157.

HARRINGTON, L. & JOHNSON, E. A. (1973). Voltage clamp of cardiac muscle in a double sucrose gap. A feasibility study. *Biophys. J.* **13**, 626–647.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 449–472.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 497–506.

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**, 424–428.

IIJIMA, T. & PAPPANO, A. J. (1979). Ontogenetic increase of the maximal rate of rise of chick embryonic heart action potentials: relationship to voltage, time and tetrodotoxin. *Circulation Res.* **44**, 358–367.

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

JOHNSON, E. A. & SOMMER, J. R. (1967). A strand of cardiac muscle. Its ultrastructure and the electrophysiological implications of its geometry. *J. cell Biol.* **33**, 103–129.

KASS, R. S. & TSIEN, R. W. (1975). Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. *J. gen. Physiol.* **66**, 169–192.

KASS, R. S., SIEGELBAUM, S. A. & TSIEN, R. W. (1979). Three-microelectrode voltage clamp experiments in calf cardiac Purkinje fibres: is slow inward current adequately measured? *J. Physiol.* **290**, 201–225.

KIMURA, J. E. & MEVES, H. (1979). The effect of temperature on the asymmetrical charge movement in squid giant axons. *J. Physiol.* **289**, 479–500.

KOOTSEY, J. M. & JOHNSON, E. A. (1972). Voltage clamp of cardiac muscle. A theoretical analysis of early currents in the single sucrose gap. *Biophys. J.* **12**, 1496–1508.

LEDERER, W. J. & TSIEN, R. W. (1976). Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. *J. Physiol.* **263**, 73–100.

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

McAllister, R. E. & Noble, D. (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. *J. Physiol.* **186**, 632–662.

McAllister, R. E., Noble, D. & Tsien, R. W. (1975). Reconstruction of the electrical activity of cardiac Purkinje fibres. *J. Physiol.* **251**, 1–59.

Moore, L. E. (1971). Effect of temperature and calcium ions on rate constants of myelinated nerve. *Am. J. Physiol.* **221**, 131–137.

Miura, D. S., Hoffman, B. F. & Rosen, M. R. (1977). The effect of extracellular potassium on the intracellular potassium ion activity and transmembrane potentials of beating canine cardiac Purkinje fibers. *J. gen. Physiol.* **69**, 463–474.

Mrose, H. E. & Chiu, S. Y. (1979). Is the steady state inactivation curve for the sodium channel temperature dependent? *Biophys. J.* **25**, 193*a*.

Nathan, R. D. & DeHaan, R. L. (1979). Voltage clamp analysis of embryonic heart cell aggregates. *J. gen. Physiol.* **73**, 175–198.

Okada, T. (1976). Effect of verapamil on electrical activities of SA node, ventricular muscle and Purkinje fibers in isolated rabbit hearts. *Jap. Circul. J.* **40**, 329–341.

Ramon, F., Anderson, N., Joyner, R. W. & Moore, J. W. (1975). Axon voltage-clamp simulations. IV. A multicellular preparation. *Biophys. J.* **15**, 55–69.

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.

Reuter, H. & Seitz, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.* **195**, 451–470.

Schneider, M. F. & Chandler, W. K. (1976). Effects of membrane potential on the capacitance of skeletal muscle fibers. *J. gen. Physiol.* **67**, 125–163.

Schoenberg, M. & Fozzard, H. A. (1979). The influence of intercellular clefts on the electrical properties of sheep cardiac Purkinje fibers. *Biophys. J.* **25**, 217–234.

Schoenberg, M., Dominguez, G. & Fozzard, H. A. (1975). Effect of diameter on membrane capacity and conductance of sheep cardiac Purkinje fibers. *J. gen. Physiol.* **65**, 441–458.

Sommer, J. R. & Johnson, E. A. (1968). Cardiac muscle. A comparative study in Purkinje fibers and ventricular fibers. *J. cell Biol.* **36**, 497–526.

Sommer, J. R. & Johnson, E. A. (1979). Ultrastructure of cardiac muscle. In *Handbook of Physiology: The Cardiovascular System*, ed. R. M. Berne & N. Sperelakis. Baltimore: Williams & Wilkins, Co.

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

Thompson, S. M. (1975). Na^+ channel permeability to metallic and organic cations in voltage clamped *trabeculae carneae cordis* of the rabbit heart. Ph.D. Thesis, University of Iowa, U.S.A.

Tsien, R. W. (1974). Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. *J. gen. Physiol.* **64**, 320–342.

Vitek, M. & Trautwein, W. (1971). Slow inward current and action potential in cardiac Purkinje fibers. The effect of Mn ions. *Pflügers Arch.* **323**, 204–218.