J. Physiol. (1978), **281**, *pp.* 209–226
With 10 *text-figures*
Printed in Great Britain

IONIC BASIS OF TRANSIENT INWARD CURRENT INDUCED BY STROPHANTHIDIN IN CARDIAC PURKINJE FIBRES

BY R. S. KASS* R. W. TSIEN AND R. WEINGART†

From the Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510, U.S.A.

(*Received 30 November 1977*)

SUMMARY

1. Voltage clamp experiments studied the ionic basis of the strophanthidin-induced transient inward current (TI) in cardiac Purkinje fibres.

2. The reversal potential of TI (E_{rev}) was determined in the presence of various bathing solutions. E_{rev} averaged -5 mV in the standard modified Tyrode solution (Kass, Lederer, Tsien & Weingart, 1978). E_{rev} was displaced toward more negative potentials when the external Na concentration (Na_o) was reduced by replacement of NaCl with Tris Cl, choline Cl or sucrose.

3. A sudden reduction of Na_o evoked a temporary increase in TI, followed after a few minutes by a sustained diminution. The initial increase was closely paralleled by an enhanced aftercontraction and could be explained by an indirect effect of Na_o on internal Ca. The subsequent fall in TI amplitude could be accounted for by the reduced driving force, $E - E_{rev}$.

4. E_{rev} was not significantly changed by replacing extracellular Cl with methylsulphate, or by limited variations in external Ca (2·7–16·2 mM) or external K (1–8 mM).

5. These results are consistent with an increase in membrane permeability to Na and perhaps K.

6. TI was not directly affected by TTX, which blocks excitatory Na channels, or by Cs, which inhibits inwardly rectifying K channels. TI may be distinguished from the slow inward current by its kinetic, pharmacological and ionic properties.

7. TI might be carried by a pre-existing ionic pathway such as the 'leak' channel which provides inward current underlying normal pace-maker depolarization. Another possibility is that TI reflects Ca extrusion by an electrogenic Ca–Na exchange.

INTRODUCTION

This paper is concerned with the ionic basis of the transient inward current which is produced when Purkinje fibres are intoxicated with cardiotonic steroids such as strophanthidin or ouabain. The transient inward current (TI) is of considerable interest because it underlies the transient depolarization (TD) which is associated with spontaneous impulses in digitalis arrhythmias (Ferrier, 1977). Previous voltage

* Present address: Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14602.

† Present address: Physiological Institute, University of Bern, Switzerland.

clamp experiments have characterized some kinetic properties of TI (Lederer & Tsien, 1976) and suggested a dependence on intracellular calcium (Kass *et al.* 1978). Here, the aim is to identify the ions which participate as charge carriers.

Our approach to this problem relied heavily on determinations of the reversal potential (E_{rev}) of TI. E_{rev} averaged $-4{\cdot}8$ mV in the standard modified Tyrode solution (Kass *et al.* 1978). The ionic nature of the TI pathway was investigated by changing the ion concentrations in the bathing fluid and studying their influence on the E_{rev}. The results suggest that the transient inward current is carried by a pathway that is permeable to both Na and K ions. Other ions such as Ca or Cl are not major charge carriers, although they may have indirect effects on the amplitude of TI which are mediated by changes in intracellular Ca release.

Some of the results have already been briefly reported (Weingart, Kass & Tsien, 1977).

METHODS

Membrane current and contractile force were measured in short calf Purkinje fibres under voltage clamp using procedures described previously (Kass *et al.* 1978). The composition of the standard modified Tyrode solution was as follows (mM): 150 Na, 4 K, 5·4 Ca, 0·5 Mg, 155·8 Cl, 5 glucose 10 Tris maleate (pH 7·2–7·4). Solutions were pregassed with 100 % O_2. In experiments with reduced external Na concentration, NaCl was replaced by equimolar amounts of choline Cl or Tris Cl, or twice-equimolar amounts of sucrose. Low Cl solutions contained Na methylsulphate (K & K Chemicals) in place of NaCl. Ca, K and Cs concentrations were varied by including appropriate amounts of the Cl salts without compensatory change in other salt concentrations.

Determinations of the reversal potential of the transient inward current used a strong prepulse (V_1) followed by repolarizing steps to various levels (V_2), as in Fig. 10 of Kass *et al.* (1978). The strength and duration at V_2 of the prepulse were adjusted to evoke a subsequent aftercontraction which appeared well separated from components of phasic or tonic tension accompanying the prepulse itself. Usually the best results were obtained with a 0·2–0·5 sec clamp to about +25 mV. The combination of fixed prepulse and variable post-pulse was applied repetitively during changes of bathing solution. In some experiments, the repolarizing step was followed by more than one transient current (Fig. 3). In these cases, the first transient current and the corresponding aftercontractions were taken for purposes of analysis. Transient amplitudes were measured with respect to a sloping base line as in Kass *et al.* 1978.

RESULTS

Influence of Na on reversal potential

Fig. 1 illustrates the basic approach used in this paper. Force and total membrane current were recorded from a short Purkinje fibre preparation under voltage clamp. The preparation was intoxicated by exposure to 1 μM-strophanthidin during the repetitive stimulation of action potentials or the repetitive application of depolarizing clamp pulses. After roughly 10–15 min the preparation reached a fairly steady toxic state where clearcut TI and aftercontraction could be evoked by the break of strong depolarizing clamp pulses (see Methods). Changes in bathing solution were then used to alter the chemical gradient for ions which might help carry the transient current.

TI was strongly influenced by changes in the external Na ion concentration, Na_o. Fig. 1 compares control signals obtained in 150 mM-Na with records obtained at various times after replacement of threequarters of the external Na with Tris. Before Na_o is reduced (A), the repolarizing step to -17 mV produced small but

clearly inward TI (an additional transient is evoked upon return to the holding potential, − 61 mV, but is less important for this illustration). The change in bathing solutions led first to the disappearance of inward TI at − 17 mV (*B*), and then to the appearance of TI in the outward direction (*C*, *D*). Each of the associated force records shows an aftercontraction during the repolarizing step to − 17 mV. The after-contractions indicate that the transient Ca release which activates TI remains qualitatively the same and is not responsible for the disappearance and eventual inversion of the transient current. In fact, as column *D* indicates, the outward TI and the associated aftercontraction have very similar wave forms.

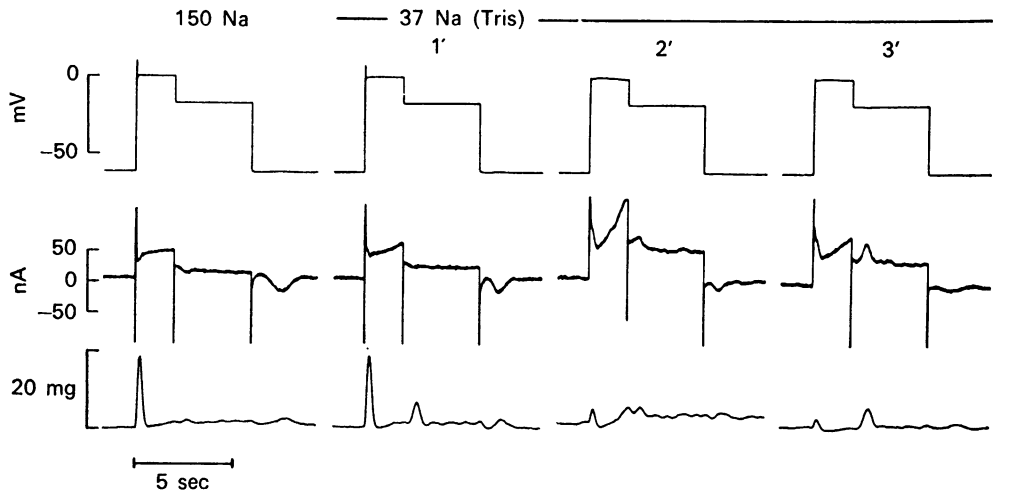

Fig. 1. Effect of lowering the external Na concentration on TI and aftercontraction. *A*, membrane potential (top), membrane current (middle) and force (bottom) in the presence of the normal external Na concentration. *B*, *C*, *D*, corresponding signals obtained 1, 2 and 3 min after replacing 75 % of the external sodium by Tris. Preparation R12-2, 1 μM-strophanthidin, apparent cylindrical area 0·007 cm^2.

Outward TI was described in the previous paper and was interpreted in terms of a transient conductance change Δg, with a driving force, $(E - E_{rev})$. The results in Fig. 1 could be accounted for by a progressive change in E_{rev} which alters the polarity of the driving force. In this respect, E_{rev} would lie positive to the test potential − 17 mV in the control (*A*) but would shift to a new level negative to the test potential when Na_o was reduced (*D*). This explanation is supported by the direct determination of E_{rev} in the presence of varying Na_o.

Fig. 2 shows records from an experiment where the reversal potential was measured in three consecutive runs in 150 Na, 75 Na (choline), and 150 Na. The top panel in each column shows four superimposed voltage traces with step repolarizations to varying levels. As the level was displaced in the positive direction, the transient current changed from inward to outward (see Kass *et al.* 1978). The reversal potential was determined graphically in Fig. 3 for each of the three runs; reversal potential values are indicated in Fig. 2 by horizontal arrows. Reversal took place at + 4 mV in the first run in 150 Na, and at − 16 mV in 75 Na (choline). The last run was taken

after the preparation had been reintoxicated with repetitive stimulation in 150 Na, and gave a reversal potential close to the original value.

Fig. 3 illustrates the analysis of the results from the three runs. Panel *A* plots the amplitude of TI as a function of the repolarization level. This relationship is shifted along the voltage axis by the reduction in bathing Na concentration. Continuous curves were drawn through the data points and the voltage intercepts were used as determinations of E_{rev} (arrows in Fig. 2). Panel *B* shows the amplitude of the associated aftercontractions as a function of repolarization level. In this experiment, changes in repolarization level or sodium concentration had relatively little

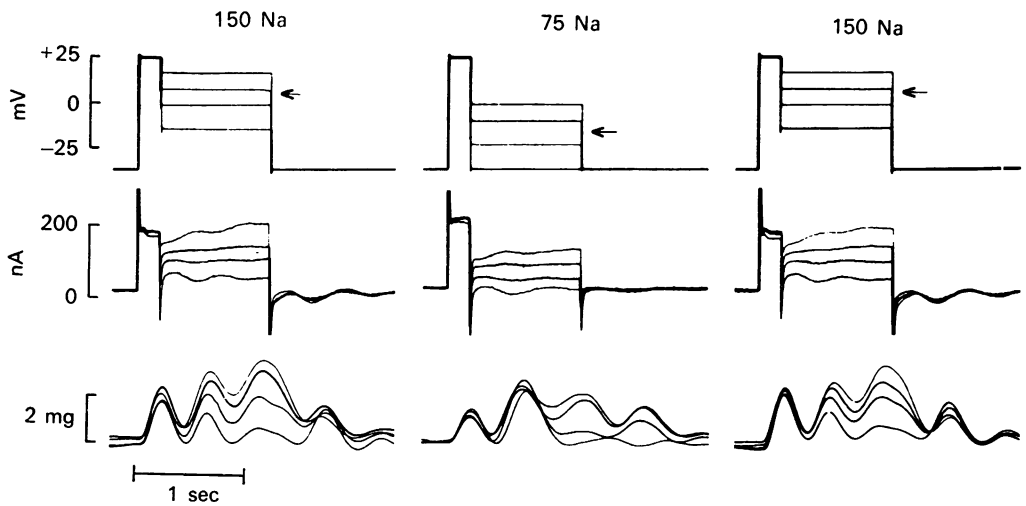

Fig. 2. Effect of Na concentration on TI reversal. In each column step repolarizations to varying potentials are superimposed (top). Membrane current (middle) and contractile force (bottom) follow the same vertical sequence as the voltage traces. Horizontal arrows indicate reversal potential values obtained by graphical analysis of data from the same runs (Fig. 3). *A*, control run in 150 Na. *B*, records taken from 3–5 min after changing external solution to 75 Na (choline). *C*, records taken 19–21 min after readmitting 150 Na. Preparation was stimulated between runs *B* and *C* to restore intoxication. Preparation R22-3, 1 μM-strophanthidin, apparent cylindrical area, 0·009 cm^2.

effect on the size of the aftercontractions, and presumably, no great influence on the degree of TI activation by Ca_i. Thus, it is likely that the observed voltage-dependence of TI (panel *A*) largely reflects the characteristics of the conducting pathway itself.

Similar experiments were carried out with varying degrees of reduction of the external Na concentration. Fig. 4 plots the change in reversal potential, ΔE_{rev}, as a function of the test Na_o. As in Fig. 2, the test run in reduced Na was bracketed by control runs in 150 Na, and average of the bracketing control values was used in calculating ΔE_{rev}. This procedure emphasizes voltage changes, and avoids possible errors in potential that arise when the micro-electrode tip enters or leaves the site of impalement. By convention, ΔE_{rev} is zero in 150 Na (filled circle). Fig. 4 presents data from thirteen determinations, with a different symbol for each individual

preparation. NaCl was wholly or partially replaced by choline Cl (open symbols), Tris Cl (filled symbols) or sucrose (crosses). There was no obvious difference between results obtained with the various Na substitutes. Sodium replacement by Tris and choline were directly compared in one experiment (triangles) and gave very similar values of E_{rev}.

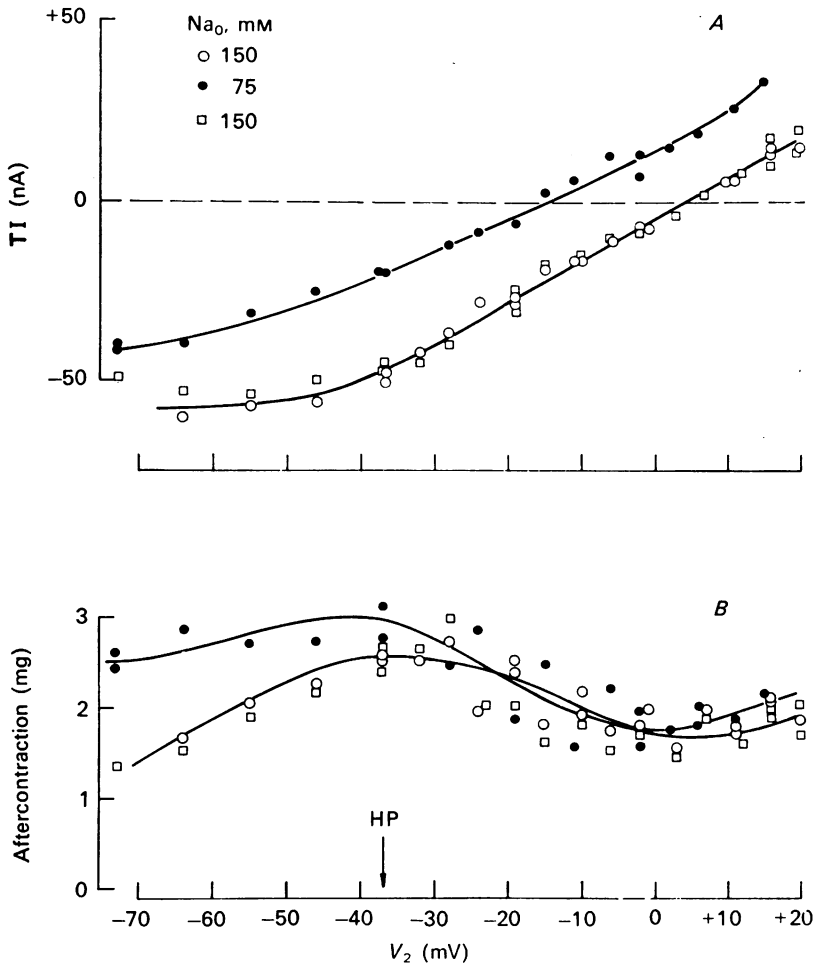

Fig. 3. Influence of repolarization potential on transient current (TI) (*A*) and after-contraction (*B*) in the presence of varying Na. Data from experimental runs illustrated in Fig. 2 using first transient current peak following repolarizing step. Continuous curves drawn by eye. Voltage intercepts in *A* were taken as reversal potentials.

The measurements in Fig. 4 may be referred to absolute values of membrane potential by using a pooled value for E_{rev} in 150 Na, −4·8 mV (Kass *et al.* 1978). The reliability of the pooled value was increased by including data from experiments where sodium removal was not carried out. The average value of E_{rev} in 150 Na provided the basis for the absolute potential scale on the right side of Fig. 4.

Other effects of sodium removal

Reduction of external Na concentration has other effects which must be distinguished from changes in E_{rev}. Using holding potentials ranging from -70 to -40 mV, Lederer (1976) found that sudden decreases in Na_o promptly enhanced the magnitude of TI. The enhancement is contrary to the change expected from a displacement of the reversal potential in the negative direction as found in this study. The apparent conflict may be resolved by measurements of aftercontractions along with the current transients. It turns out that the early increase in TI magnitude at potentials negative to -40 mV may be explained by the influence of Na deprivation on intracellular Ca.

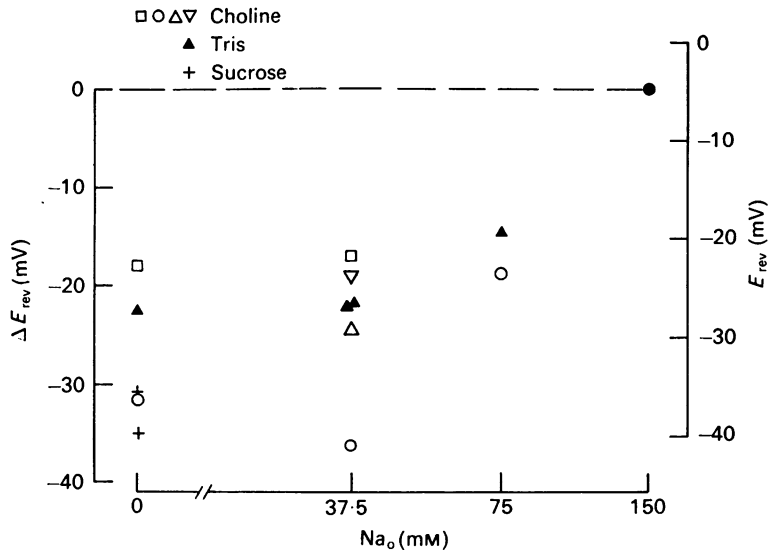

Fig. 4. Displacement of TI reversal potential by reduction of external Na concentration. Pooled results from Fig. 3 and similar experiments using various Na substitutes (inset). Each symbol indicates a different preparation: R12-2 (△), R20-3 (□), R22-3 (○), R37-4 (▽), R40-4 (+). Scale on right side refers measurements to absolute membrane potential (see text for explanation).

Fig. 5 illustrates the progressive changes in TI and aftercontraction during a prolonged exposure to Na-free solution. Representative current and force records are shown and the time course of changes in the transient amplitudes are plotted below. The holding potential was -40 mV and 5 sec clamp pulses to -4 were applied at regular intervals. The clamp pulses were followed by TI and a very small aftercontraction in 150 Na (record *a*). Complete replacement of the external Na with choline began at zero time and evoked a rapidly developing increase in both TI and aftercontraction (*b*). Beyond this point the time courses diverged. The enhancement of TI gave way to a rather steep decline which was nearly complete within 2 min after the initiation of the solution change (*c*). At this time, the after-contraction had just risen to its maximal amplitude. With further exposure to 0 Na, TI remained small while the amplitude of the aftercontraction fell off slowly (*d*). Both transients were completely abolished when the external Na was restored (*e*).

Similar effects of Na removal were observed in a repeat run and in two earlier experiments where force was not recorded. In all cases, TI was initially enhanced and later depressed by reducing the external Na concentration.

The comparison between the current and force transients in Fig. 5 leads to the following interpretation of the effects of Na removal. The early augmentation of TI (*b*) is attributed to a greater amount of Ca release and a greater degree of activation of TI conductance (see discussion of Kass *et al.* 1978). The increase in Ca release

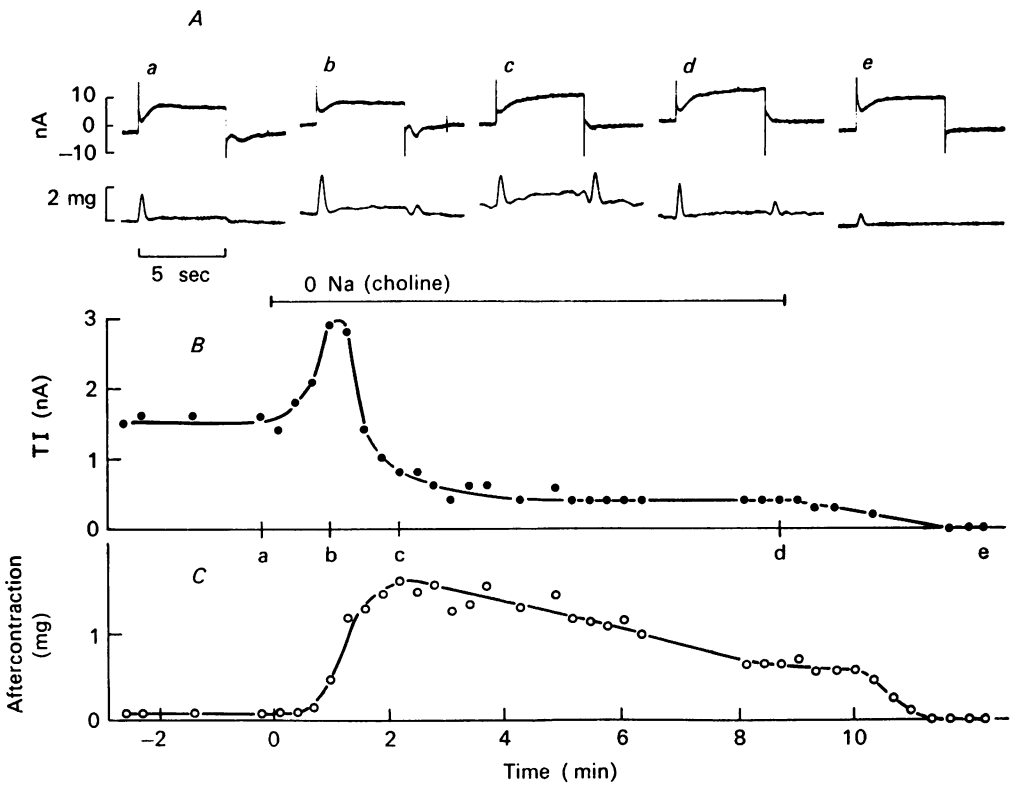

Fig. 5. Opposing effects of Na removal on TI. *A*, current and force records associated with a standard 5 sec clamp pulse from −40 to −4 mV. Records *a–e* were taken at various times indicated on the abscissa below. *B*, amplitude of TI. *C*, amplitude of aftercontraction. Preparation R34-1, no strophanthidin present.

is signalled by the enhanced aftercontraction and it seems consistent with the larger twitch and the development of a partial contracture (*b*, *c*). All of these inotropic effects may be due to a change in Na-dependent Ca movements via the Ca–Na exchange (Reuter & Seitz, 1968; Baker, Blaustein, Hodgkin & Steinhardt, 1969). Evidence on the Ca–Na exchange (Glitsch, Reuter & Scholz, 1970) suggests that intracellular calcium may vary directly with $(Na_o)^2$, or possibly some higher power of the external Na concentration (Horackova & Vassort, 1976). It is not surprising then that the enhancement of Ca_i is the dominant factor immediately following the solution change.

The divergence between TI and aftercontraction (*c*) is compatible with an altered

reversal potential and a decrease in the magnitude of the driving force, $(E - E_{rev})$. Presumably TI does not disappear completely because E_{rev} remains positive to the potential at which TI is recorded (-40 mV). This interpretation is consistent with determination of E_{rev} in 0-Na (choline) solutions (Fig. 4).

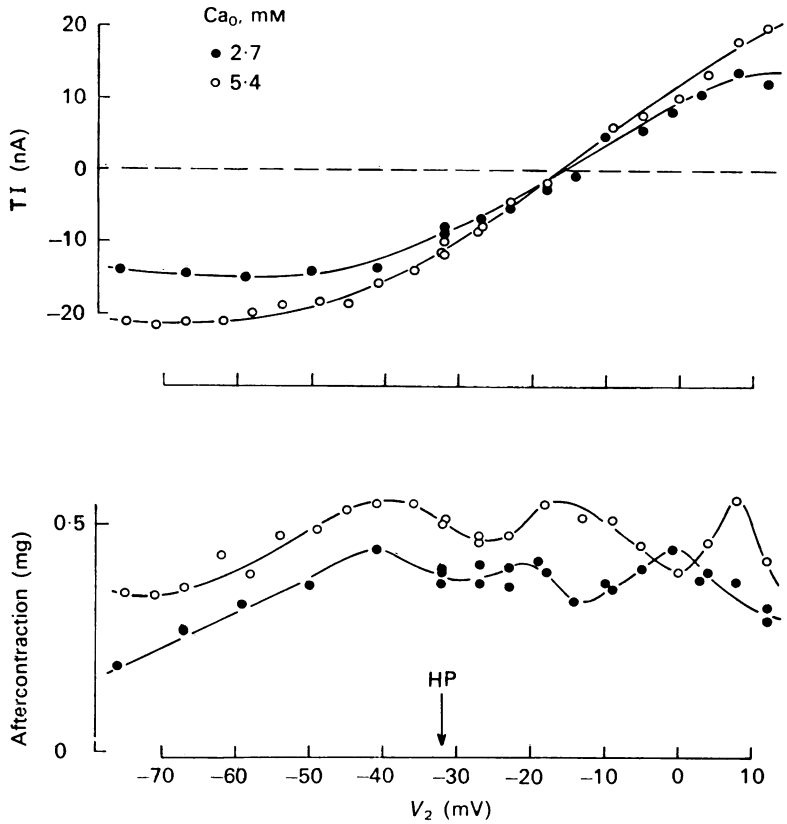

Fig. 6. Influence of extracellular Ca concentration on TI and aftercontraction at varying repolarization levels. Same protocol as used in Figs 2 and 3. Repolarizing steps followed 0·2 sec prepulse to +30 mV. Preparation R29-2, 1 μM-strophanthidin.

Influence of Ca on the reversal potential

The evidence presented so far supports the involvement of Na ions in carrying the transient inward current while leaving open the possible participation of other ions. It is particularly important to consider Ca ions since Ferrier & Moe (1973) have already suggested the existence of a transient Ca^{2+} influx under digitalis intoxication. Reversal potential measurements provide a measure of the extent to which Ca^{2+} ions serve as charge carriers.

Fig. 6 shows how TI and aftercontraction depend on membrane potential as the extracellular Ca concentration is varied. Increasing Ca_o from 2·7 to 5·4 mM produced a modest enhancement of the magnitude of TI and aftercontraction at all potentials as expected from the results of Kass *et al.* (1978). But the reversal potential for TI remained essentially unchanged. This type of experiment was

carried out in a total of six runs in four individual preparations, using twofold or threefold changes in Ca_o within the range between 2·7 and 16·2 mM. In no instance was there a clear-cut change in E_{rev}. The results indicate that Ca ions do not carry substantial amounts of charge during the transient current. The importance of Ca ions lies instead in the activation of the conductance change which generates the transient current (see Kass *et al.* 1978).

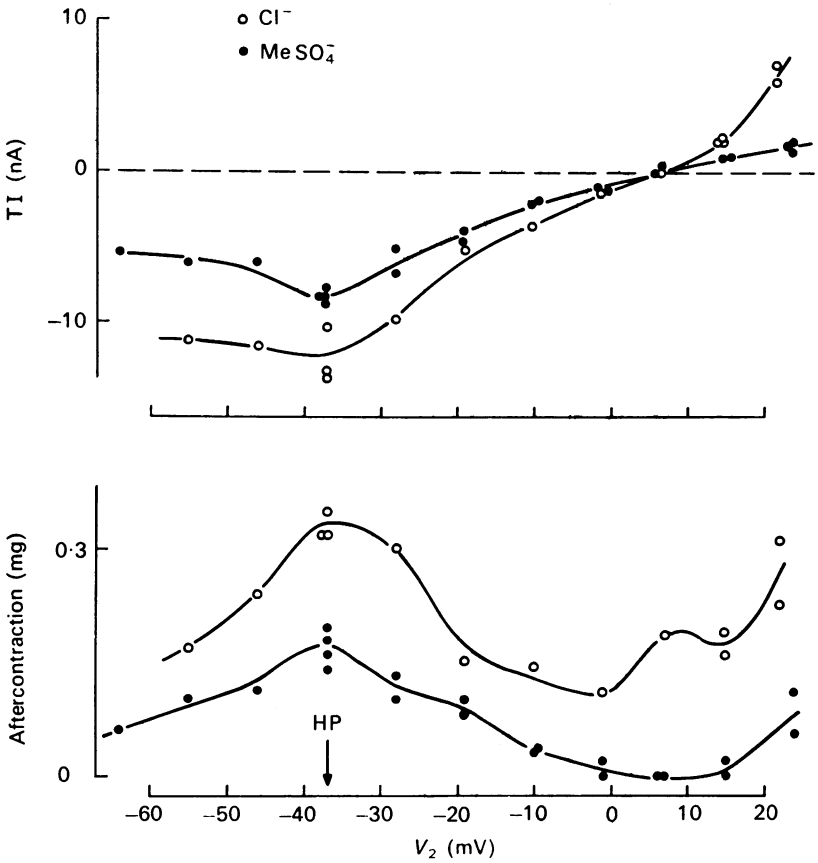

Fig. 7. Effect of Cl replacement by methylsulphate on TI and aftercontraction at various levels of repolarization. Repolarizing steps followed 0·45 sec prepulse to +33 mV. Preparation R25-1, 1 μM-strophanthidin.

Influence of extracellular concentrations of Cl and K

TI cannot be a Na-specific current since E_{rev} is close to zero membrane potential and E_{Na} is approximately +70 mV (Ellis, 1977). This suggests that some ion with a negative equilibrium potential participates as a charge carrier. Cl and K ions are obvious candidates.

Fig. 7 illustrates the effects of removing Cl from the bathing solution, using methylsulphate as the main anion substitute. The solution change brought about reductions in the amplitudes of TI and the aftercontraction over the entire potential range. The reversal potential of TI remains unchanged. Thus, Cl substitution fails to

support the involvement of this ion as a charge carrier, although it leaves open the possibility of indirect effects on the steps leading up to the TI conductance change.

We were particularly interested in exploring the involvement of K ions because of possible analogies between TI and motor end-plate currents. Both currents involve Na ions and show reversal potentials near 0 mV. K ions are known to pass through end-plate channels and there is some evidence that changes in K_0 alter

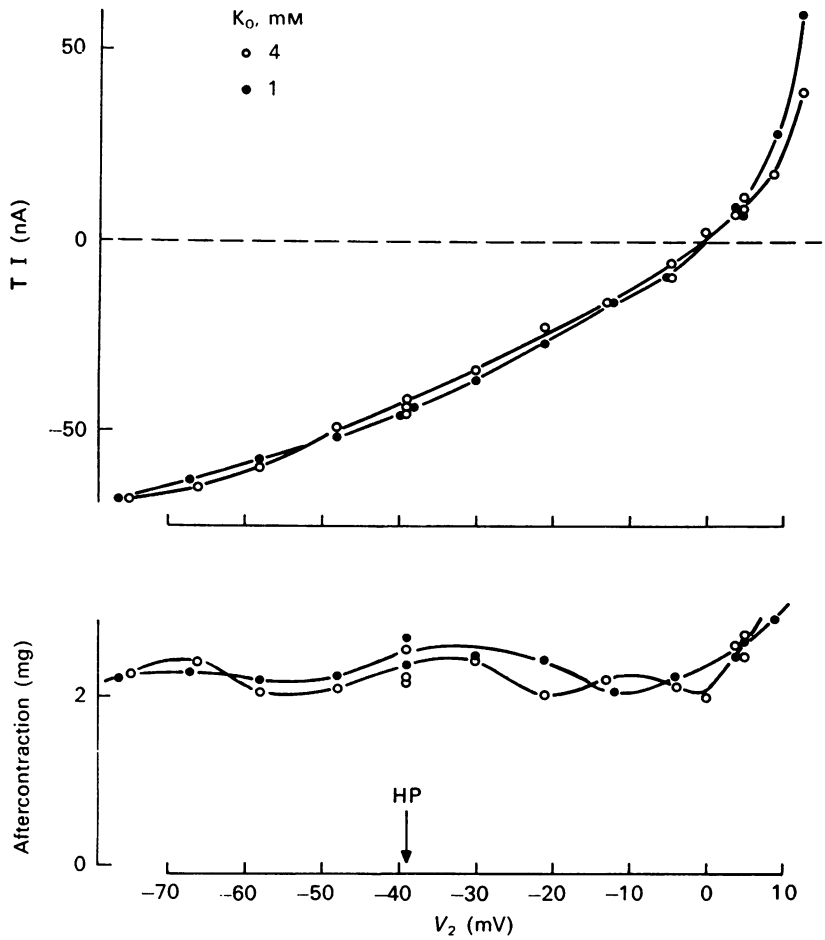

Fig. 8. Influence of external K concentration on TI and aftercontraction at various repolarization levels. Repolarizing steps followed 0·3 sec pulse to +29 mV. Preparation R37-4, 1 μM-strophanthidin.

E_{rev} (Takeuchi, 1963*a*), although this was not found in other experiments (see Discussion). Fig. 8 illustrates the effect of K_0 on TI. Data was obtained between 4 and 6 min after reducing the bulk K concentration from 4 to 1 mM. In this example, there was no significant alteration in the amplitude of TI or aftercontraction at any of the potentials studied. The reversal potential also remained essentially unchanged. This point may be reinforced by giving results from two earlier runs from the same experiment which are not illustrated in the Figure. The over-all sequence

of solutions was 4 K, 1 K, 4 K, 1 K. E_{rev} was obtained as the voltage intercept in these runs and was respectively, +1, +2, −0·5, +0·5 mV. The variation in these values lies within the experimental error of the E_{rev} determinations. Similar results were obtained in three other preparations. In all cases, changes in the bulk potassium concentration over the range between 1 and 8 mM failed to evoke a clear-cut displacement of E_{rev}. These experiments neither support nor exclude the involvement of potassium ions as charge carriers during TI (see Discussion).

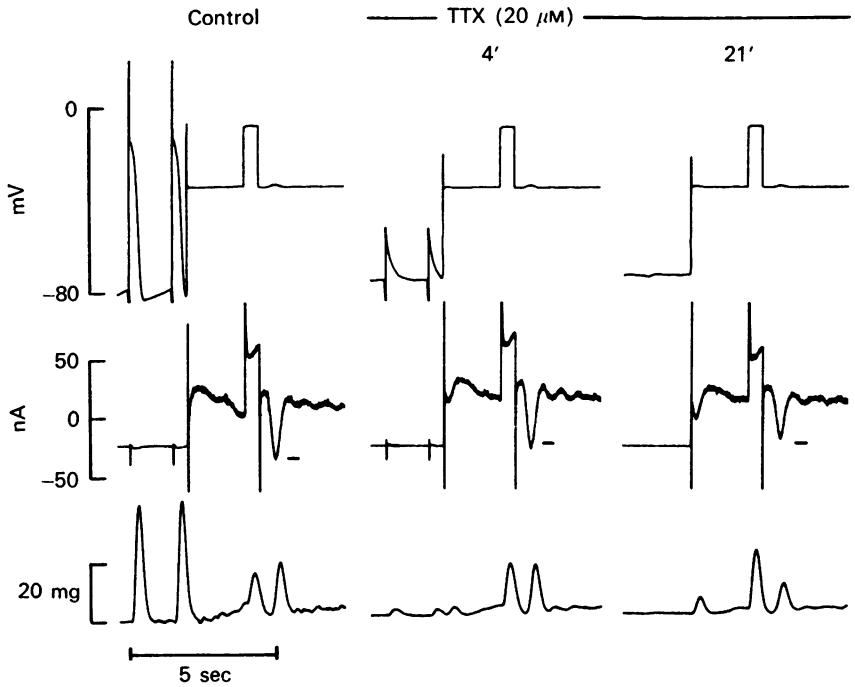

Fig. 9. Effect of tetrodotoxin on electrical activity, TI and aftercontraction. Records of membrane potential (upper row), membrane current (middle row) and contraction (bottom row) were obtained before (left column), 4 min after (middle column) and 21 min after (right column) exposure to 20 μM-TTX. Current traces show steady hyperpolarizing current and shock artifacts before application of voltage clamp. External stimulation was discontinued soon after disappearance of full-blown action potentials at 4 min exposure to TTX. Horizontal bars indicate the amplitude of the TI measured during a prior control run, before (left), 4 min after (middle), and 21 min after (right) discontinuation of external stimulation. Preparation R12-2, 1 μM-strophanthidin.

Effect of tetrodotoxin (TTX)

The ion replacement experiments raised questions about the nature of the pathway for the transient current. Can TI be explained by the modulation of the conductance of pre-existing channels? Since sodium appears to be the principal carrier of the inward current, one must consider the pathways for inward Na movement which are already known in Purkinje fibres. Rapid Na channels are one obvious possibility. The inactivation of these channels is normally complete at potentials positive to −55 mV (Weidmann, 1955), that is, over a range where inward TI may

be observed. The participation of Na channels cannot be ruled out, however, since it is conceivable that channel gating may be modified in the intoxicated state. Alterations in Na channel gating have already been found for nerve membranes under the influence of scorpion venom (see Cahalan, 1975).

TTX provides a simple test for the involvement of Na channels since it is known to block the rapid excitatory Na-current in Purkinje fibres (Dudel, Peper, Rüdel & Trautwein, 1967; Carmeliet & Vereecke, 1969). Fig. 9 illustrates the effect of TTX on TI in a preparation intoxicated by evoked activity in the presence of 1 μM-strophanthidin. The protocol was designed to compare changes in excitability with possible alterations in the amplitudes of TI or the aftercontraction. The upper panels are records of the membrane potential in the absence of TTX (*A*) and during a continuous exposure to 20 μM TTX (*B*, *C*). The control record shows action potentials in response to externally applied stimuli, followed by the application of the voltage clamp at a holding potential of -34 mV. A depolarizing pulse from this holding potential produced TI which was registered on the current trace (middle row, *A*). The lower panel shows the associated force record. Twitches accompany the action potentials and the 'make' of the depolarizing pulse, and an aftercontraction is associated with TI which follows the 'break' of the pulse.

This protocol was repeated during exposure to TTX. After 4 min, the drug almost completely abolished the response to the extracellular shocks, while TI and the associated aftercontraction remained unchanged. This result indicates that TI is not directly affected by blockade of a majority of the excitatory sodium channels.

Panel *C* Fig. 9 shows the influence of prolonged exposure to TTX. After 21 min there was a decline in the amplitude of both TI and aftercontraction. We suspected that the decline might be a result of the absence of action potentials during the period in TTX, and not a direct effect of the drug itself. This explanation was tested by a control run in the same experiment, where action potentials were eliminated, not by TTX, but by omitting the external stimuli. The TI amplitude at corresponding states during the control run is indicated by the horizontal markings in the middle panel. It is evident that the long-term effect of TTX is well matched by the long term effect of withdrawal of stimulation. Both of these interventions may act indirectly through reduction of sodium influx and the degree of intoxication (see Vassalle, Karis & Hoffman, 1962; Lin & Vassalle, 1976). This experiment re-emphasizes the importance of distinguishing between direct effects on the current pathway and indirect effects which are brought about by an altered amount of Ca release. The correlation between the slow decline in TI and the aftercontraction in Fig. 9 provides support for an indirect mechanism.

Effect of Cs

Another pharmacological approach was prompted by the level of the reversal potential and the possible participation of K ions. According to Isenberg (1976), the inwardly rectifying K currents in Purkinje fibres are completely abolished by exposure to 20 mM-CsCl. Fig. 10 illustrates the effect of Cs ions on TI. Apart from a gradual loss of toxicity, there is no dramatic change in the voltage dependence of either TI or aftercontraction. The lack of displacement of the reversal potential suggests that cesium-sensitive K currents do not contribute to the transient current.

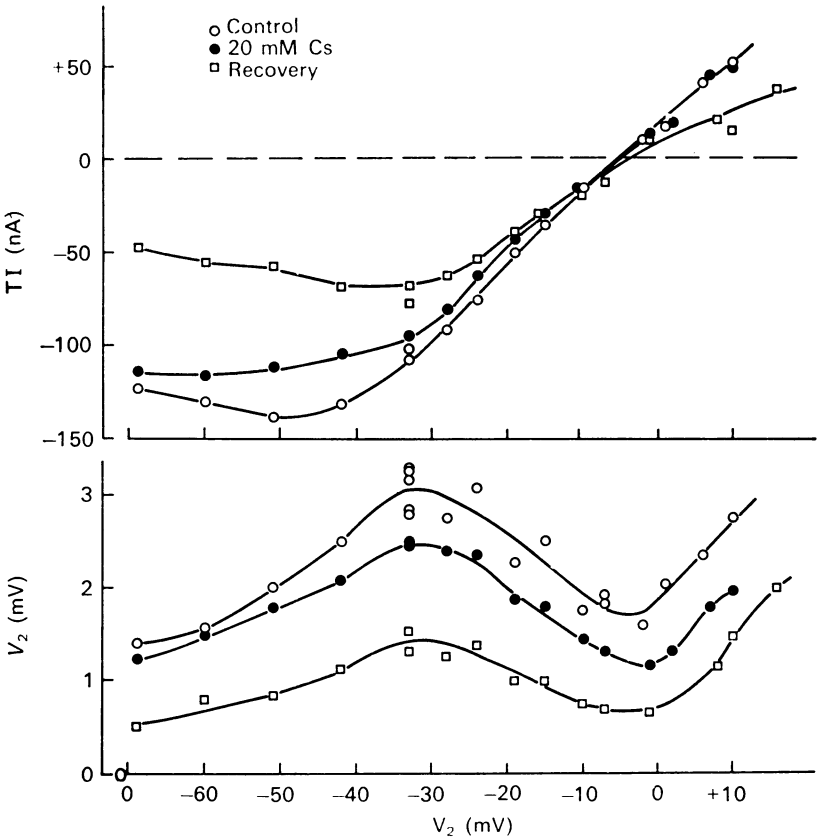

Fig. 10. Effect of cesium on TI and aftercontraction. Dependence of TI and after-contraction on repolarization potential in standard Tyrode (○), after exposure to 20 mM-CsCl for 5 min or more (●), and after restoration of Cs-free solution for 8 min or more (□). Repolarizing steps followed 0·2 sec prepulse to + 13 mV. Preparation R40-4, 1 μM-strophanthidin.

DISCUSSION

Which ions carry TI?

Changes in external ion concentration may affect the transient current in at least two ways: by directly altering the availability of charge carriers or by indirectly influencing events leading to the transient change in membrane permeability. Reversal potential measurements are important because they offer a method for deciding whether or not an ion carries current. However, indirect effects on intracellular Ca are of practical importance because the reversal potential cannot be measured if the transient Ca release is suppressed.

The main conclusion of this paper is that Na ions play a major role in carrying TI. Na cannot be the only permeant species, however, since E_{rev} lies far negative to reasonable estimates of E_{Na} in the presence of cardiotonic steroid. The difference suggests the involvement of an ion with a negative reversal potential – either Cl or K. Participation of Cl seems unlikely since its replacement by methylsulphate, a much larger anion, produces no change in E_{rev}. This leaves K as the most likely

possibility. Our experiments provide no direct evidence for K permeability. The main problem is that increases in external K were restricted by the well known antagonistic effect of K on digitalis toxicity. Over the range of concentrations tested (1–8 mM) no significant variation of E_{rev} was detected.

The lack of effect of K_o could be consistent with significant potassium permeability. If one assumes arbitrarily that E_{rev} can be described by the potential equation of Goldman (1943) and Hodgkin & Katz (1949), and takes published values for the internal and external activities of Na and K (Miura, Rosen & Hoffman, 1977; Ellis, 1977), the average value of E_{rev} ($-4{\cdot}8$ mV) corresponds to $P_{Na}/P_K = 0{\cdot}99$. Changing the external K concentration from 1 to 8 mM would be expected to displace E_{rev} by only $+1{\cdot}23$ mV, a value well within the range of experimental error in determining E_{rev}. According to the Goldman equation, it is the *intracellular* K concentration which is important in determining E_{rev}. Thus it might be worthwhile to look for changes in E_{rev} when internal K is varied by changes in bathing solution osmolarity (see, for example, Akiyama & Fozzard, 1975).

The reversal potential showed no detectable sensitivity to changes in external Ca concentration within the range of 2·7 and 16·2 mM. Thus Ca cannot be the predominant carrier of inward current at potentials near E_{rev}, contrary to earlier suggestions by Ferrier & Moe (1973) and Lederer & Tsien (1975). The present experiments do not rule out the idea that Ca ions carry some small fraction of the inward current. This seems plausible since E_{rev} was only displaced to about -35 mV, a level significantly positive to E_K, when sucrose replaced NaCl in the bathing solution (Fig. 4). It is difficult to use the results in 0-Na (sucrose) to estimate the Ca permeability without additional information about possible magnesium permeability (see Jan and Jan, 1976) or trapping of Na ions in narrow extracellular spaces (Johnson & Lieberman, 1971). It is also possible that substitution of sucrose for NaCl might facilitate Ca movements by minimizing any competition between Ca and Na ions for channel binding sites and by reducing ionic strength and increasing negative surface potential.

Similarities between TI and transmitter-induced currents

Reversal potential results provide a basis for comparing TI with acetylcholine (ACh)-activated currents in membranes of frog muscle (Takeuchi & Takeuchi, 1960), cultured rat myotubes (Ritchie & Fambrough, 1975) eel electroplaques (Lassignal & Martin, 1977), and other excitable cells (see Rang, 1975 for review). In many systems, the ACh-induced current reverses polarity at a membrane potential near 0 mV with normal external Na. Cl replacement by large, presumably impermeant anions leaves the reversal potential unchanged; sodium replacement by Tris or sucrose displaces E_{rev} in the negative direction but never as far as E_K. The deviations from E_K reflect the fact that the ACh-sensitive channel is somewhat permeable to Tris (Steinbach, 1975; Ritchie & Fambrough, 1975; Maeno, Edwards & Anraku, 1977) and Ca (Jenkinson & Nicholls, 1961; Takeuchi, 1963*b*; Evans, 1974; Lassignal & Martin, 1977; Lewis, 1977). In most studies E_{rev} showed little sensitivity to external potassium concentration in the presence of normal Na_o (Ritchie & Fambrough, 1975; Steinbach, 1975; Lassignal & Martin, 1977; but see Takeuchi, 1963*a*).

TI differs from ACh-induced currents in its mode of activation. According to the results in the previous paper (Kass *et al.* 1978), Ca acts as an internal 'transmitter' in controlling the transient electrical change. Work in other multicellular preparations provides some precedent for the idea that Ca_i may activate a depolarizing ionic current. In *Chironomus* salivary gland cells, injection of Ca ions causes a depolarization that has been attributed to increased permeability to sodium and possibly other ions (Loewenstein, 1975, p. 54). Injection of Ca into pancreatic acinar cells also causes membrane depolarization and increased membrane conductance (Iwatsuki & Petersen, 1977), with a reversal potential for the conductance change near -15 mV (Petersen & Iwatsuki, 1978). Internal Ca increases membrane conductance in many other cell types, but as a general rule the permeability change is rather selective for potassium ions (see, for example, Meech & Standen, 1975).

What ionic pathway carries TI?

Can TI be explained as a phasic change in the current carried by a normally functional ionic pathway? The results in this paper do not settle this question but they narrow the range of possibilities.

Slow inward current channels? Effects of Ca and Mn on TD prompted Ferrier & Moe (1973) to consider the idea that slow inward current (I_{si}) channels might carry a transient depolarizing current. But subsequent voltage clamp experiments have suggested that I_{si} channels do not carry TI but merely contribute to the state of 'Ca overload' which underlies TI (Kass *et al.* 1978). The slow inward current and TI differ in several respects: (1) the slow inward current is activated by depolarizing voltage changes, while TI is triggered by repolarization following a previous depolarization (Lederer & Tsien, 1976); (2) although both currents are inhibited by Mn ions, there are dramatic dissimilarities in the degree of inhibition and its rate of onset and removal (Kass *et al.* 1978); (3) the slow inward current reverses polarity at a strongly positive potential (Reuter, 1973; Trautwein, 1973) while TI inverts at a weakly negative potential; (4) The reversal potential of the slow inward current is quite sensitive to Ca concentration (Reuter & Scholz, 1977) over a range where no change in E_{rev} for TI could be detected (this paper). Taken together, these considerations argue strongly against the idea that TI is carried by normal slow inward current channels.

Excitatory Na channels or inward rectifier K channels? Tetrodotoxin and Cs ions are accepted as rather selective inhibitors of certain ionic channels in Purkinje fibres (Dudel *et al.* 1967; Isenberg, 1976). Experiments with TTX show no direct effect on TI and therefore suggest that excitatory Na channels probably do not participate in carrying inward current. The involvement of inwardly rectifying K current channel is also unlikely since E_{rev} was not changed by exposure to 20 mM-CsCl.

'Leak' channels? Tracer flux experiments have demonstrated the existence of TTX-insensitive Na movements and Cs-insensitive K movements. Resting Purkinje fibres show a large Na exchange at potentials where TTX-sensitive channels are presumably closed (Bosteels & Carmeliet, 1972). Almost half the normal K efflux in resting preparations remains in the presence of 20 mM-CsCl (Carmeliet, 1977). Very little is known about these cation fluxes; it is possible that they are mediated by rather non-selective 'leak' channels whose function is to provide the inward

'background' current that underlies the normal pace-maker depolarization in Purkinje fibres. TI could be accounted for if the 'leak' conductance were enhanced by internal Ca. This hypothesis might help explain why ventricular muscle has a greater tendency than Purkinje tissue to show aftercontractions without transient depolarizations (Ferrier, 1976). Ventricular muscle has relatively little inward 'leak' current, judging from the proximity of the resting potential to E_K (Lee & Fozzard, 1975). If all other things were equal, smaller TI would be expected in ventricular muscle for a given amount of Ca release.

Ca–Na exchange? As an alternative hypothesis, we have considered the possibility that the transient inward current might be carried by an electrogenic Ca–Na exchange, which couples the entry of more than two Na ions with the exit of a single Ca ion. An electrogenic stoichiometry of this nature has not been firmly established, but would be expected if the resting Ca gradient was derived from the Na gradient without direct expenditure of metabolic energy (see Baker, 1972; Reuter, 1974; or Blaustein, 1974 for reviews). Horackova & Vassort (1976) have used tonic force measurements to argue that four or more Na ions might be exchanged for each Ca ion in frog atrial muscle. The evidence for an electrogenic mechanism is somewhat stronger in squid giant axon, where the Na dependent Ca efflux varies with membrane potential in a manner consistent with electrogenic exchange (Blaustein, Russell & DeWeer, 1974; Mullins & Brinley, 1975; Baker & McNaughton, 1976).

Participation of the Ca–Na exchange would readily account for the apparent relationship between internal Ca and inward current. A transient rise in Ca_i would promote a phasic Ca efflux, Na entry, and a resulting net entry of the positive charge. TI would be a consequence of Ca homeostasis. The main drawback of the hypothesis is that it does not readily explain transient currents in the outward direction. If the stoichiometry of the Ca–Na exchange is independent of membrane potential, phasic increases of internal Ca can only promote transient currents in the inward direction. Observations of outward transients require some additional mechanism, such as a Ca-activated K channel of the type proposed by Isenberg (1975) and Bassingthwaighte, Fry & McGuigan (1976). Reversal of TI can be accounted for, but only by invoking another mechanism in parallel with the Ca–Na exchange.

Conclusion. We have offered two alternative explanations of TI which involve normally functional ionic pathways. Both hypotheses depend upon unproven assumptions – for example, the degree of selectivity of 'leak' channels, or the electrogenic nature of the Ca–Na exchange. Future tests of these hypotheses would be greatly aided by the discovery of pharmacological agents which specifically inhibit the various pathways for Na entry.

We are grateful to Dr Carol A. Lewis and Mr S. A. Siegelbaum for helpful discussions. This work was supported by grants HL 13306 and AM 17433 from the U.S. Public Health Service. R. Weingart was supported by the Swiss National Science Foundation. R. W. Tsien is an Established Investigator of the American Heart Association.

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