Origin of the Background Sodium Current and Effects of Sodium Removal in Cultured Embryonic Cardiac Cells

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SUMMARY. Cardiac automaticity is partly due to a diastolic sodium current. Possible mediators of this include tetrodotoxin-sensitive "fast" channels, cesium-sensitive time-dependent pacemaker current channels, calcium-gated nonspecific channels, and electrogenic sodium-calcium exchange. We have studied the effects of abrupt sodium removal on membrane current and conductance in voltage-clamped chick embryonic myocardial cell aggregates, in the presence of various sodium flux inhibitors. Total replacement of sodium by lithium, Tris, or tetraethylammonium ions in aggregates clamped in the pacemaker range caused a brief outward current followed by a sustained net inward current. The outward current reached a peak value of 1.1 ± 0.5 μA/cm² at a mean latency of 5.4 ± 1.2 sec. (n = 6; V = −70.5 ± 8.9 mV; Tris). Conductance often decreased during the outward current. The inward current developed exponentially (τ = 19 ± 5 sec) and reached a steady state value of −1.6 ± 0.4 μA/cm². This current was reversed by depolarization (mean reversal potential = −13 ± 13 mV), and was accompanied by increased conductance and spontaneous mechanical activity. Neither of the sodium-removal currents was affected by 20 μM tetrodotoxin. Cesium (up to 20 mM) had no effect on the late inward current or the mechanical activity, but decreased the early outward current by 80 ± 12%. Manganese (25 mM), which blocks sodium-calcium exchange, abolished the late inward current and the mechanical activity. Manganese also reduced the early outward current by 27 ± 10%. Manganese and cesium together blocked all the effects of sodium removal. We conclude that removal of extracellular sodium interrupts a cesium-sensitive "background" current, that may be related to the time-dependent pacemaker current, If. Sodium removal also causes gradual activation of a nonspecific conductance, which can ultimately depolarize the cells, and which may be gated by cytoplasmic calcium. (Circ Res 55: 67-77, 1984)

SPONTANEOUS beating in cardiac cells requires the presence of a "background" inward sodium current, which causes the membrane to depolarize when the plateau potassium current decays. Interruption of background sodium current is at least partly responsible for the hyperpolarization (Noma and Irisawa, 1975; Seyama, 1977; Boyden et al., 1983) or reduction in spontaneous beat frequency (Clusin, 1981) that occurs when various cardiac tissues are exposed to sodium-free fluid.

Several molecular mechanisms may contribute to the background sodium current. Attwell et al. (1979) described a "window" current in sheep Purkinje fibers that is sensitive to tetrodotoxin (TTX), and is ascribable to cross-over of the activation and inactivation curves for the "fast" sodium channels. However, this current is present only at the positive end of the potential range where cardiac pacemaking occurs. A second mechanism that could carry background sodium current is the time-dependent I_f or I_{K2} current, which develops in various cardiac pacemaker cells when they are hyperpolarized between −60 and −90 mV (Noble and Tsien, 1968; DiFrancesco and Ojeda, 1980; Clay and Shrier, 1981). This current is now thought to be carried by channels that are permeable to both sodium and potassium (DiFrancesco, 1981a, 1981b), and blocked by cesium ions (Isenberg, 1976; DiFrancesco, 1982; Clusin, 1983a).

The study of background currents in the heart is complicated by the fact that replacement of extracellular ions may cause rapid changes in the ionic content of the cytoplasm—changes that may, in turn, affect membrane permeability. Removal of extracellular sodium causes net influx of calcium ions via sodium-calcium exchange (Jundt et al., 1975; Langer et al., 1976; Marban et al., 1980; Lee et al., 1980; Sheu and Fozzard, 1982; Barry and Smith, 1982). If the exchange is electrogenic, there will be greater hyperpolarization than would be expected from interruption of background sodium current alone (Mullins, 1979; Fischmeister and Vassort, 1981; Coraboeuf et al., 1981). Furthermore, the increase in intracellular calcium could lead to activation of calcium-gated channels. The known calcium-gated channels in the heart are nonspecific
channels, which produce depolarization during most of the cardiac cycle (Kass et al., 1978a, 1978b; Colquhoun et al., 1980). They admit sodium freely, but may also produce depolarization when sodium is absent (Kass et al., 1978b).

One method of distinguishing between primary and secondary effects of extracellular ion substitutions is to use a ‘fast-flow’ system (Gadsby and Cranefield, 1977; Boyden et al., 1983). With such a system, one maximizes the temporal separation between effects that directly reflect extracellular conditions, and those resulting from cytoplasmic alteration. An extension of this strategy is to use tissue-cultured cells, where diffusion delays are minimized (Clusin, 1983b; Clusin et al., 1983).

In the present study, we have examined the effects of abrupt removal of extracellular sodium on membrane current in chick embryonic myocardial cell aggregates when they are voltage clamped in the normal pacemaker potential range. Sodium removal is found to elicit a rapidly developing outward current, which is followed by a sustained inward current. The outward current is due primarily to interruption of background sodium current that flows through a cesium-sensitive pathway. The late inward current is due to a nonspecific conductance increase whose properties suggest that it is gated by intracellular calcium. A preliminary communication has appeared (Mead and Clusin, 1983).

Methods

Ventricular fragments were obtained by sterile dissection of 9- to 12-day-old chick embryos, and dissociated into single cells by cyclic trypsinization, as previously described (Clusin 1980, 1981). Cells were cultured in Sykes Moore chambers (Belco Glass Inc.). The bottom of each chamber was coated with a thin layer of Sylgard elastomer (Dow Corning) to prevent adhesion of the tissue prior to equilibration of the extracellular fluid. Spontaneously beating aggregates 90–150 μm in diameter formed within 1–3 days. Aggregates were maintained in culture for 3–10 days until used for experiments, when they were transferred to a Sylgard-free glass surface, and immersed in physiological saline on the stage of an inverted microscope.

The culture medium contained 20% nutrient medium M-199 (GIBCO), 6% heat-inactivated fetal calf serum (GIBCO), 1% penicillin-gentamicin solution, and 73% potassium-free salt solution, containing 116 mM NaCl, 26.2 mM NaHCO3, 0.8 mM MgSO4, 0.9 mM NaH2PO4, 1.8 mM CaCl2, and 5.5 mM dextrose, adjusted to pH 7.4. All recordings were obtained in physiological saline consisting of 137 mM NaCl, 0.4 mM NaH2PO4, 2.7 mM KCl, 1.0 mM MgCl2, 1.8 mM CaCl2, 5.5 mM dextrose, and 5 mM HEPES, at pH 7.4 and 37°C. Sodium-free solutions were prepared by substitution of 146 mM Tris base [Tris-(hydroxymethyl)-aminomethane], lithium chloride, or tetraethylammonium chloride (TEA) for NaCl and NaH2PO4. The Tris solution was truly sodium-free, as pH could be adjusted with concentrated HCl. The pH of the lithium and TEA solutions was adjusted with KOH (which replaced KCl) plus small amounts of NaOH. The final sodium concentration in these solutions was about 5 mM.

Intracellular recordings were obtained with microelec-

trodes made from fiber-filled glass capillaries that had tip resistances of 20–50 MΩ when filled with 3 M KCl. Recordings were obtained in either current or voltage clamp mode with a Dagan 8100 single electrode voltage clamp at a sampling frequency of 500 Hz. Adequacy of membrane potential control has been demonstrated previously in similar aggregates with an independent intracellular electrode (Clusin, 1983b).

Aggregates were superfused with a Buchler peristaltic pump whose effluent catheters had an inner diameter of 280 μm, and were positioned about 100 μm from the aggregates. Two such catheters were sometimes used to permit serial superfusions. When the pump was activated at a flow rate of 1.6 cm/sec (60 μl/min), the aggregate was engulfed by the superfusate stream in less than 1 second. The time required to completely replace the extracellular fluid was therefore limited by diffusion of the superfusate into the interior of the preparation (Clusin et al., 1983). This diffusion delay was estimated by abruptly superfusing aggregates with a solution containing 3 mM TTX plus 2 mM manganese, during regular stimulation with hyperpolarizing current pulses. Reduction of action potential upstroke velocity was usually apparent within 1 second, and there was loss of regenerative anode-break responses after 8–15 seconds (Fig. 1). Replacement of extracellular fluid by the superfusate was therefore completed within this time.

A possible source of error in these experiments would be the occurrence of liquid junction potentials within the tissue prior to equilibration of the extracellular fluid (Spitzer and Walker, 1979). The magnitude of these junction potentials was estimated by superfusion of an agar bridge containing physiological saline with the various sodium-free solutions. Resulting changes in junction potential were 0.7 mV for the Tris-containing solution and <0.1 mV for the lithium solution. Based on the current-voltage relation obtained from a typical cell aggregate (Fig. 5), the spurious current produced by the larger of these junction potentials would be about 40 pA, which is insignificant compared with the current shifts actually recorded.

Data were recorded on a Gould Brush 2400 strip chart recorder. Membrane currents were normalized to mem-

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**FIGURE 1.** Rapid suppression of electrical excitability by tetrodotoxin (TTX) plus manganese. A myocardial cell aggregate is stimulated by hyperpolarizing current pulses (bottom trace) and then abruptly superfused with 3 mM TTX plus 2 mM manganese (arrow). Action potential amplitude is reduced within 1–2 seconds, and anode break excitation is abolished within 12 seconds. This gives an estimate of the time required for complete replacement of extracellular space by the superfusate.
brane area, which was estimated as $k \cdot a \cdot b^2$, where $a$ and $b$ are the major and minor hemiaxes of the aggregate (in $\mu m$), and $k$ is $1.85 \times 10^{-8} \text{ cm}^2/\mu \text{m}^3$, a constant derived from the histological data of Nathan and DeHaan (1979). Group data are given as mean ± SD.

**Results**

**Effect of Sodium Removal on Membrane Current**

Abrupt removal of extracellular sodium from cell aggregates clamped in the pacemaker range produced a transient outward shift in membrane current (Fig. 2). The outward current reached a mean peak value of $1.1 \pm 0.5 \mu A/cm^2$ in six aggregates that were clamped at $-70.5 \pm 8.9 \text{ mV}$ and superfused with Tris. The outward current began without perceptible delay, and was maximal after $5.4 \pm 1.2$ seconds of superfusion. Comparable membrane currents were obtained when either lithium (Fig. 7) or TEA was used as a sodium substitute.

The initial outward current was followed by a sustained net inward current (Figs. 2 and 4). The steady state inward current (relative to the initial holding current) was $-1.6 \pm 0.4 \mu A/cm^2$ in aggregates superfused with Tris. The inward current developed exponentially, as shown by the logarithmic plot in Figure 3A, and its onset was considerably slower than the equilibration of extracellular fluid (c.f. Fig. 1). The mean time constant with which the inward current developed was $19 \pm 5$ seconds in Tris. The inward current also decayed slowly when extracellular sodium was abruptly restored. In the experiment of Figure 2B, reintroduction of physiological saline caused additional inward current, which peaked after a few seconds. Current then returned to the original baseline exponentially (Fig. 3B). The mean time constant with which the inward current decayed was $26 \pm 11$ seconds. These experiments suggest that the inward current was an indirect consequence of sodium removal, whereas the outward current was a direct consequence.

Development of the late inward current was accompanied by spontaneous, disorganized mechanical activity, which began after the outward current peak, and persisted as the inward current reached steady state. The mean duration of sodium removal needed for spontaneous activity to become perceptible was $8.9 \pm 3.8$ seconds ($n = 7$, Tris). The mechanical activity was presumably due to cyclic release and reuptake of sequestered intracellular calcium, because it resembled the spontaneous activity that occurs in other situations producing calcium overload (Akselrod et al., 1979; Clusin et al., 1982; Kass and Tsien, 1982; Wier et al., 1983).

**Voltage-Dependence of the Sodium Removal Currents**

To determine the origin of the currents arising with sodium removal, we studied the accompanying variation in apparent membrane conductance. Brief (300-msec) hyperpolarizing and depolarizing voltage steps of up to $25 \text{ mV}$ were introduced at 1500-msec intervals, prior to and during sodium removal (Fig. 4). The initial effects of sodium removal on conductance were variable, but a slight conductance decrease was often evident at the peak of the early outward current (e.g., Fig. 4). In contrast, development of the late inward current was always accom-
FIGURE 3. Exponential onset and decay of the late inward current induced by sodium removal. Panel A is a logarithmic plot of membrane current during exposure of a cell aggregate to Tris solution. Current is plotted as the difference from the steady state value for each of three recurring potential steps, beginning 24 seconds after sodium removal (same experiment as Fig. 4). Each plot is fitted by an exponential with a time constant of 26 seconds. Panel B demonstrates exponential decay of the inward current when sodium is abruptly restored (same experiment as Fig. 2B). The near identity of the time constants in panels A and B is fortuitous (see text for range of values observed).

Companied by a conductance increase of up to 100%. The time course of the conductance increase was similar to that of the inward current, so that currents recorded at different potentials approached steady-state simultaneously (Fig. 4). In Figure 3A, the late inward current at each of the three potentials in Figure 4 has been plotted logarithmically as a function of time. Although the amplitude of the current varied with potential, current at each potential was described by the same exponential. This would be expected if the current were caused by a single, exponentially developing conductance increase.

There was often a further increase in conductance when extracellular sodium was abruptly restored (not illustrated). This was followed by a gradual decrease in conductance back to the initial value. Disappearance of the inward current after sodium restitution (c.f. Figs. 2B and 3B) occurred with the same time course as the conductance decrease.

The reversal potential of the late inward current was determined in two ways. In Figure 5, the reversal potential was obtained by linear extrapolation of two current-voltage relations from the experiment in Figure 4. Both of these current-voltage relations were obtained after a substantial period of sodium removal, when extracellular ionic concentrations (and hence E_{Na}) would be expected to have stabilized. The plot shown by filled circles was obtained 80 seconds after sodium removal, when the inward current had approached steady state. The linearity of this plot indicates that the normal inward rectifier had been shunted by parallel addition of a large linear conductance. The plot shown by X's was obtained prior to the steady state (i.e., 30 seconds after sodium removal), but at a time when membrane conductance had also increased enough to linearize the current-voltage relation. Extrapolation of the two plots gives an estimate of the equilibrium potential of the additional conductance increase that occurred between 30 and 80 seconds. This value was $-18 \text{ mV}$. Similar plots were constructed for seven other aggregates superfused with Tris, and gave a mean extrapolated equilibrium potential of $-13 \pm 13 \text{ mV}$.

In three experiments, it was possible to demonstrate reversal of the late current directly, by performing a series of sodium-free superfusions at different potentials in the same aggregate. Our most complete experiment is shown in Figure 6, where the aggregate was briefly superfused with Tris six times, allowing about 5 minutes for recovery between trials. The initial current shift was outward at all potentials from $-2$ to $-52 \text{ mV}$. However, the late current, which was inward at more negative potentials, had clearly reversed at $-2$ and $-8 \text{ mV}$. Reversal of the late current between $-8$ and $-13 \text{ mV}$ would be consistent with the result in Figure 5, and suggests that this current is carried by a channel that is not especially selective for any of the ion species present.

**Insensitivity of the Early Outward Current to TTX**

The transient conductance decrease in Figure 4 suggests that the initial effect of sodium removal was interruption of a background inward current, rather than activation of new outward current. To characterize this background current further, sodium removal was performed in the presence of tetrodotoxin. The effects of tetrodotoxin were studied in 21 aggregates, at concentrations as high as 20 $\mu\text{M}$. TTX suppressed spontaneous beating, and markedly diminished action potential upstroke velocity, but did not alter the early outward current or late
FIGURE 4. Effects of sodium removal on conductance. Membrane current at three different potentials is recorded every 1500 msec, before and after superfusion with sodium-free Tris solution (arrow). There is initially a slight conductance decrease that is coincident with the early outward current. This is followed by a 2-fold conductance increase, which accompanies the late inward current. The predominant holding potential is −74 mV, with alternating 300-msec steps to −95 and −55 mV. Since TTX is absent, there is a brief, inward current during depolarizing steps prior to sodium removal. Aggregate hemi-axes are 50 μm × 50 μm.

FIGURE 5. Current voltage relations from the experiment in Figure 4. Current is plotted for each of the three potential steps at two points in time. The first plot (X’s) is after 30 seconds of sodium-free superfusion, at which time extracellular ionic concentrations would have stabilized. The second plot (filled circles) is after 80 seconds, when current at all three potentials has approached steady state. The change in current indicated by the two plots is consistent with development of an additional linear conductance, whose equilibrium potential is −18 mV.

FIGURE 6. Membrane currents elicited by sodium removal at six different potentials. The cell aggregate is clamped at the potential indicated in the right column during, and for several seconds before, superfusion with sodium-free Tris solution. Sodium removal always evokes an initial outward current. The subsequent change in current is inward between −13 and −52 mV (bottom four records) and outward at −2 and −8 mV (top two records). Absolute current at each potential is indicated by the vertical scales. The top record was obtained at reduced gain, and is redrawn in expanded form. Aggregate hemi-axes are 50 μm × 50 μm.

**Suppression of the Early Outward Current by Cesium**

A second possible explanation for the early outward current is interruption of sodium influx through "I_r" channels (DiFrancesco, 1981b). This possibility was tested by application of cesium ions,
which block the $I_f$ current in a variety of tissues, including embryonic myocardial cell aggregates (Clusin, 1983a). Figures 7–9 show membrane currents induced by sodium removal before and after application of 20 mM cesium. Cesium markedly depressed the outward current, irrespective of whether lithium ($n = 5$) or Tris ($n = 7$) was used as a sodium substitute. The effects of cesium were fully reversible (Figs. 7 and 8), and were also observed at lower cesium concentrations (down to 1.5 mM). Although the outward current was often completely abolished by cesium (e.g., Fig. 7), a small remnant of this current usually persisted (Figs. 8 and 9). The mean amplitude of the persisting outward current was $20 \pm 12\%$ of the pre-cesium current in seven aggregates studied in Tris. Cesium had no effect on the magnitude or time course of the late inward current (Figs. 8 and 9), nor did it prevent the associated conductance increase (Fig. 9). Cesium also failed to alter the character or time course of spontaneous mechanical activity after sodium removal.

**Suppression of the Late Inward Current by Manganese**

As noted in the introduction, some of the effects of sodium removal on membrane current might be ascribable to calcium influx through the sodium-calcium exchange. To test this possibility, sodium removal was performed in the presence of high concentrations of manganese, which blocks sodium-calcium exchange in nerve (Baker, 1972; Blaustein, 1977) and abolishes the sodium-free contracture in potassium-deprived canine Purkinje fibers (Coraboeuf et al., 1981).

The effects of 25 mM manganese on the sodium removal current are shown in Figure 10. The principal effect of manganese was to abolish completely the late inward current, and the associated conductance increase. The spontaneous asynchronous mechanical activity was also abolished by manganese. These observations suggest that the depolarizing conductance increases in low sodium is dependent upon calcium influx.

Manganese did not abolish the early outward current (Fig. 10), but in most experiments, a moderate reduction of this current was discernible. In eight aggregates superfused with Tris, manganese reduced the early outward current by $27 \pm 10\%$. The ability of manganese to suppress a component of the early outward current was more apparent when cesium and manganese were applied together. In Figure 11B, pretreatment with 20 mM cesium plus 30 mM manganese produced a marked reduction in membrane conductance. Subsequent removal of sodium had practically no effect on membrane current at any of the three potentials. Similar results were obtained in three other aggregates. The fact that the early outward current was largely resistant to manganese (despite abolition of mechanical activity) suggests that this current is not carried primarily by electrogenic sodium-calcium exchange. However, the component of outward current that is resistant to cesium may, in fact, involve this mechanism.

**Discussion**

Existence of a background sodium current implies that a cell should hyperpolarize if extracellular so-
The sodium-removal currents that we measure are true transmembrane currents. They cannot be ascribed to junction potentials within the tissue, nor do they result from electrical uncoupling. Kameyama (1983) studied uncoupling in pairs of guinea pig ventricular cells exposed to low sodium and found that junctional resistance was not increased during the first 20 seconds. Although some uncoupling probably does occur in our experiments, this would not invalidate our conclusions for several reasons. The major effects of sodium removal—hyperpolarization followed by depolarization with
FIGURE 11. Failure of sodium removal to affect membrane current significantly after treatment with manganese plus cesium. The experimental protocol is similar to that in Figure 9. Panel B is in the presence, and panel A, in the absence of 20 mM cesium plus 30 mM manganese. Cesium and manganese drastically reduce membrane conductance. Subsequent removal of sodium (arrow) causes almost no change in membrane current or conductance. Tris serves as the sodium substitute, and 20 μM TTX has been added to all solutions. Membrane potential is cycled between -61, -69, and -84 mV. Aggregate hemiapses are 50 μm × 50 μm.

loss of membrane resistance—are all observable in current clamp. The initial hyperpolarization is also confirmed by slowing or cessation of synchronized beating in aggregates that are not impaled with microelectrodes (Clusin, 1981). Finally, true reversal of the late inward current could not occur if this current were an artifact of uncoupling. Uncoupling would produce an apparent conductance decrease rather than an increase.

Origin of the Early Outward Current

There are several potential explanations for the rapidly developing outward current. One possibility is that sodium removal causes activation of potassium-selective channels that are gated by intracellular calcium (Isenberg, 1975; Clusin, 1980; Eisner and Vaughan-Jones, 1983). This hypothesis is not supported by the present results. As noted above, there is usually no conductance increase coincident with the early outward current, and often a small conductance decrease. The outward current does not diminish, disappear, or invert with hyperpolarization to the expected vicinity of E_k (Figs. 4 and 10). Moreover, the outward current precedes any spontaneous mechanical activity. Thus, there is no reason to postulate activation of outward current channels by intracellular calcium.

A second possible source of the outward current is electrogenic sodium-calcium exchange. Sodium-dependent calcium influx has been amply demonstrated during sodium removal in the heart, and, undoubtedly, leads to the spontaneous mechanical activity that we observe (Wier et al., 1983). This hypothesis is not supported by the present results. As noted above, there is usually no conductance increase coincident with the early outward current, and often a small conductance decrease. The outward current does not diminish, disappear, or invert with hyperpolarization to the expected vicinity of E_k (Figs. 4 and 10). Moreover, the outward current precedes any spontaneous mechanical activity. Thus, there is no reason to postulate activation of outward current channels by intracellular calcium.

A second possible source of the outward current is electrogenic sodium-calcium exchange. Sodium-dependent calcium influx has been amply demonstrated during sodium removal in the heart, and, undoubtedly, leads to the spontaneous mechanical activity that we observe (Wier et al., 1983). There is evidence that the exchange is electrogenic in various cardiac tissues including embryonic myocardial cell aggregates (Clusin et al., 1983). Suppression of outwardly directed exchange current would explain the reduction in outward current that we observe with manganese. Cesium might also diminish outward sodium-calcium exchange current, since cesium stimulates the sodium-potassium pump, and could therefore reduce intracellular sodium.

However, two observations indicate that sodium-calcium exchange is not the primary mediator of the outward current in our experiments. First, a substantial outward current persists in manganese, even though spontaneous mechanical activity is abolished. Second, spontaneous mechanical activity develops normally when the early outward current is suppressed by cesium. This observation suggests that significant sodium-calcium exchange can occur with relatively little outward current.

Having considered the alternatives, one concludes that the outward current is mainly due to the reduction in inward current through patent nonspecific channels. First, is the TTX-sensitive sodium channel (Attwell et al., 1979), which is clearly excluded by our data, because the outward current is insensitive to TTX, and because it can be observed at strongly negative potentials. Boyden et al. (1983) reached similar conclusions in canine coronary sinus.

A second channel that has been considered to carry background current is the calcium-activated nonspecific channel that was first described in connection with the digitalis-induced transient inward current (TI; Kass et al., 1978a, 1978b; Colquhoun et al., 1981). If sodium removal produced an immediate decline in inward current through patent nonspecific channels, but also caused a gradual increase in the number of open channels, then an outward-
inward current sequence would result. The problem with this explanation is that it fails to account for the suppression of the early outward current by cesium. Kass et al. (1978b) found that the digitalis-induced TI is practically unaffected by 20 mM cesium. The cesium-sensitive background sodium current must therefore flow through a different pathway.

The third type of channel that could carry background sodium current is the sodium permeant 'I_s' channel that has been postulated based on studies of the pacemaker current in sheep Purkinje fibers (DiFrancesco, 1981a, 1981b). This mechanism is consistent with our observations, particularly since the corresponding pacemaker current and its sensitivity to cesium have already been described in myocardial cell aggregates (Clay and Shrier, 1981; Clusin, 1983a). Our finding of a cesium-sensitive diastolic sodium current in pacemaker cells could be viewed as important confirmation of the 'I_s' hypothesis.

There is, however, one property of the sodium-removal current that is not consistent with the 'I_s' formulation, namely, the voltage-dependence of the cesium effect. As seen in Figure 9, cesium suppresses the initial sodium-removal current even during voltage steps to —50 mV, which is outside the potential range where 'I_s' occurs. In order for the cesium-sensitive sodium-removal current and the 'I_s' current to involve identical channels, some of the channels would have to remain open at potentials where no time-dependent current is seen. Analogous behavior has been described with other ion channels (e.g., Kass et al., 1976).

Origin of the Late Inward Current

The late inward current during sodium removal clearly results from a depolarizing conductance increase. The inward current has the same time course as the conductance change, and its reversal at positive potentials proves that the current is caused by the conductance. The reversal potential of the late current is such that it cannot be carried by any one ionic species. The current is also not due to membrane breakdown, because the reversal potential is distinctly negative to zero. Activation of a channel that is permeable both to potassium and other ions would explain the observed reversal potential, as well as the increased potassium loss that occurs when cardiac fibers are exposed to low sodium (Carmeliet and Vereecke, 1979).

A nonspecific conductance increase has also been observed when sodium is removed from voltage-clamped Purkinje fibers after inhibition of the sodium pump (Lederer et al., 1983). In these fibers, sodium removal elicits a membrane current that is closely correlated with the accompanying contraction, and reverses at moderate negative potentials. These observations differ from ours in only two respects. First, no initial outward current is described. Second, the conductance increase is not observed in Purkinje fibers unless the sodium pump is inhibited. These discrepancies could be due to greater diffusion barriers in Purkinje fibers. It is also possible that sodium removal alone produces less calcium 'overload' in Purkinje fibers because of better sequestration by the sarcoplasmic reticulum.

The most likely mediator of the conductance increase in low sodium is the calcium-activated nonspecific channel ('TI channel'). This interpretation is suggested by the fact that there is accompanying mechanical activity under voltage clamp. The mechanical activity and the conductance increase develop with the same time course, and are simultaneously abolished by 25 mM manganese, which prevents sodium-dependent calcium influx (Fig. 10).

The voltage-dependence of the sodium-removal current also resembles that of the digitalis TI, at least as it is described in sheep Purkinje fibers (Kass et al., 1978a, 1978b). Kass et al. found that the digitalis TI normally reverses at about zero mV, which suggests equal permeability of the 'TI channels' to sodium and potassium. Removal of extracellular sodium shifts the reversal potential to about —25 mV. This observation shows that the channels prefer sodium over other extracellular ions (e.g., calcium, magnesium, and the various sodium substitutes), but that one or more of these ions can traverse the channels to some extent. Otherwise, the TI would reverse at E_K in zero sodium. Our measurements of the reversal potential of the sodium-removal current are in good agreement with what Kass et al. obtained for the digitalis-induced TI. Although we measured the reversal potential only in sodium-free solution, we would expect reversal to occur at a more positive value (i.e., near zero mV), when sodium is abruptly restored. This prediction is consistent with the increased inward current seen after sodium restitution in Figure 2B.

One problem with the above interpretation is that some calcium-activated TIs fail to reverse with strong depolarization (Karagueuzian and Katzung, 1982). In embryonic myocardial cells, the TI induced by abrupt exposure to 10 mM caffeine remains inward at potentials near E_{calc} (Clusin et al., 1983). This response may therefore be mediated primarily by electrogenic sodium-calcium exchange, which can never generate outward current in response to intracellular calcium release.

The reversal of the sodium-removal current and the nonreversal of the caffeine TI are not, in fact, contradictory. When the caffeine TI is recorded in the diastolic potential range in normal sodium, it is about four times larger, relative to membrane area, than the inward sodium-removal current. Total calcium-activated current could therefore be dominated by sodium-calcium exchange in the presence of sodium, whereas, in sodium-free solution, nonspecific channel current would predominate. Coexistence of these currents has already been proposed,
based on the failure of the caffeine TI to increase at large positive potentials, as predicted for a pure sodium-calcium exchange current (Clusin et al., 1983).

**Possible Role of the Electrogenic Sodium Pump**

A traditional explanation for the depolarization of cardiac fibers in low sodium is cessation of the electrogenic sodium pump. Electrogenicity of the sodium-potassium-pump is well established in the heart (see Glitsch, 1982), so there is little doubt that a portion of the sodium-removal current must be due to this mechanism. However, as pointed out by Wiggins and Cranefield (1974), only a fraction of the sodium ions entering a cell are extruded electrogenically. Interruption of the pump by sodium removal could not, therefore, produce net inward current unless most of the resting sodium influx were by way of an electroneutral carrier. Prevailing evidence is now against such a carrier. A more serious limitation of the sodium pump explanation is that the pump current is insensitive to voltage. The voltage-dependence of the sodium-removal current in Figures 3–6 requires that the current be mediated by a channel rather than a pump. Our evidence suggests that this channel is activated by cytoplasmic calcium, and that its influence is especially conspicuous in sodium-free solutions because other currents are diminished.

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