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# THE ROLE OF INTRACELLULAR SODIUM ACTIVITY IN THE ANTI-ARRHYTHMIC ACTION OF LOCAL ANAESTHETICS IN SHEEP PURKINJE FIBRES

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SUMMARY

1. The effects of lidocaine have been examined on the arrhythmogenic transient inward current ($I_{TI}$) in voltage-clamped sheep cardiac Purkinje fibres. Tension and intracellular Na activity ($a^i_{Na}$) were measured simultaneously.

2. The addition of lidocaine (200–300 $\mu$M) produced an immediate decrease of inward holding current and a gradual fall of $a^i_{Na}$. The relative magnitudes of the changes of current and $a^i_{Na}$ were shown to be consistent with the outward shift of current representing principally a reduction of inward Na current.

3. The Na pump was inhibited by reducing the external Rb concentration in a K-free solution. This produced an after-contraction and transient inward current ($I_{TI}$) along with a rise of $a^i_{Na}$. The subsequent addition of lidocaine decreased the magnitude of $I_{TI}$ and the after-contraction while decreasing $a^i_{Na}$.

4. Tetrodotoxin (TTX) had qualitatively similar effects to lidocaine on inward holding current, $a^i_{Na}$, $I_{TI}$ and the after-contraction.

5. When $a^i_{Na}$ was changed by (i) lidocaine, (ii) TTX or (iii) small changes of external Rb concentration, a hysteresis was seen in the relationship between $a^i_{Na}$ and $I_{TI}$ or after-contraction. The hysteresis was similar to that previously found between $a^i_{Na}$ and contraction (Eisner, Lederer & Vaughan-Jones, 1981). Despite this hysteresis, neither lidocaine nor TTX affected the relationship between the magnitudes of $I_{TI}$ and the after-contraction.

6. It is suggested that the fall of $a^i_{Na}$ is a major factor in the reduction of $I_{TI}$ by lidocaine.

7. These results are discussed in relation to the anti-arrhythmic actions of lidocaine.

## INTRODUCTION

An arrhythmogenic oscillatory transient inward current ($I_{TI}$) is seen in mammalian cardiac muscle when the intracellular Ca concentration ($[Ca^{2+}]_i$) is elevated. This current is produced on repolarization and appears to be activated by an oscillatory increase of $[Ca^{2+}]_i$ (Kass, Lederer, Tsien & Weingart, 1978*a*) which may activate a mixed Na and K conductance (Kass, Tsien & Weingart, 1978*b*; Colquhoun, Neher,

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Reuter & Stevens, 1981). $I_{TI}$ can be produced experimentally by interventions such as the application of cardiotonic steroids (Lederer & Tsien, 1976) and the removal of external K (Eisner & Lederer, 1979*a*, *b*). These manoeuvres inhibit the Na pump and increase intracellular Na activity ($a^i_{Na}$) which then acts to elevate $[Ca^{2+}]_i$ (Bers & Ellis, 1982; Sheu & Fozzard, 1982) presumably via a Na/Ca exchange (Reuter & Seitz, 1968; Baker, Blaustein, Hodgkin & Steinhardt, 1969).

$I_{TI}$ can produce transient depolarizations which follow the action potential and have been implicated in the genesis of ectopic pace-maker activity in some ventricular arrhythmias, in particular those associated with digitalis intoxication (Rosen, Gelband & Hoffman, 1973; Ferrier, Saunders & Mendez, 1973; Ferrier & Moe, 1973). Local anaesthetic anti-arrhythmic agents such as lidocaine and procainamide are often used in the initial treatment of such arrhythmias although their mechanism of action is still unclear. Various possibilities have, however, been suggested. (i) Lidocaine decreases the Na current and can decrease the excitability of the cell (Weidmann, 1955). A given arrhythmogenic stimulus such as a transient depolarization will therefore be less likely to produce an action potential. (ii) Lidocaine may decrease the magnitude of the transient depolarization itself. In support of this latter hypothesis, Rosen & Danilo (1980) found that lidocaine decreased the transient depolarization. They concluded, however, that lidocaine had no effect on $I_{TI}$, the effect on the transient depolarization being produced by an action on the background conductance of the membrane. On the other hand, Eisner & Lederer (1979*c*) found that lidocaine could abolish $I_{TI}$.

In the present paper we examine the mechanism of this reduction of $I_{TI}$ by lidocaine. One possible explanation is suggested by the observation of Deitmer & Ellis (1980) that lidocaine decreases $a^i_{Na}$. Since an increase of $a^i_{Na}$ underlies the production of $I_{TI}$ by Na pump inhibition, we have investigated whether this reduction of $a^i_{Na}$ is responsible for the effects of lidocaine on $I_{TI}$. An alternative possibility suggested by Tsien, Weingart & Kass (1978) is that local anaesthetics abolish $I_{TI}$ by interfering directly with Ca metabolism (Almers & Best, 1976). The present paper shows that most of the effects of lidocaine on $I_{TI}$ can indeed be attributed to the fall of $a^i_{Na}$. Preliminary results have been presented to the Biophysical Society (Sheu, Lederer & Eisner, 1982).

## METHODS

The methods used in the present study are similar to those described previously (Eisner *et al.* 1981) with the exception that a liquid ion-exchanger Na-sensitive micro-electrode was used (see below). In summary, free-running Purkinje fibres were dissected from the hearts of freshly killed sheep obtained from a local abbatoir. The Purkinje fibres were shortened to $< 2$ mm in length and mounted in the experimental bath, one end being attached to a tension transducer. Membrane potential was controlled with a two-micro-electrode voltage clamp while intracellular Na activity ($a^i_{Na}$) was measured.

### *Liquid ion-exchanger Na-sensitive micro-electrodes*

The construction and calibration of Na-sensitive micro-electrodes using the resin ETH 227 (Steiner, Oehme, Ammann & Simon, 1979) have been described in detail elsewhere (Sheu & Fozzard, 1982). The typical response of the electrodes to a change of $[Na^+]$ from 3 to 30 mM (substituted by KCl such that $[Na^+]+[K^+] = 150$ mM) was 30–50 mV. Thus, over the range of $a^i_{Na}$ encountered in the present study, a 1 mM change of $a^i_{Na}$ gives a voltage change of 1–3 mV. Na-sensitive micro-electrodes are affected by $Ca^{2+}$ (Steiner *et al.* 1979). As estimated previously (Sheu & Fozzard, 1982), at an $a^i_{Na}$ of 7·5 mM, a rise of $[Ca^{2+}]$ from 0 to 1 $\mu$M gave an apparent extra Na activity of

1·2 mM. The Na-sensitive micro-electrodes were unaffected by tetrodotoxin (TTX) or lidocaine at the concentrations used.

*Solutions*

The standard modified Tyrode solution consisted of: 145 mM-NaCl, 4 mM-KCl, 2 mM-$CaCl_2$, 1 mM-$MgCl_2$, 10 mM-Tris HCl, 10 mM-glucose. Modifications to this standard solution are indicated in the text. The pH of all solutions was 7·4 ± 0·1. All experiments were performed between 35–37 °C and, in any given experiment, the temperature was maintained constant to within 0·5 °C. Rb is frequently used as a substitute for K in the present experiments. It should be noted that Rb and K are equivalent in their ability to activate the Na pump (Eisner & Lederer, 1980; Eisner *et al.* 1981; Glitsch, Kampmann & Pusch, 1981).

## RESULTS

### *$I_{TI}$ and $a^i_{Na}$ during Na pump inhibition*

Fig. 1 *A* shows the effects of inhibiting the Na pump, by removing external $K^+$ ions, on membrane current, tension and $a^i_{Na}$. There is a rapid decrease of outward current (cf. Eisner & Lederer, 1979*a*; Gadsby & Cranefield, 1979) which is followed by a slower increase of $a^i_{Na}$ and twitch tension. Original records of current and tension are shown on an expanded scale in Fig. 1 *B*. The first record *a*, was obtained at a low $a^i_{Na}$ (6·3 mM) and a control current record is seen. By record *b*, $a^i_{Na}$ had risen to 10·8 mM and $I_{TI}$ and an after-contraction are present. With a further increase of $a^i_{Na}$ to 15·8 mM (*c*) both the after-contraction and $I_{TI}$ increased. The time course of these phenomena is shown in Fig. 1 *C*. Both $I_{TI}$ and the after-contraction are steeply dependent on $a^i_{Na}$; comparatively small increases of $a^i_{Na}$ produce large increases of $I_{TI}$ and after-contraction (see also Fig. 6). The steep dependence of after-contraction on $a^i_{Na}$ has been observed before (cf. Fig. 5 of Eisner, Lederer & Vaughan-Jones, 1983). It should be noted that when $a^i_{Na}$ approaches its steady-state value there is little subsequent change in the magnitude of either $I_{TI}$ or the after-contraction. Qualitatively similar results were found in experiments in which the Na pump was inhibited by exposing the preparation to solutions containing strophanthidin (0·1–10 μM).

### *The effects of lidocaine on $a^i_{Na}$ and membrane current*

Before examining the effects of lidocaine on $I_{TI}$ it was necessary to characterize its effects on $a^i_{Na}$ in voltage-clamped Purkinje fibres. The Na pump had been inhibited in the experiment of Fig. 2 by exposure to a K-free superfusing solution. This produced an increase of $a^i_{Na}$. Lidocaine (300 μM) was then added producing a fall of $a^i_{Na}$ and an outward shift of holding current. These changes of current and $a^i_{Na}$ were reversed on removing lidocaine. This shift of current has been observed previously in rabbit and sheep cardiac Purkinje fibres (Colatsky, 1982; Carmeliet & Saikawa, 1982) and is attributed to the decrease of an inward steady-state Na current. The present experiment shows both the decrease of this Na current and its effects on $a^i_{Na}$. It should therefore be possible to compare the magnitudes of these effects on $a^i_{Na}$ and current.

The initial rate of fall of $a^i_{Na}$ produced by adding lidocaine $(da^i_{Na}/dt)_{initial}$ should be related to the change of Na current ($\Delta I$) by the relation:

$$\frac{V_c F}{\gamma}\left(\frac{da^i_{Na}}{dt}\right)_{initial} = \Delta I, \qquad (1)$$

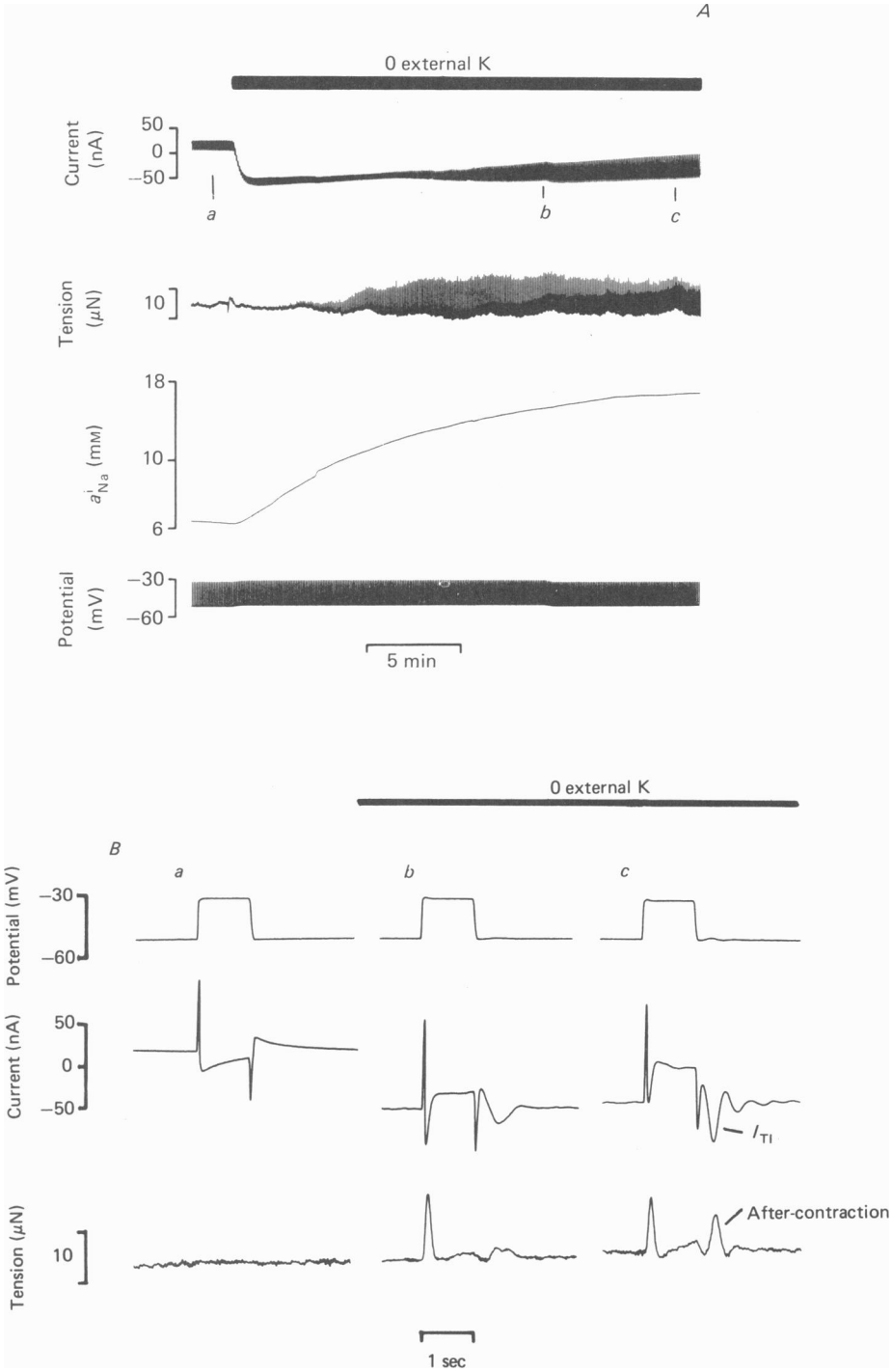


**Fig. 1. For legend see facing page.**

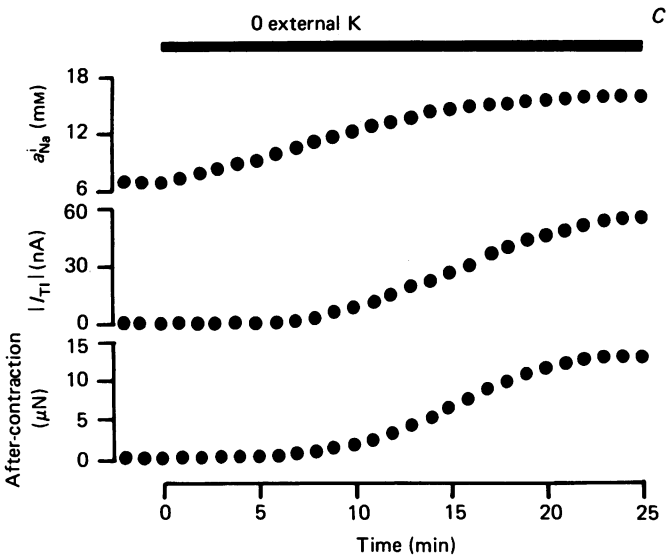


Fig. 1. The effects of Na pump inhibition on membrane currents, tension and $a^i_{Na}$. *A*, time course of development of the effects of Na pump inhibition. Traces show (from top to bottom): current, tension, $a^i_{Na}$ and membrane potential. The preparation was initially superfused with control solution containing 4 mM-external K and, at the time shown, external K was removed. The membrane potential was held at −51 mV and 1 sec duration pulses to −31 mV were applied at 0·2 Hz. *B*, specimen current and tension records. Traces show (from top to bottom): potential, current and tension. The records were obtained at the times shown on *A*. *c*, time course of the effects of exposure to K-free solution.

where $V_c$ is the intracellular volume of the preparation, $\gamma$ the activity coefficient for $Na^+$ ions and $F$ the Faraday constant. If the values of $V_c$ and $\gamma$ were known it would be possible to compare the changes of $a^i_{Na}$ and current. The problems of estimating these values have been discussed elsewhere (Eisner *et al.* 1981) and complicate this comparison. It is possible, however, to compare the relationship between the effects of lidocaine on current and $a^i_{Na}$ with that produced by the Na pump. In this experiment we also added 1–10 mM-Rb to reactivate the Na pump after exposure to a K-free, Rb-free solution. This resulted in an electrogenic Na pump current transient accompanying the fall of $a^i_{Na}$. The relationship between the charge transported by the Na pump during this transient ($Q$) and the fall of $a^i_{Na}$ ($\Delta a^i_{Na}$) is given by Eisner *et al.* (1981) as:

$$Q = \frac{r V_c F}{\gamma} \Delta a^i_{Na}, \tag{2}$$

where $r$ is the fraction of Na extruded by the Na pump as net charge transfer rather than exchanged for $K^+$ ions entering the cell.

$V_c/\gamma$ can be eliminated from 1 and 2 and the following result obtained:

$$r = \frac{Q\left(\frac{da^i_{Na}}{dt}\right)_{initial}}{\Delta I \, \Delta a^i_{Na}}. \tag{3}$$

It is therefore possible to calculate $r$ from the available measurements without making assumptions about $V_c$ or $\gamma$. A value of $0{\cdot}38 \pm 0{\cdot}07$ (S.E. of the mean, $n = 4$) is

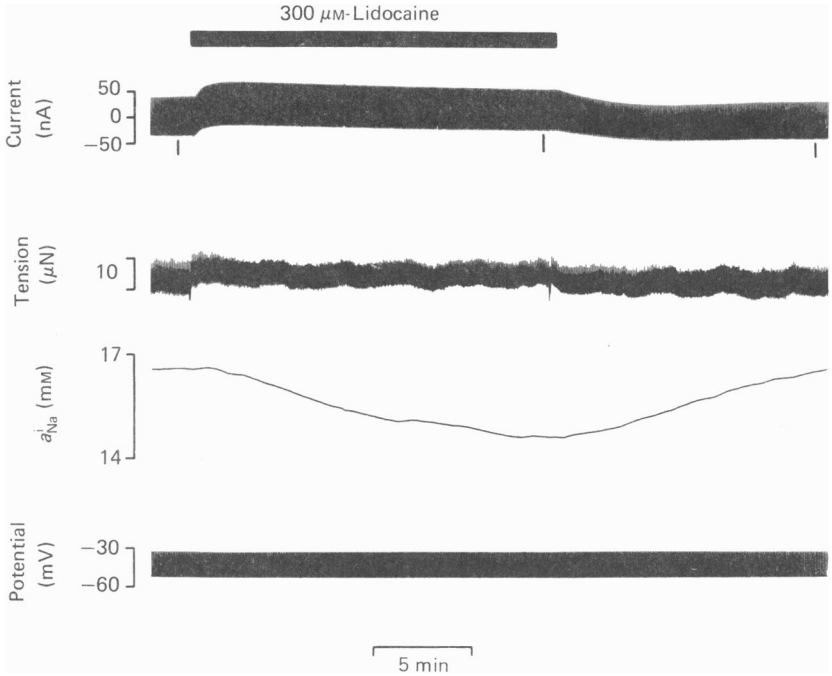


Fig. 2. Comparison of the effects of lidocaine on $a^i_{Na}$ and membrane current. Traces show (from top to bottom): current, tension, $a^i_{Na}$ and membrane potential. The preparation had been exposed to a K-free solution to elevate $a^i_{Na}$ for 21 min before the beginning of the record. Lidocaine (300 $\mu$M) was applied for the period shown. The membrane potential was held at −51 mV and 1 sec duration pulses to −31 mV were applied to 0·2 Hz.

obtained from the present experiments. This does not differ significantly from that of $0{\cdot}26 \pm 0{\cdot}06$ obtained with a completely different method (Eisner *et al.* 1981). The fact that the value for $r$ obtained from the present method agrees with previous measurements in a variety of tissues (Thomas, 1972) implies that most of the change of current produced by lidocaine is due to inhibition of Na entry into the cell. It suggests that, under the conditions of the present experiments even at high concentrations, lidocaine does not have significant effects on other steady-state currents.

*Effects of lidocaine on $I_{TI}$ and $a^i_{Na}$*

The experiment of Fig. 3 was designed to examine the effects of lidocaine on $I_{TI}$. In this experiment the Na pump was partly inhibited by exposing the preparation to a K-free solution containing 0·25 mM-Rb. This has the advantage over a completely Rb-free solution, that a steady state is reached at a lower $a^i_{Na}$. Fig. 3*A* shows that the subsequent addition of lidocaine (300 $\mu$M) produced a fall of $a^i_{Na}$. Specimen records of current and tension are shown in Fig. 3*B*. At *a*, there is a large $I_{TI}$ and after-contraction whereas the addition of lidocaine (*b*) abolishes both $I_{TI}$ and the after-contraction.

There are two possible explanations for the abolition of $I_{TI}$ by lidocaine: (i) lidocaine decreases $I_{TI}$ by decreasing $a^i_{Na}$; (ii) some other action is responsible. For

example, lidocaine could block the $I_{TI}$ channel directly or interfere with Ca metabolism. These possible explanations can be partly separated by examining the time course of the effects of lidocaine (Fig. 3*C*). This shows that the action of lidocaine to block the resting Na current, as shown by the outward shift of holding current, is very rapid in onset whereas the abolition of $I_{TI}$ is slower and comparable with that of the fall of $a^i_{Na}$. This result is at least consistent with the hypothesis that the abolition of $I_{TI}$ is mediated by the fall of $a^i_{Na}$ since, if it were due to some other action, this alternative mechanism would also have to be only slowly affected by lidocaine. Since lidocaine acts at the inside of the membrane to block the Na channel (Strichartz, 1973) the rapid abolition of the Na current suggests that lidocaine enters the cell rapidly. Therefore, the slow time course of the abolition of $I_{TI}$ cannot be attributed to the time taken for lidocaine to enter the cell.

The involvement of changes of $a^i_{Na}$ in the actions of lidocaine can be examined more rigorously by comparing the effects of lidocaine with those of two other interventions which change $a^i_{Na}$: (i) the application of TTX; (ii) the addition of low concentrations of Rb.

(*i*) *The effects of TTX.* Fig. 4 shows the effects of TTX, a specific blocker of the Na channel (Narahashi, 1974). TTX was applied while the preparation was exposed to a K-free, Rb-free solution and, like lidocaine, produces an outward shift of current and a fall of $a^i_{Na}$ (Fig. 4*A*). Fig. 4*B* shows that TTX decreases the magnitude of $I_{TI}$ and the after-contraction. These effects of TTX were reversible. TTX can therefore mimic all the effects of lidocaine on current and tension. This result is therefore consistent with the idea that lidocaine removes $I_{TI}$ by decreasing $a^i_{Na}$. TTX had less effect than lidocaine on both $a^i_{Na}$ and $I_{TI}$. Deitmer & Ellis (1980) similarly found TTX to have less effect than lidocaine on $a^i_{Na}$.

(*ii*) *The effects of low concentrations of $Rb^+$ ions.* In the experiment of Fig. 5, 0·5 mM-Rb was added to a K-free, Rb-free solution to reactivate the Na pump partially and to produce a slow fall of $a^i_{Na}$ (Fig. 5*A*). The fall of $a^i_{Na}$ is associated with an electrogenic Na-pump current transient. $Rb^+$ was then removed and $a^i_{Na}$ increased. Fig. 5*B* shows specimen current and tension records. The reduction of $a^i_{Na}$ produced by external Rb abolishes both the after-contraction and $I_{TI}$ reversibly.

*The relationship betwen $I_{TI}$, after-contractions and $a^i_{Na}$*

Fig. 6*A* shows the relationship between $I_{TI}$ and $a^i_{Na}$ during the interventions discussed above. The circles show the relationship both during the onset of Na-pump inhibition produced by removing external Rb (open circles) and on reactivating the Na pump by adding back external Rb (filled circles). Although increasing $a^i_{Na}$ is associated with increased $I_{TI}$, there is not a unique relationship between $I_{TI}$ and $a^i_{Na}$. A hysteresis is present with a given level of $a^i_{Na}$ being associated with a larger $I_{TI}$ when $a^i_{Na}$ is increasing compared to the relationship when $a^i_{Na}$ is decreasing. The effects of lidocaine are also shown from the same experiment in this Figure. The relationship between $I_{TI}$ and $a^i_{Na}$ produced by adding lidocaine shows a similar hysteresis to that produced by removing and adding external Rb. Fig. 6*B* demonstrates that hystereses are also seen between after-contraction and $a^i_{Na}$ both while changing Rb and during the application and removal of lidocaine. An identical hysteresis is seen between either $I_{TI}$ or after-contraction and $a^i_{Na}$ during the onset and removal of TTX (not shown).

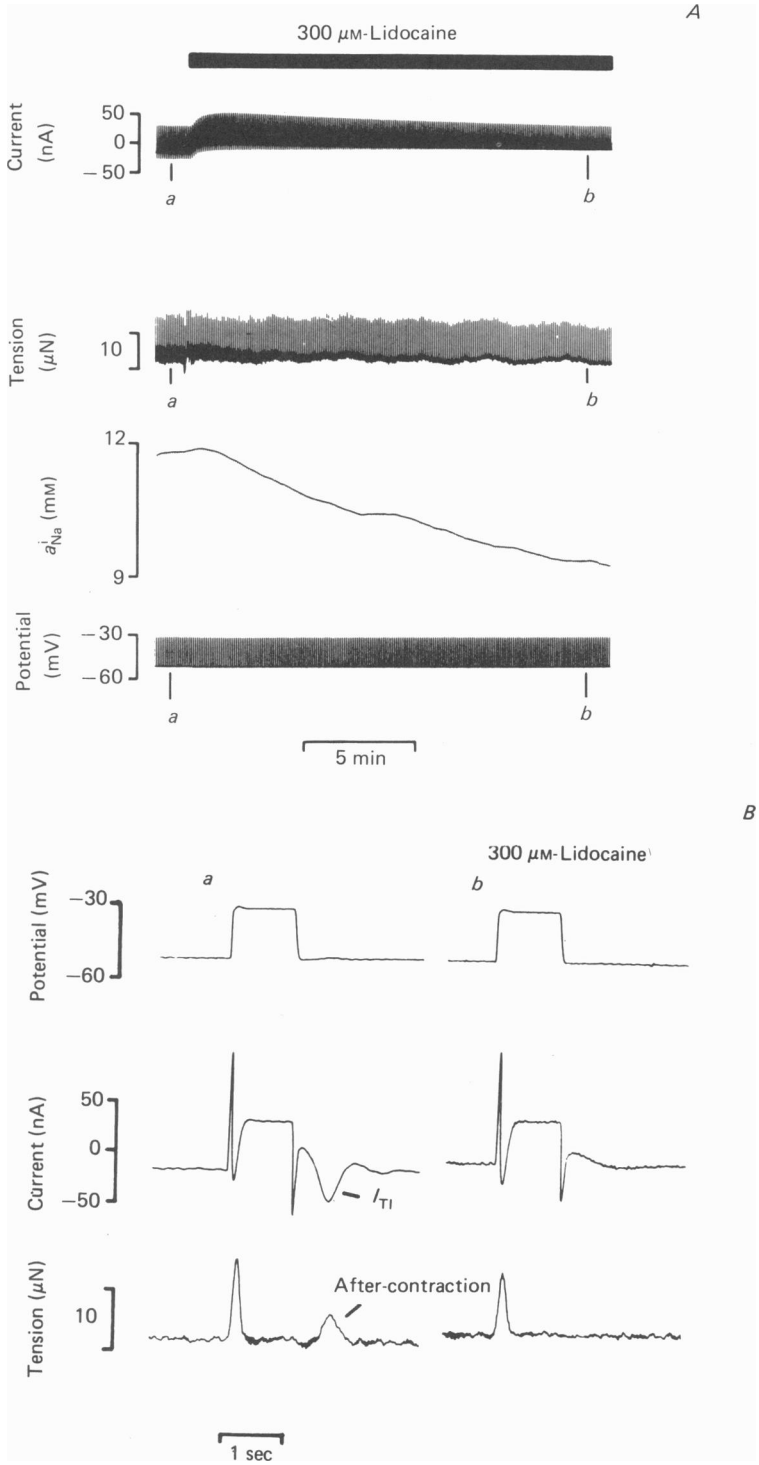


**Fig. 3. For legend see facing page.**

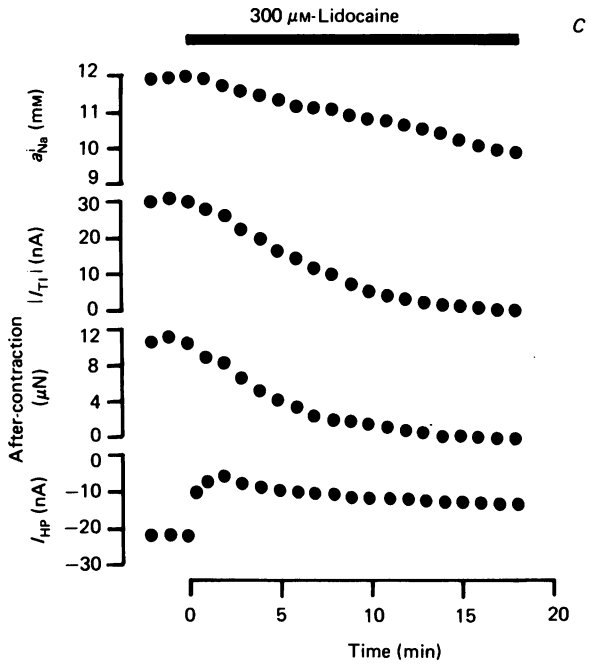


Fig. 3. The effects of lidocaine on membrane current, tension and $a^i_{Na}$. *A*, time course of onset of the effects of lidocaine. Traces show (from top to bottom): membrane current, tension, $a^i_{Na}$ and membrane potential. The preparation was exposed to a K-free solution containing 0·25 mM-Rb to inhibit the Na pump partially and elevate $a^i_{Na}$. At the time indicated lidocaine (300 μM) was added. The membrane potential was held at −51 mV and 1 sec duration pulses were applied to −31 mV at 0·2 Hz. *B*, specimen records of current and tension. Traces show (from top to bottom): potential, current, tension. The records were obtained at the times indicated on *A*. *C*, comparison of the fast rate of onset of the effects of lidocaine on holding current ($I_{HP}$) with its slower effects on $a^i_{Na}$, $I_{TI}$ and after-contraction.

A similar hysteresis is seen between tension and $a^i_{Na}$ during Na pump reactivation by external Rb (Eisner *et al.* 1981). Further information can be obtained from this experiment if $I_{TI}$ magnitude is plotted as a function of after-contraction magnitude. This has been done in Fig. 7 which shows that, although both TTX and lidocaine decrease the magnitude of $I_{TI}$ and the after-contraction, they do not affect the relationship between the two. Changing external Rb produces some hysteresis in the relationship between $I_{TI}$ and after-contraction but less than that seen in Fig. 6. The fact that lidocaine and TTX do not affect the relationship between $I_{TI}$ and after-contraction is consistent with the hypothesis that these agents do not interfere directly with the $I_{TI}$ channel.

*The effects of external Na on $I_{TI}$ and the after-contraction*

The experiments presented above have shown that manipulations of $a^i_{Na}$ can change the magnitude of $I_{TI}$. The results are consistent with the hypothesis that a rise of $a^i_{Na}$ acts by decreasing the gradient of Na ions across the membrane and thereby increases internal Ca. The effects of changes of external Na were investigated in the experiment

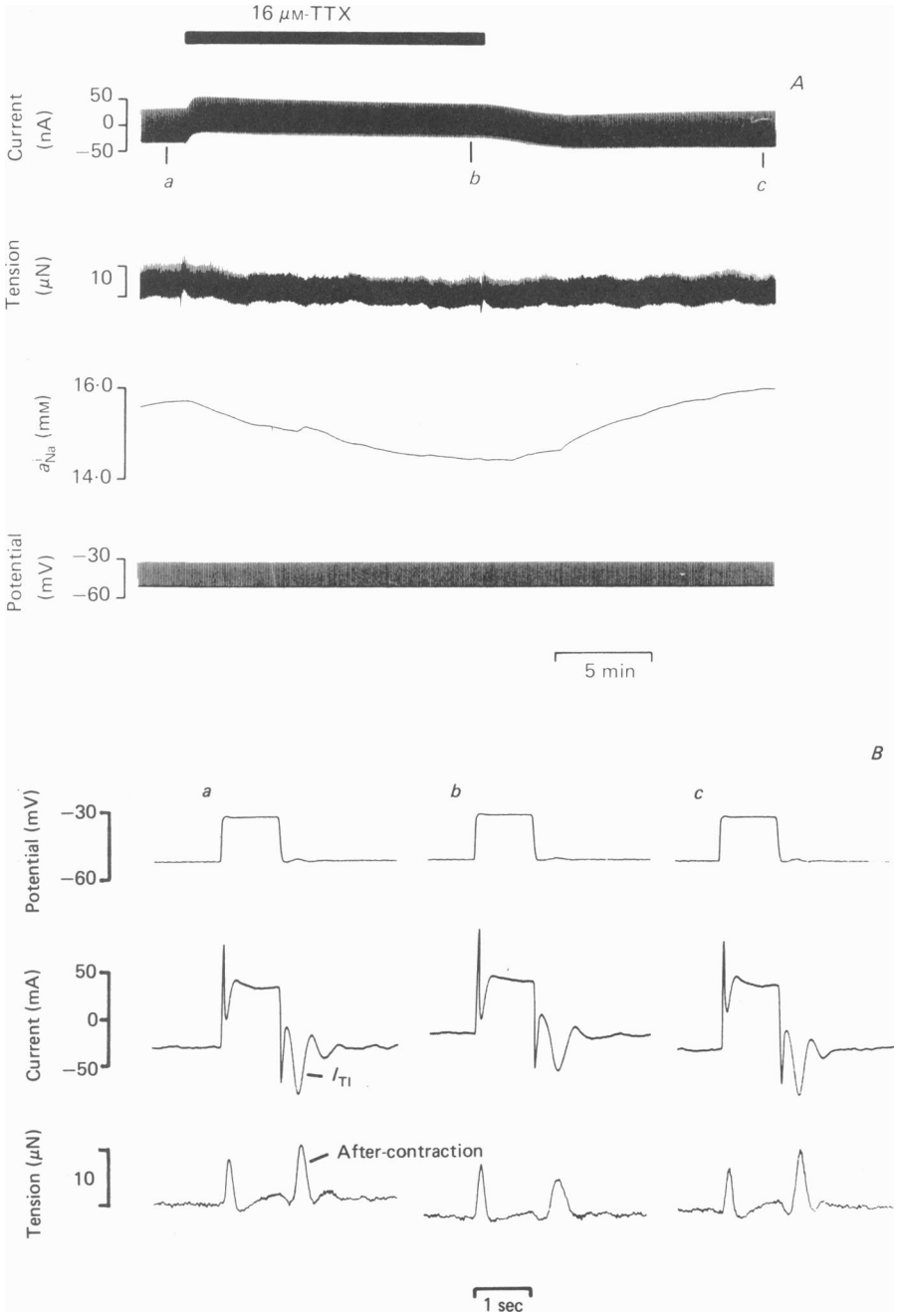


Fig. 4. The effects of TTX on current, $a^i_{Na}$ and tension. *A*, time course of onset of the effects of TTX. Traces show (from top to bottom): current, tension, $a^i_{Na}$ and membrane potential. TTX (16 μM) was applied for the period shown. The membrane potential was held at −51 mV and 1 sec duration pulses were applied to −31 mV at 0·2 Hz. The bathing solution was K-free, Rb-free throughout. *B*, specimen records of current and tension. Traces show (from top to bottom): potential, current and tension. The records were obtained at the times indicated on *A*.

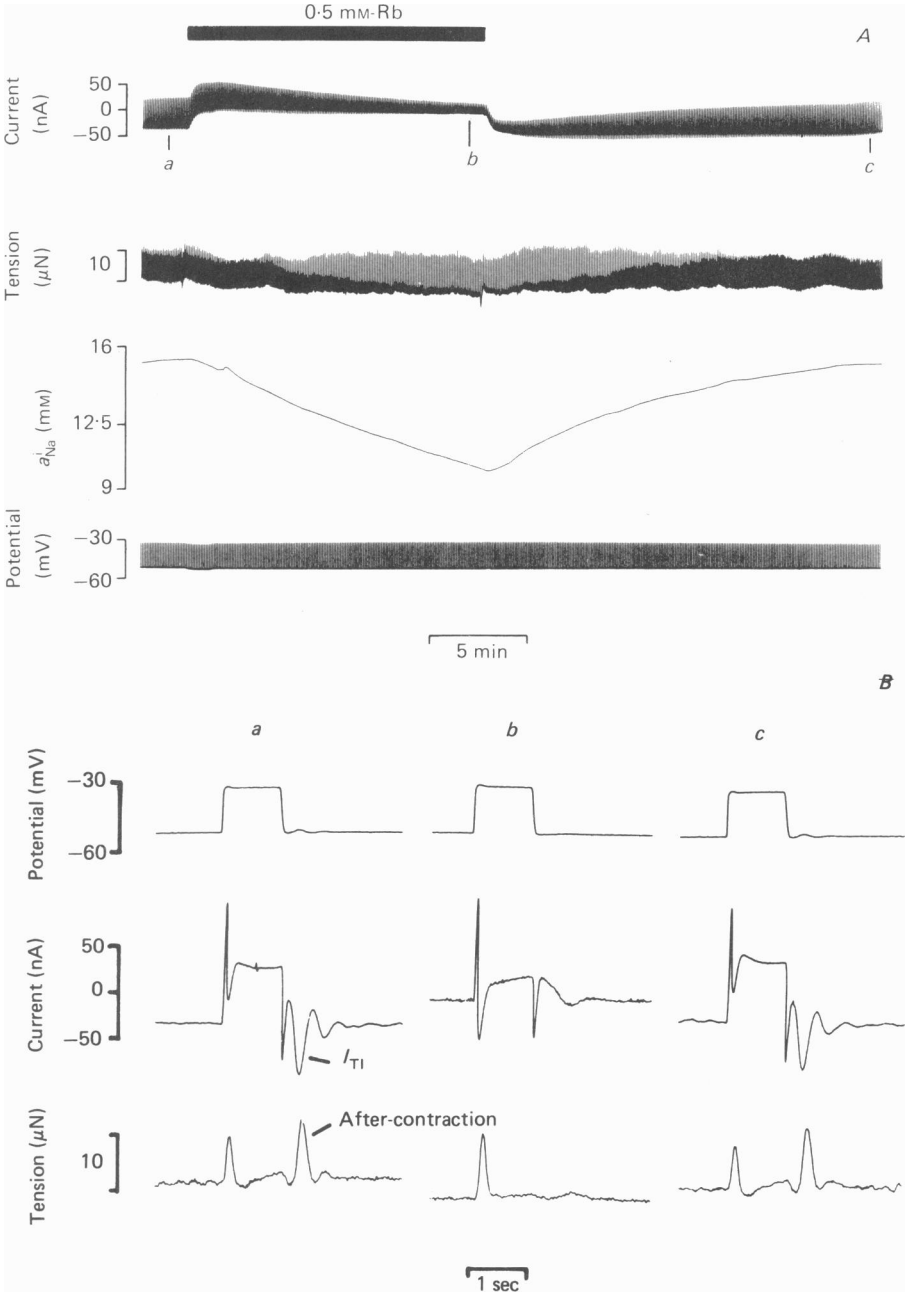


**Fig. 5. The effects of low concentrations of external Rb on membrane current, tension and $a^i_{Na}$. *A*, time course of effects. Traces show (from top to bottom): current, tension, $a^i_{Na}$ and membrane potential. The preparation was initially superfused with a K-free, Rb-free solution to elevate $a^i_{Na}$ and 0·5 mM–Rb was applied for the period shown. The membrane potential was held at −51 mV and 1 sec duration voltage-clamp pulses were applied to −31 mV at 0·2 Hz. *B*, specimen records of current and tension. Traces show (from top to bottom): potential, current, tension. The records were obtained at the times indicated on *A*.**

of Fig. 8. Removal of all the external Na produces a slow decrease of $a^i_{Na}$ (Fig. 8*A*). Specimen current and tension records are shown in Fig. 8*B*. The control record *a*, shows no after-contraction or $I_{TI}$ whereas *b* (obtained after 1 min in Na-free solution) demonstrates a clear $I_{TI}$ and after-contraction. As previously noted (Kass *et al.* 1978*b*), $I_{TI}$ and the after-contraction eventually decay away, *c*. The fall of $I_{TI}$ and the after-contraction has a similar time course to that of $a^i_{Na}$ (Fig. 8). Thus the decay of $I_{TI}$, like the phenomena reported earlier in the paper, shows that $a^i_{Na}$ exerts a powerful controlling influence on $I_{TI}$.

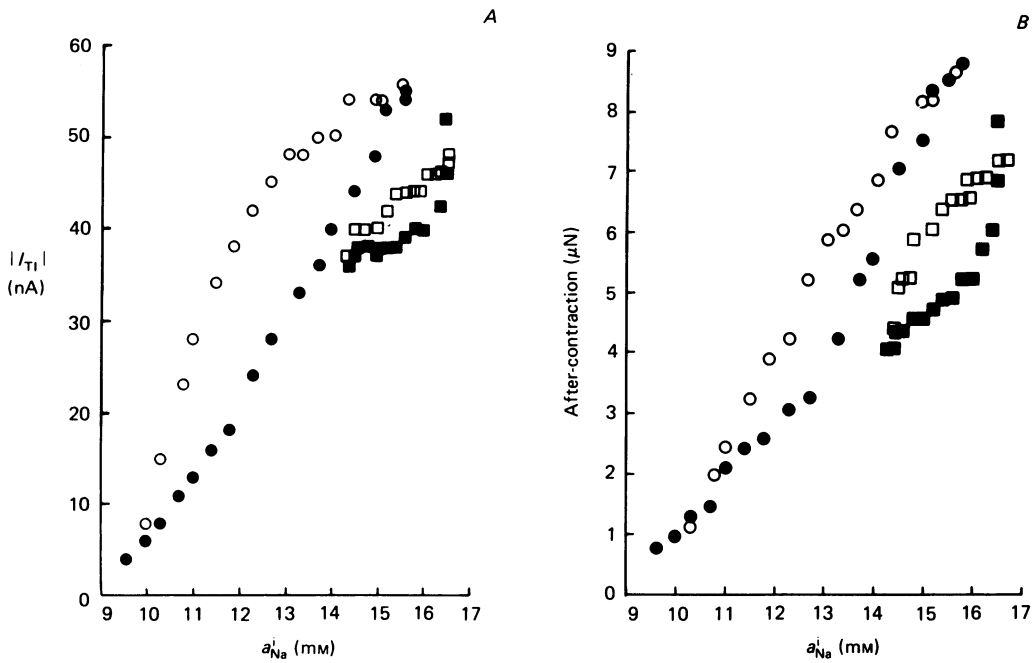


Fig. 6. The dependence of $I_{TI}$ and after-contraction magnitude on $a^i_{Na}$. Data from Figs. 3 and 5. *A*, relationship between $I_{TI}$ and $a^i_{Na}$. The circles show the relationship during the exposure to 0·5 mM–Rb (●) and while removing external Rb (○) (Fig. 5). The squares show the relationship during the onset of the effects of 300 μM-lidocaine (■) and while washing off lidocaine (□) (see Fig. 3). *B*, relationship between after-contraction and $a^i_{Na}$. Same symbols as *A*.

There is a significant difference between the result of Fig. 8 and that reported previously by Kass *et al.* (1978*b*). These authors found that the decay of $I_{TI}$ after removing external Na was much faster than the decay of the after-contraction. The faster fall of $I_{TI}$ was explained as representing the effects of external Na on the $I_{TI}$ conductance, whereas the time course of the after-contraction was taken to be governed by the fall of $a^i_{Na}$ and thence of internal Ca. Such a clear dissociation of the time courses of $I_{TI}$ and the after-contraction was not evident in the present experiments. It is, however, worth noting that: (i) the after-contraction magnitude decayed much more rapidly in the present experiments than in the study of Kass *et al.* (1978*b*) and (ii) the fibre illustrated by Kass *et al.* had $I_{TI}$ under control conditions whereas those used in the present work did not.

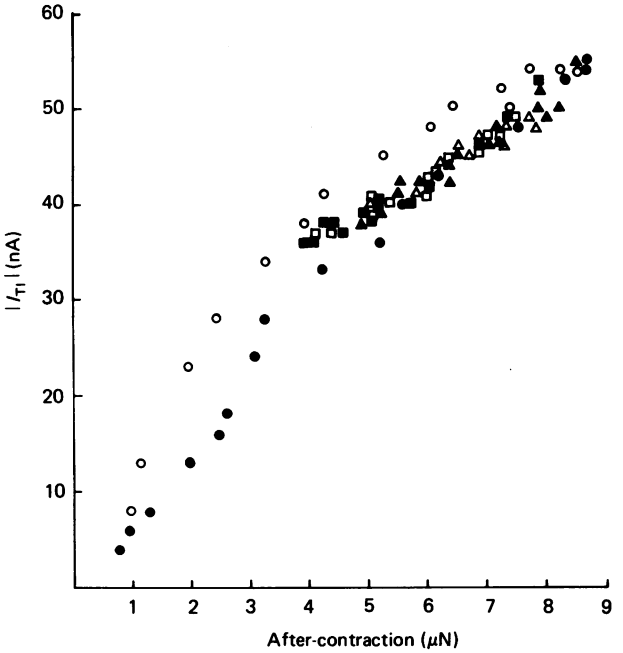


Fig. 7. The relationship between $I_{TI}$ and after-contraction magnitude. In this Figure the data from Fig. 6 have been re-plotted to show $I_{TI}$ as a function of after-contraction magnitude. In addition the triangles show this relationship for the data of Fig. 5 during the application of 15·7 μM-TTX (▲) and while washing off TTX (△). ●, 0·5 mM-Rb; ○, 0 Rb; ■, 300 μM-lidocaine; □, washoff of lidocaine.

## DISCUSSION

### *The effects of lidocaine on* $a^i_{Na}$

The present work is in agreement with that of Deitmer & Ellis (1980) in showing that lidocaine decreases $a^i_{Na}$. At the same time lidocaine produces an outward shift of current attributed to the reduction of a steady-state inward Na current (Attwell *et al.* 1979; Carmeliet & Saikawa, 1982; Colatsky, 1982). The present combination of measurements of $a^i_{Na}$ and current confirms this identification of the current since the fall of $a^i_{Na}$ produced by lidocaine is quantitatively consistent with the magnitude of the outward shift of current. This conclusion is important since it demonstrates that lidocaine decreases $a^i_{Na}$ by decreasing the inward Na current ($I_{Na}$). This suggests that any concentration of lidocaine which can decrease $I_{Na}$ should also decrease $a^i_{Na}$ and therefore both mechanisms must always be considered for the anti-arrhythmic actions of lidocaine (see below).

### *The effects of lidocaine on* $I_{TI}$

The present experiments indicate that lidocaine decreases both $I_{TI}$ and the after-contraction. TTX similarly decreases both of these events. The inhibitory effects of TTX on $I_{TI}$ have been attributed to an indirect consequence of the decreased Na current (Kass *et al.* 1978*b*). Rosen & Danilo (1980) showed that both lidocaine and TTX decreased the transient depolarization which originates from the transient

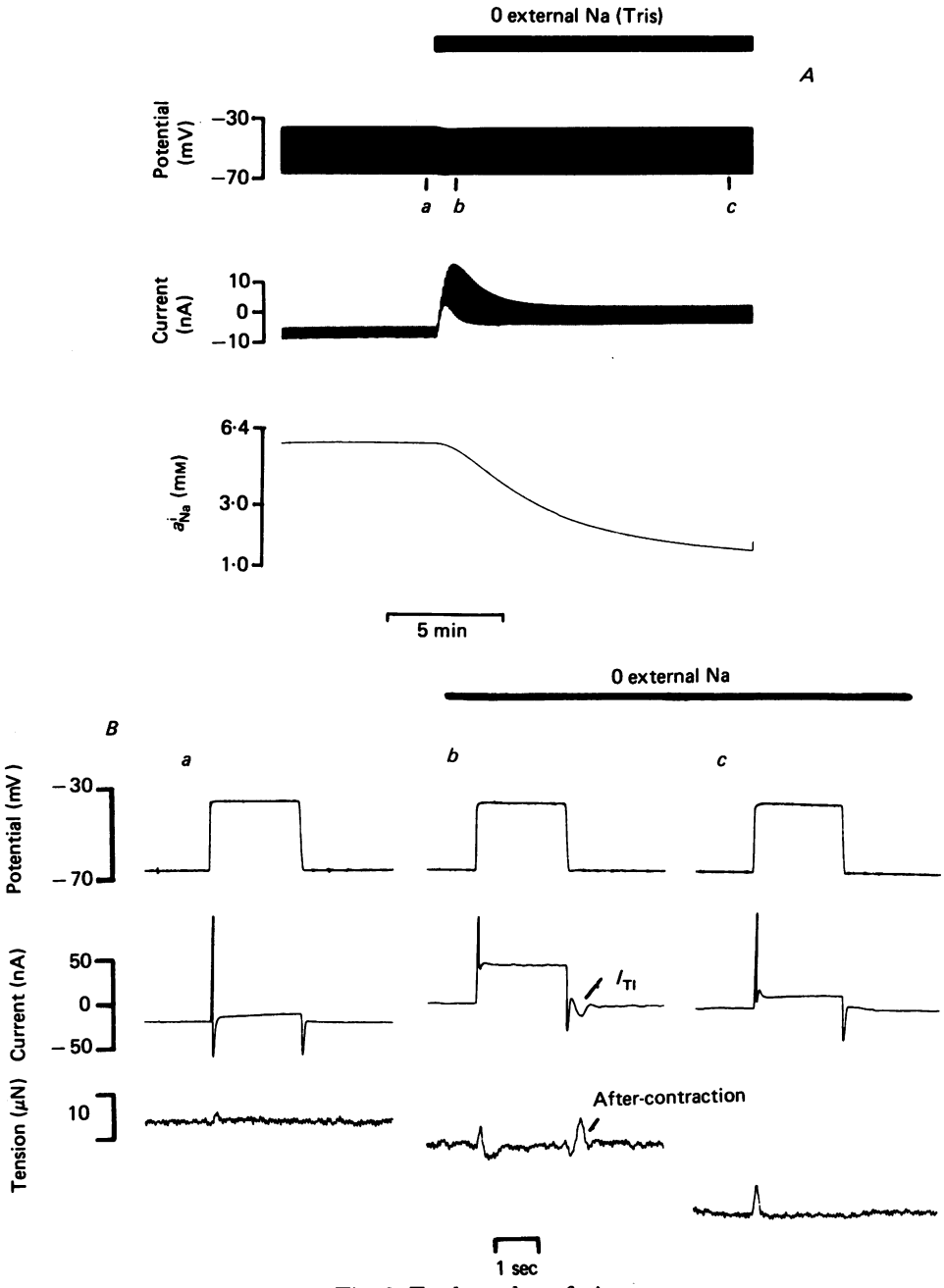


Fig. 8. For legend see facing page.

inward current. Although no voltage-clamp measurements were made, they concluded that the effects of lidocaine were not on $I_{TI}$ but were mediated by changes of the background membrane conductance. The present work, however, confirms that of Eisner & Lederer (1979*c*) in demonstrating that lidocaine abolishes $I_{TI}$.

*The mechanism of the effects of lidocaine on* $I_{TI}$. There are several possible ways in

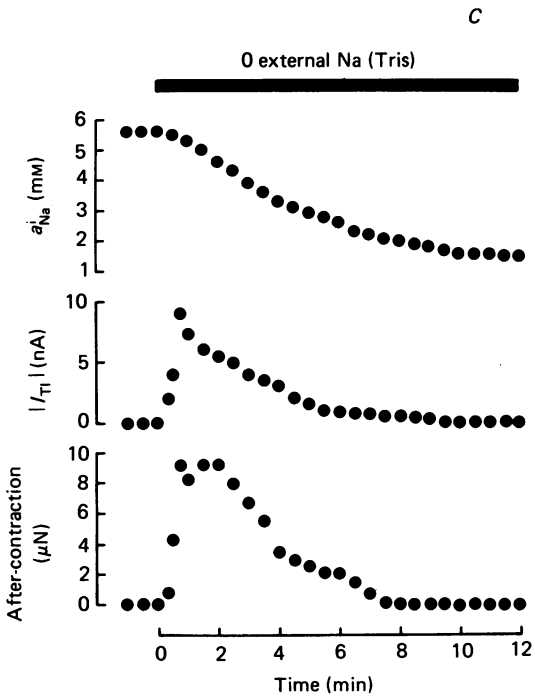


Fig. 8. The effects of removal of external Na on current, $a^i_{Na}$ and tension. *A*, time course of the effects of 0 external Na. Traces show (from top to bottom): potential, current and $a^i_{Na}$. External Na was removed and replaced by Tris at the time indicated above the record. The membrane potential was held at −65 mV and 2 sec duration pulses to −36 mV were applied at 0·2 Hz. *B*, specimen current and tension records. Traces show (from top to bottom): potential, current and tension. The records were obtained at the times indicated on *A*. *C*, comparison of the time-course of change of $a^i_{Na}$, $I_{TI}$ and after-contraction on removing external Na.

which lidocaine could decrease $I_{TI}$. These are best discussed with respect to the scheme below which is taken from the work of Kass, Lederer, Tsien & Weingart (1978*a*).

$$\downarrow\text{Na–K pump} \xrightarrow{(1)} \uparrow a^i_{Na} \xrightarrow{(2)} \uparrow[Ca^{2+}]_i \xrightarrow{(3)} \text{oscillatory Ca release} \xrightarrow{(4)} I_{TI}, \text{ after-contraction}$$

Any agent which decreases $I_{TI}$ could, *a priori*, act at any of the above steps. For example, it has been suggested that manoeuvres such as decreasing external Ca which abolish $I_{TI}$ do so by acting at step 2 to prevent the increase of $[Ca^{2+}]_i$ (Kass *et al.* 1978*a*) whereas those such as caffeine (Eisner & Lederer, 1982; Karagueuzian & Katzung, 1982) or tetracaine (Tsien *et al.* 1978) act at step 3 to stop the oscillatory release of $Ca^{2+}$ ions from the sarcoplasmic reticulum (s.r.). We must therefore consider the possibility that lidocaine acts at any or all of these steps. The experiments with TTX provide an interesting comparison since this is a specific inhibitor of the Na channel and is therefore most likely to act at step 1 to decrease the rise of $a^i_{Na}$ produced by Na pump inhibition. This hypothesis is supported

qualitatively by the fact that TTX decreases $a^i_{Na}$. Due to the hysteresis between $a^i_{Na}$ and $I_{TI}$ magnitude it was impossible to pursue this point quantitatively and establish that TTX had no action on the relationship between $I_{TI}$ and $a^i_{Na}$, a necessary result of this hypothesis.

Lidocaine has been suggested to have many actions on muscle, any of which might explain its actions. Lidocaine decreases the Na permeability in cardiac muscle (Colatsky, 1982; Bean, Cohen & Tsien, 1981) and could therefore produce a reduction of $I_{TI}$ at step 1. Lidocaine could act at step 3 to decrease the amount of Ca oscillation by interfering with the s.r. This explanation has been suggested to explain the abolition of $I_{TI}$ by tetracaine (Tsien *et al.* 1978). In skeletal muscle at least, lidocaine has very much less effect than tetracaine on the s.r. release of Ca (Bianchi & Bolton, 1967) which would not support this mechanism. Such an action would also not explain the fact that lidocaine can abolish the after-contraction without greatly decreasing the twitch (Fig. 3) since agents such as tetracaine and caffeine abolish both. However, this is exactly the behaviour to be expected if lidocaine acts by decreasing $a^i_{Na}$ (cf. Eisner & Lederer, 1979*a*, Fig. 9). It also seems unlikely that lidocaine acts at step 4 since such an action would be expected to change the relationship between the magnitude of $I_{TI}$ and that of the after-contraction. Fig. 7 demonstrates that this relationship is unaffected by lidocaine. Apart from the simple possibility that lidocaine acts by decreasing Na influx at step 1 the only alternative left is that it interferes with step 2 and decreases Ca entry into the cell. One possibility would be that lidocaine can block the slow inward current as has been shown to be the case for tetracaine (Eisner, Lederer & Noble, 1979; Chapman & Leoty, 1981). We have found small effects of lidocaine on $I_{si}$ (unpublished experiments). It is however not clear whether these represent direct effects of lidocaine on $I_{si}$ or are mediated via a fall of $a^i_{Na}$ produced by lidocaine (cf. Lederer & Eisner, 1982; Marban & Tsien, 1982).

In the present experiments we have deliberately used high concentrations of lidocaine in order to reveal any actions this drug may have in addition to the well-documented reduction of $I_{Na}$. Even with these high concentrations no evidence has been found for effects of lidocaine on $I_{TI}$ other than those which can be attributed to the fall of $a^i_{Na}$. It is therefore likely that at lower concentrations of lidocaine the abolition of $I_{TI}$ (Eisner & Lederer, 1979*c*) can be attributed to a fall of $a^i_{Na}$ which must accompany the observed decrease of $I_{Na}$ produced by therapeutic concentrations of lidocaine (Carmeliet & Saikawa, 1982; Colatsky, 1982).

*The mechanism of the anti-arrhythmic actions of lidocaine*

Various explanations have been suggested for the anti-arrhythmic actions of lidocaine. The classic explanation is based on the observation that lidocaine and other anti-arrhythmic agents decrease the excitability of cardiac muscle by decreasing the excitatory Na current (Weidmann, 1955). This has been called the class I mechanism of anti-arrhythmic action (Vaughan-Williams, 1970). There has been some disagreement about whether this mechanism operates at therapeutic levels (2–5 mg/l. or 8–20 $\mu$M) but it has been demonstrated recently that lidocaine can decrease $I_{Na}$ (Bean *et al.* 1981) at a concentration of 20 $\mu$M).

The alternative explanation for the anti-arrhythmic action of lidocaine is that it acts, not by decreasing the excitability of the cell, but by decreasing the arrhythmo-

genic stimulus, in this case the transient depolarization. There are two ways in which lidocaine could interfere with the transient depolarization: (i) by interfering with $I_{TI}$ directly; (ii) by affecting the background conductance of the membrane so that a given $I_{TI}$ produces a smaller transient depolarization. The latter mechanism has been suggested by several results. First, Weld & Bigger (1976) reported that lidocaine (1 mg/ml.) decreased the inward background current. As suggested by Attwell, Cohen, Eisner, Ohba & Ojeda (1979) and demonstrated by Colatsky (1982) this effect merely reflects the action of lidocaine to block the steady-state component of $I_{Na}$ and will therefore account for the fall of $a^i_{Na}$ seen in the present work. Rosen & Danilo (1980) found that lidocaine (4 mg/l.) decreased the size of the transient depolarization and, although they suggested that this was an effect on the background conductance, their results are compatible with lidocaine decreasing $I_{TI}$. Eisner & Lederer (1979*c*) found that a slightly greater concentration of lidocaine (35 $\mu$M) abolished $I_{TI}$ suggesting that concentrations of lidocaine in the therapeutic range would have significant effects on $I_{TI}$. It therefore appears likely that a reduction of $I_{TI}$ contributes to the therapeutic anti-arrhythmic actions of lidocaine.

This paper has shown that part of the anti-arrhythmic actions of lidocaine is mediated via a reduction of $I_{TI}$. Therefore both of the proposed mechanisms for lidocaine's action depend ultimately on its interaction with the Na channel. This will directly and rapidly decrease the Na current and thence the excitability of the cell. This reduction of the Na current produces a fall of $a^i_{Na}$, a decrease of $I_{TI}$ and thereby abolishes the abnormal pace-maker activity.

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## REFERENCES

ALMERS, W. & BEST, P. M. (1976). Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *J. Physiol.* **262**, 583–611.

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

BAKER, P. F., BLAUSTEIN, M. P., HODGKIN, A. L. & STEINHARDT, R. A. (1969). The influence of calcium on sodium efflux in squid axons. *J. Physiol.* **200**, 431–458.

BEAN, B. P., COHEN, C. J. & TSIEN, R. W. (1981). Lidocaine binding to resting and inactivated cardiac sodium channels. *Biophys. J.* **33**, 208a.

BERS, D. M. & ELLIS, D. (1982). Intracellular calcium and sodium activity in sheep heart Purkinje fibres. Effects of changes of external sodium and intracellular pH. *Pflügers Arch.* **393**, 171–178.

BIANCHI, C. P. & BOLTON, T. C. (1967). Action of local anaesthetics on coupling systems in muscle. *J. Pharmac. exp. Ther.* **157**, 388–405.

CARMELIET, E. & SAIKAWA, T. (1982). Shortening of the action potential and reduction of pacemaker activity by lidocaine, quinidine and procainamide in sheep cardiac Purkinje fibers – an effect of Na or K currents? *Circulation Res.* **50**, 257–272.

CHAPMAN, R. A. & LEOTY, C. (1981). The effects of tetracaine on the membrane currents and contraction of frog atrial muscle. *J. Physiol.* **317**, 475–486.

COLATSKY, T. J. (1982). Mechanism of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibers – an effect on steady state sodium currents? *Circulation Res.* **50**, 17–27.

COLQUHOUN, D., NEHER, E., REUTER, H. & STEVENS, C. F. (1981). Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature, Lond.* **294**, 752–754.

DEITMER, J. W. & ELLIS, D. (1980). The intracellular sodium activity of sheep heart Purkinje fibres: effects of local anaesthetics and tetrodotoxin, *J. Physiol.* **300**, 269–282.

EISNER, D. A. & LEDERER, W. J. (1979*a*). Inotropic and arrhythmogenic effects of potassium-depleted solutions on mammalian cardiac muscle. *J. Physiol.* **294**, 255–277.

EISNER, D. A. & LEDERER, W. J. (1979*b*). The role of the sodium pump in the effects of potassium-depleted solutions on mammalian cardiac muscle. *J. Physiol.* **294**, 279–301.

EISNER, D. A. & LEDERER, W. J. (1979*c*). A cellular basis for lidocaine's anti-arrhythmic action. *J. Physiol.* **295**, 25–26*P*.

EISNER, D. A. & LEDERER, W. J. (1980). The relationship between sodium pump activity and twitch tension in cardiac Purkinje fibres. *J. Physiol.* **303**, 475–494.

EISNER, D. A. & LEDERER, W. J. (1982). Effects of caffeine on the transient inward current in cardiac Purkinje fibres. *J. Physiol.* **322**, 48–49*P*.

EISNER, D. A., LEDERER, W. J. & NOBLE, D. (1979). Caffeine and tetracaine abolish the slow inward calcium current in cardiac Purkinje fibres. *J. Physiol.* **293**, 76–77*P*.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981). The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep Purkinje fibres. *J. Physiol.* **317**, 163–187.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1983). The control of tonic tension by membrane potential and intracellular Na activity in the sheep cardiac Purkinje fibre. *J. Physiol.* **335**, 723–743.

FERRIER, G. R. & MOE, G. K. (1973). Effect of calcium on acetylstrophanthidin-induced transient depolarizations in canine Purkinje tissue. *Circulation Res.* **33**, 508–515.

FERRIER, G. R., SAUNDERS, J. H. & MENDEZ, C. (1973). A cellular mechanism for the generation of ventricular arrhythmias by acetylstrophanthidin. *Circulation Res.* **33**, 508–515.

GADSBY, D. C. & CRANEFIELD, P. F. (1979). Direct measurement of changes in sodium pump current in canine cardiac Purkinje fibres. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1783–1787.

GLITSCH, H. G., KAMPMANN, W. & PUSCH, H. (1981). Activation of active Na transport in sheep Purkinje fibres by external K or Rb ions. *Pflügers Arch.* **391**, 28–34.

KARAGUEUZIAN, H. S. & KATZUNG, B. G. (1982). Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. *J. Physiol.* **327**, 255–271.

KASS, R. S., LEDERER, W. J., TSIEN, R. W. & WEINGART, R. (1978*a*). Role of calcium ions in transient inward currents and after contractions induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol.* **281**, 187–208.

KASS, R. S., TSIEN, R. W. & WEINGART, R. (1978*b*). Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol.* **281**, 209–226.

LEDERER, W. J. & EISNER, D. A. (1982). The effects of sodium pump activity on the slow inward current in sheep cardiac Purkinje fibres. *Proc. R. Soc.* **214**, 249–262.

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

MARBAN, E. & TSIEN, R. W. (1982). Enhancement of cardiac calcium current during digitalis inotropy: positive feedback regulation by intracellular calcium? *J. Physiol.* **329**, 589–614.

NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**, 814–889.

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

ROSEN, M. R. & DANILO, P. (1980). Effects of tetrodotoxin, lidocaine, verapamil and AHR-2666 on ouabain-induced delayed afterdepolarizations in canine Purkinje fibers. *Circulation Res.* **46**, 117–124.

ROSEN, M. R., GELBAND, H. & HOFFMAN, B. F. (1973). Correlation between effects of ouabain on the canine electrocardiogram and transmembrane potentials in isolated Purkinje fibers. *Circulation* **47**, 65–72.

SHEU, S-S. & FOZZARD, H. A. (1982). Transmembrane $Na^+$ and $Ca^{2+}$ electrochemical gradients in cardiac muscle and their relationship to force development. *J. gen. Physiol.* **80**, 325–351.

SHEU, S-S., LEDERER, W. J. & EISNER, D. A. (1982). How does lidocaine reduce the oscillatory transient inward current in sheep cardiac Purkinje fibers? *Biophys. J.* **37**, 343a.

STEINER, R. A., OEHME, M., AMMANN, D. & SIMON, W. (1979). Neutral carrier sodium ion-selective microelectrode for intracellular studies. *Analyt. Chem.* **51**, 351–353.

STRICHARTZ, G. R. (1973). The inhibition of sodium currents in myelinated nerve by quarternary derivatives of lidocaine. *J. gen. Physiol.* **62**, 37–57.

THOMAS, R. C. (1972). Electrogenic sodium pump in nerve and muscle. *Physiol. Rev.* **52**, 563–594.

TSIEN, R. W., WEINGART, R. & KASS, R. S. (1978). Digitalis: Inotropic and arrhythmogenic effects on membrane currents in cardiac Purkinje fibers. In *Biophysical Aspects of Cardiac Muscle*, ed. MORAD, M., pp. 345–368. N.Y.: Academic Press.

VAUGHAN-WILLIAMS, E. M. (1970). Classification of antiarrhythmic drugs. In *Symposium on Cardiac Arrhythmias*, ed. SANDO, E., FLENSTED, JENSEN, E. & OLESEN, K. H., pp. 449–472. Sodertalje, Sweden: AB Astra.

WEIDEMANN, S. (1955). Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. *J. Physiol.* **129**, 568–582.

WELD, F. M. & BIGGER, J. T. (1976). The effect of lidocaine on diastolic transmembrane currents determining depolarization in cardiac Purkinje fibers. *Circulation Res.* **38**, 203–208.