J. Physiol. (1980), **300**, *pp.* 269–282
With 8 text-figures
Printed in Great Britain

THE INTRACELLULAR SODIUM ACTIVITY OF SHEEP HEART PURKINJE FIBRES: EFFECTS OF LOCAL ANAESTHETICS AND TETRODOTOXIN

BY JOACHIM W. DEITMER* AND DAVID ELLIS†

From the Department of Physiology, Medical School, University of Bristol, Bristol BS8 1TD

(*Received 2 February 1979*)

SUMMARY

1. The intracellular Na activity (a^i_{Na}) of quiescent sheep heart Purkinje fibres has been measured using Na^+-sensitive glass micro-electrodes. The effects of local anaesthetics (procaine and lidocaine) and tetrodotoxin (TTX) have been investigated.

2. Local anaesthetics reduced the steady-state level of the intracellular Na activity in a dose-dependent manner. The highest concentrations used (10^{-2} M) reduced the intracellular Na activity by about 25 %.

3. TTX decreased the steady-state level of the intracellular Na activity. At a concentration of 10^{-6} g/ml. ($3{\cdot}13 \times 10^{-6}$ M), TTX produced a decrease in intracellular Na activity of approximately 10 %.

4. The initial rate of rise of the intracellular Na activity upon addition of the cardioactive steroid strophanthidin (10^{-5} M) was used to estimate the net passive Na influx.

5. Procaine (5×10^{-4} M) caused a 50 % reduction of this rate of rise of the intracellular Na activity. The highest concentration of procaine used (10^{-2} M) decreased the rate of rise by approximately 80 %.

6. Procaine (5×10^{-3} M) also reduced the rate of rise of intracellular Na produced by the removal of external K (K_o), and prevented the large depolarization associated with the absence of K_o.

7. TTX also produced a decrease in the rate of rise of the intracellular Na activity that occurs upon addition of strophanthidin. A maximum effect was produced in our experiments at a TTX concentration of 10^{-6} g/ml. At this concentration the rate of rise of intracellular Na activity was reduced by approximately 40 % at a membrane potential of -70 mV.

8. We conclude from our experiments that the effects of local anaesthetics and TTX on the intracellular Na activity are brought about by a reduction of the Na permeability of the cell membrane, and that at the normal resting potential, Na entry through TTX-sensitive channels contributes greatly to the total net Na influx.

* Present address: Abteilung Biologie, Ruhr-Universität, D-4630 Bochum 1, West Germany.
† Present address: Department of Physiology, University Medical School, Teviot Place, Edinburgh, EH8 9AG.

0022-3751/80/7340-0053 $07.50

INTRODUCTION

The intracellular Na activity (a^i_{Na}) of sheep heart Purkinje fibres is maintained at a low level primarily by the action of the Na–K pump (Ellis, 1977; Deitmer & Ellis, 1978*b*). The rate at which the pump works depends, among other factors, upon the rate of Na influx into the cells. When the Na–K pump is inhibited (e.g. by high concentrations of cardioactive steroids), the intracellular Na activity rapidly increases. In quiescent Purkinje fibres the initial rate of this rise of internal Na presumably reflects the net passive Na influx, which depends on the Na permeability of the cell membrane and the Na electrochemical gradient. For *small* changes in membrane potential (E_m) and/or intracellular Na activity, the Na electrochemical gradient will be essentially constant so the rate of rise of intracellular Na upon inhibition of the Na–K pump will give an indication of the Na permeability of the cell membrane. For larger changes in membrane potential the Na gradient can be easily calculated if, as in the present experiments, both E_m and a^i_{Na} are measured.

In this study we have looked at the effects of local anaesthetics and tetrodotoxin (TTX) on the intracellular Na activity of sheep heart Purkinje fibres. The intracellular Na was measured using Na^+-sensitive micro-electrodes (Thomas, 1970, 1978). It is known that local anaesthetics increase the threshold, and decrease the rate of depolarization of the action potential in Purkinje fibres (Weidmann, 1955). One of the actions of local anaesthetics is to decrease the over-all Na permeability of cell membranes (e.g. see Strichartz, 1976). TTX on the other hand is a very specific blocker of Na entry during the action potential (for references see Blankenship, 1976) and also greatly decreases the rate of Purkinje fibre depolarization (Dudel, Peper, Rüdel & Trautwein, 1967). The action of TTX is presumably due to its binding to the voltage-sensitive Na channels, whereas local anaesthetics might reduce Na influx through both TTX-sensitive Na-channels and TTX-insensitive Na pathways. These TTX-insensitive Na pathways could include K channels (although see Hille, 1973) and non-specific 'leakage' pathways.

The aims of the present experiments were: (i) to test how much the resting permeability to Na is reduced by local anaesthetics and TTX by monitoring their effects on the intracellular Na activity and the rate of rise of the Na activity produced by a high concentration (10^{-5} M) of strophanthidin, and (ii) to compare the actions of the two types of drug on the Na permeability of the membrane. Our results indicate that high concentrations of both local anaesthetics and TTX are required to produce a significant effect on the intracellular Na activity and the Na permeability of the resting membrane.

METHODS

These were essentially the same as described previously (Ellis, 1977; Deitmer & Ellis, 1978*a*, *b*) with the isolated Purkinje fibres pinned into a small superfusion chamber (flow rate of solutions approximately 4–6 bath vol./min, temperature 35 °C).

The cells were penetrated by two micro-electrodes, a conventional one, and a Na^+-sensitive micro-electrode (Thomas, 1970, 1978). All the Na^+-sensitive micro-electrodes used showed a 90% complete response to a change in $[Na]_o$ in less than 90 sec. The fastest changes of intracellular Na measured were at least ten times slower than the response-time of the Na^+-sensitive micro-electrodes used in our experiments. Therefore measurements of changes of the intracellular Na activity were not limited by the time course of the electrode response. The size of the response varied between 54 and 60 mV for a tenfold change in Na concentration.

Solutions

All solutions were equilibrated with 100% O_2. The normal saline contained (mM) Na 140, K 6, Ca 2, Mg 1, pyruvate 2, glucose 10, Cl 145, and was buffered with 10 mM of the Na salt of HEPES (2-*N*-2-hydroxyethyl piperazine-*N'*-ethanesulphonic acid) to give a pH of 7·4 (±0·05). In experiments involving the use of high procaine concentrations (a maximum of 10 mM was used in this study) all solutions were made hypertonic. The various procaine concentrations were produced by mixing a normal Tyrode solution (made hypertonic by the addition of 10 mM-Tris) with a Tyrode solution to which 10 mM-procaine had been added.

Procaine (B.D.H.) and lidocaine (a gift from Astra Chemicals Ltd) were added as their hydrochlorides. Strophanthidin (Boehringer Mannheim) was added from a stock solution of 10^{-2} M in ethanol. TTX (Calbiochem Ltd) was dissolved in Tyrode solution to give a maximum concentration of 2×10^{-5} g/ml. ($6{\cdot}26 \times 10^{-5}$ M).

RESULTS

Effects of local anaesthetics

The addition of procaine or lidocaine at concentrations between 10^{-4} and 10^{-2} M to the bathing solution produced a decrease in the intracellular Na activity to a new steady-state level. This effect was complete within 15 to 30 minutes and was fully reversible.

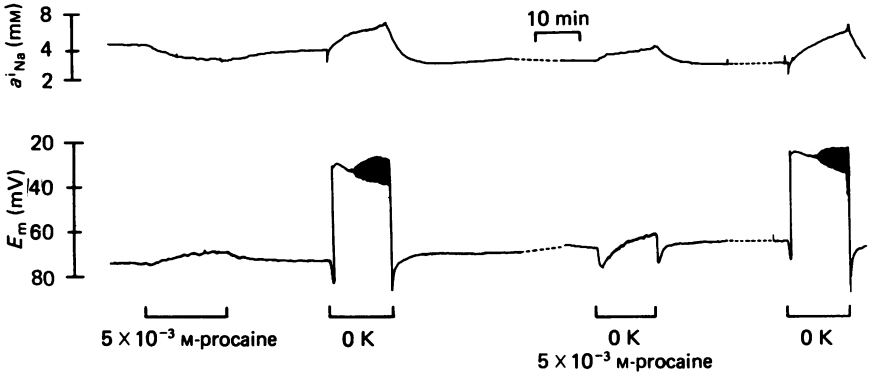

Fig. 1. Pen recording showing the effects of 5×10^{-3} M-procaine in the presence of 6 mM-$[K]_o$ and in K-free solution on the intracellular Na activity (a^i_{Na}) (top trace), and on the membrane potential (bottom trace). Isotonicity was maintained by substituting Tris for K and procaine for Tris. The breaks in the figure (interrupted lines in the traces) were for periods of 40 and 10 min respectively.

Fig. 1 shows the effect of 5×10^{-3} M-procaine on the intracellular Na and the membrane potential (E_m) and also on the changes of intracellular Na produced by K-free solution. In this experiment the steady-state intracellular Na activity decreased from 4·8 to 3·2 mM when procaine was applied. In four experiments of this type the steady-state internal Na activity decreased by 20 ± 4% (mean ± S.D.).

Removal of K_o results in a depolarizing 'switch' of the membrane potential to about −30 to −45 mV, and intracellular Na increases due to the inhibition of the Na–K pump (Ellis, 1977). As is shown in Fig. 1, 5×10^{-3} M-procaine considerably slowed the rise of internal Na produced by the removal of K_o and also prevented the depolarizing 'switch' of the membrane potential. These effects are in agreement with the finding that local anaesthetics reduce the Na permeability of cell membranes

(Shanes, Freygang, Grundfest & Amatniek, 1959; Taylor, 1959), and they support the suggestion (Carmeliet, 1961; Gadsby & Cranefield, 1977) that the depolarizing 'switch' of the Purkinje fibre membrane potential is due to an increase in the Na permeability relative to that of K. Procaine always caused a depolarization (Fig. 1) when such high concentrations were used. However, at low procaine concentrations ($< 10^{-3}$ M) a small hyperpolarization occurred.

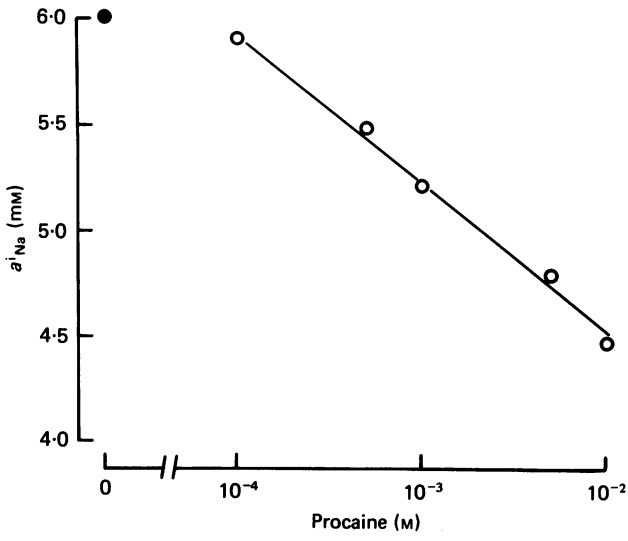

Fig. 2. The results of an experiment where the steady-state values of a^i_{Na} were measured in the absence of procaine (filled circle) and at various procaine concentrations (open circles).

Fig. 2 shows the results of an experiment where the steady-state intracellular Na activity was measured at various procaine concentrations between 10^{-4} and 10^{-2} M. Over this range, procaine caused a dose-dependent decrease in intracellular Na. At, for example, 10^{-3} M-procaine the a^i_{Na} was decreased by approximately 12 %.

The addition of 10^{-5} M-strophanthidin to the external solution resulted in a rapid rise in intracellular Na activity at an average maximum rate of about 0·5 m-mole/min (Deitmer & Ellis, 1978*b*). This strophanthidin concentration was found to have a maximal effect on the rate of rise of internal Na and was therefore suggested to be sufficient to completely block the Na–K pump. If it is assumed that there is only a small buffering of the intracellular Na activity then this rate of rise of internal Na reflects the rate of net passive Na influx into the cells. We have calculated this influx to be approximately 2·8 p mole/cm^2 sec (Deitmer & Ellis, 1978*b*). This rise of the intracellular Na activity in 10^{-5} M-strophanthidin reaches its maximum rate within three minutes and remained approximately constant for the next 8–15 min (see also Deitmer & Ellis, 1978*b*). Thus this initial maximum rate of rise has been used to estimate changes in the net Na influx under various conditions. Fig. 3*A* illustrates an experiment where the effect of various procaine concentrations on the rise of intracellular Na has been investigated. As the procaine concentration was increased, inhibition of the Na–K pump by strophanthidin resulted in slower rates of

increase of intracellular Na. Fig. 3*B* shows the effect on the maximum rate of rise of intracellular Na of procaine concentrations between 5×10^{-5} and 10^{-2} M. Over this range of concentrations, a decrease of 25 % in the rate of rise of intracellular sodium activity occurred per tenfold increase in the procaine concentration. The highest procaine concentration used (10^{-2} M) decreased the rate of rise of intracellular Na activity by about 80 %. This high procaine concentration produced a large membrane depolarization (approximately 20 mV). This depolarization would be expected to

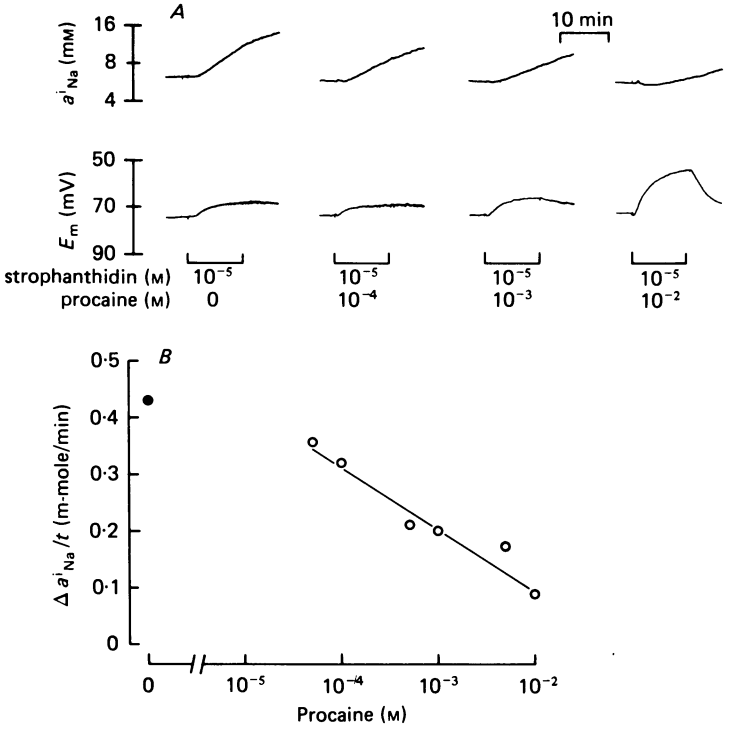

Fig. 3. *A*, the effect of various procaine concentrations on the increase of a^i_{Na} produced by the application of 10^{-5} M-strophanthidin. The record of the recovery of a^i_{Na} following removal of strophanthidin has been cut out in each case in order to shorten the figure. The breaks in the record were for periods of approximately 40 min. *B*, the maximum rate of increase of a^i_{Na} in 10^{-5} M-strophanthidin (from the same experiment as that shown in part *A*) has been plotted against the procaine concentration (open circles) and in the absence of procaine (filled circle).

decrease the net influx and thereby contribute to the large reduction in the rate of rise of intracellular Na.

On removal of strophanthidin the recovery of the intracellular Na activity is somewhat delayed (Deitmer & Ellis, 1978*b*), probably due to the slow dissociation of strophanthidin from its inhibitory binding site. When procaine and strophanthidin were removed simultaneously, the rate of rise of intracellular Na showed a *transient increase* for 5–10 min. This suggests that procaine does not interfere with the binding of strophanthidin. Thus, the results indicate that procaine can substantially reduce the net passive Na influx.

We have compared the effects of procaine, described above, with those of lidocaine (at 10^{-4} and 10^{-3} M). Both the steady-state intracellular activity and the rise of intracellular Na following Na–K pump inhibition were very similarly affected by these two local anaesthetics. Fig. 4 shows an experiment in which we have compared the effects of 10^{-3} M-procaine and lidocaine on the rise of intracellular Na produced by addition of strophanthidin. In this experiment the rate of rise of intracellular Na was reduced by 61 % with procaine and by 55 % with lidocaine compared to the rate in the absence of local anaesthetics. Under the combined influence of local anaesthetics

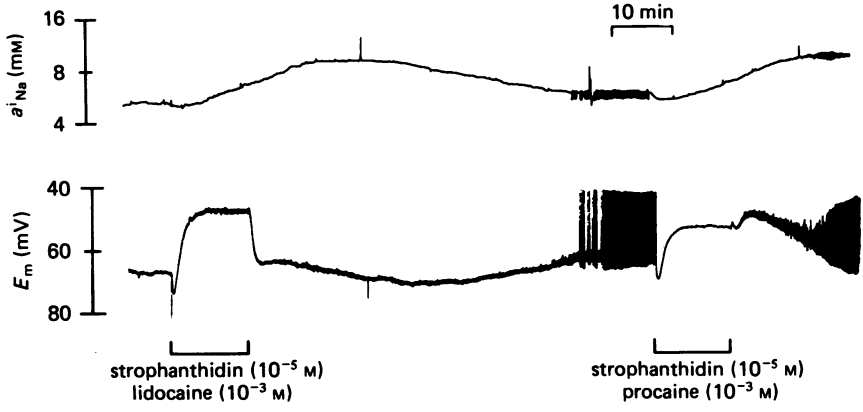

Fig. 4. Pen-recording of an experiment showing the effect of 10^{-3} M-procaine and 10^{-3} M-lidocaine on E_m and on the rise of a^i_{Na} on addition to 10^{-5} M-strophanthidin. The black areas on the E_m trace were due to membrane potential oscillations and to full action potentials which were too rapid for the pen-recorder to follow accurately.

and strophanthidin there was normally a transient hyperpolarization followed by a depolarization of 12–15 mV. In the experiment illustrated in Fig. 4 there were some spontaneous action potentials prior to, and during the recovery from, the addition of procaine plus strophanthidin. The action potentials were suppressed during exposure to these drugs. Spontaneous action potentials or action potentials elicited by electrical stimulation (up to 2/sec) changed the steady-state intracellular Na activity by between 1 and 3 mM. This rise of intracellular Na was completed within 5–15 min.

Effects of tetrodotoxin

TTX is well known for its very specific blocking action on the Na conductance during the rising phase of the action potential (Narahashi, Moore & Scott, 1964). In mammalian cardiac muscle, in contrast to nerve and skeletal muscle, very high concentrations of TTX are required to produce a marked inhibition of the amplitude and rate of rise of the action potential ($> 10^{-5}$ g/ml. in Purkinje fibres, Dudel *et al.* 1967). It has been suggested that the Na entry via these voltage- and TTX-sensitive membrane channels may contribute significantly to the total passive Na influx at the resting membrane potential (Cohen & Strichartz, 1977).

We have investigated the effect of TTX on both the steady-state level of intracellular Na activity and on the rate of rise of intracellular Na produced by 10^{-5} M-strophanthidin. Fig. 5 shows an experiment of this type. When TTX, 2×10^{-5} g/ml.,

was added, the intracellular Na activity slowly and reversibly decreased from 8·2 to 7·2 mM. The fibre was then exposed to strophanthidin for 10 min. This resulted in a relatively small increase of the intracellular Na. On the right is shown the larger increase of intracellular Na produced by strophanthidin in the absence of TTX. TTX also caused a transient hyperpolarization of the membrane potential by 1–2 mV and reduced the subsequent depolarization produced by strophanthidin. The effects of TTX on both the steady-state intracellular Na activity and the rate of rise of Na produced by strophanthidin were fully reversible within 30–60 min in our preparations.

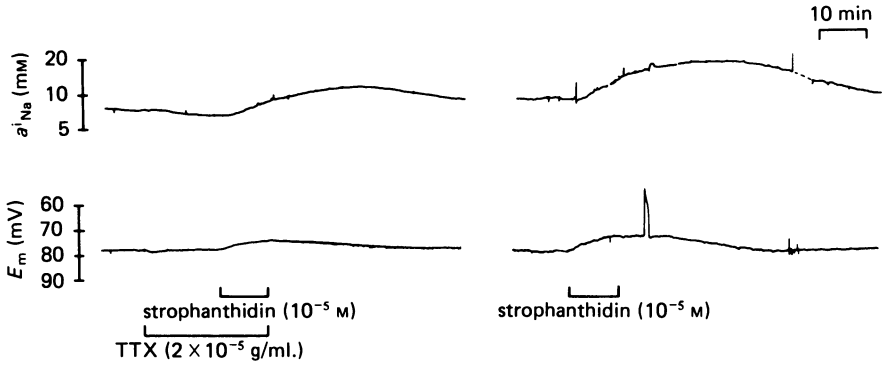

Fig. 5. The effect on a^i_{Na} and E_m of applying 10^{-5} M-strophanthidin in the presence and absence of TTX (2×10^{-5} g/ml.). The gap in the recording was for a period of approximately 190 min. During the recovery of a^i_{Na} from the second exposure to strophanthidin the amplifier connected to the Na^+-sensitive micro-electrode was switched off for 4 min (interrupted line in upper trace).

The dose dependence of the TTX effects on the steady-state intracellular Na activity and on the rate of rise of intracellular Na were studied in more detail in two experiments as shown in Fig. 6. The Purkinje fibres were exposed to the various concentrations of TTX for 15–20 min, and the steady-state intracellular Na activity measured (Fig. 6*C*). The fibres were then exposed to 10^{-5} M-strophanthidin and the rate of rise of intracellular Na was measured (Fig. 6*B*). Some of the records from one experiment are shown in Fig. 6*A*. TTX at a concentration of 10^{-6} and 2×10^{-5} g/ml. reduced the steady-state intracellular Na activity by 8–10 % and decreased the rate of rise of intracellular Na activity by up to 50 %. It should be noted, however, that the TTX purchased contained 5 parts by weight citrate per 1 part TTX. Thus at a TTX concentration of 2×10^{-5} g/ml. a small but significant amount of Ca^{2+} in our experimental solutions was chelated by citrate. The amount of Ca^{2+} chelated in this high TTX concentration was 0·3 mM out of a total Ca concentration of 2·0 mM (Hastings, McLean, Eichelberger, Hall & da Costa, 1934). This reduction in extracellular Ca^{2+} would tend to increase the intracellular Na activity and the net Na influx (Ellis, 1977; Deitmer & Ellis, 1978*a*), thereby partially offsetting the effects of TTX.

The TTX-sensitive Na channels of excitable tissues are known to be voltage dependent. They inactivate with increasing membrane depolarization according to

the 'h'-inactivation scheme (Hodgkin & Huxley, 1952; Weidmann, 1955; Noble, 1962). In one experiment we examined a Purkinje fibre that was in a depolarized state (E_m being -40 mV) soon after penetration of the cells by the micro-electrodes. This depolarized state was probably associated with some membrane injury. Since the membrane potential recovered to its normal high value later in this experiment,

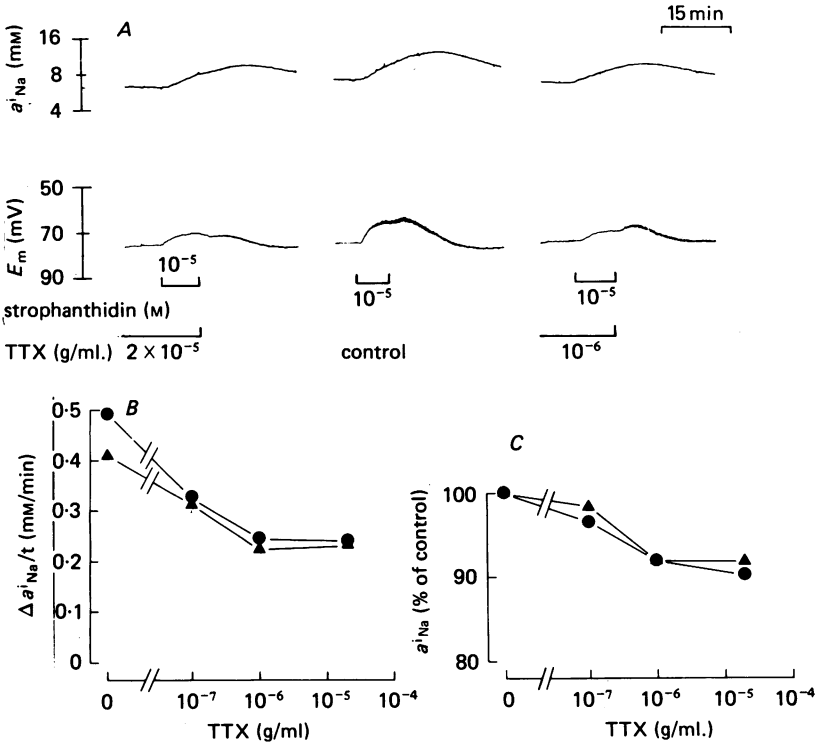

Fig. 6. The effect of various concentrations of TTX on a^i_{Na} and on the rise of a^i_{Na} on addition of 10^{-5} M-strophanthidin. *A*, pen-recordings showing the changes in a^i_{Na} (top trace) and E_m (lower trace) produced by 10^{-5} M-strophanthidin in the absence, and in the presence of three different concentrations of TTX. *B*, the rate of rise of a^i_{Na} ($\Delta a^i_{Na}/t$) on addition of 10^{-5} M strophanthidin at various TTX concentrations. Triangles and circles represent two different experiments. *C*, the steady-state a^i_{Na} levels in the presence of various TTX concentrations as a percentage of the a^i_{Na} in the absence of TTX. Triangles and circles represent the same two experiments as in (*B*).

we were able to observe the effects of TTX at low and high membrane potentials. Fig. 7 shows the beginning of this experiment, where the cells were in a depolarized state. First, the rise of intracellular Na activity during a short exposure to strophanthidin (10^{-5} M) was measured (control). When TTX (10^{-6} g/ml.) was applied, the membrane hyperpolarized within 2 min to a resting level of more than -70 mV. After another 7 min the membrane potential 'switched' spontaneously to an even more depolarized level than previously. Following the removal of both TTX and strophanthidin, the membrane regained its normal polarized level which was then maintained throughout the rest of a 9 hr experiment.

The rate of rise of the intracellular Na activity was 0·44 m-mole/min in the

control exposure (without TTX) to strophanthidin shown in Fig. 7. This rate was reduced by 27 % in the presence of 10^{-6} g TTX/ml. When the membrane had gained its normal polarized level (approximately -75 mV) later in the experiment (shown in Fig. 6*A*) the addition of strophanthidin produced a somewhat smaller rate of rise of intracellular Na activity (0·41 m-mole/min) than before. This rate was now

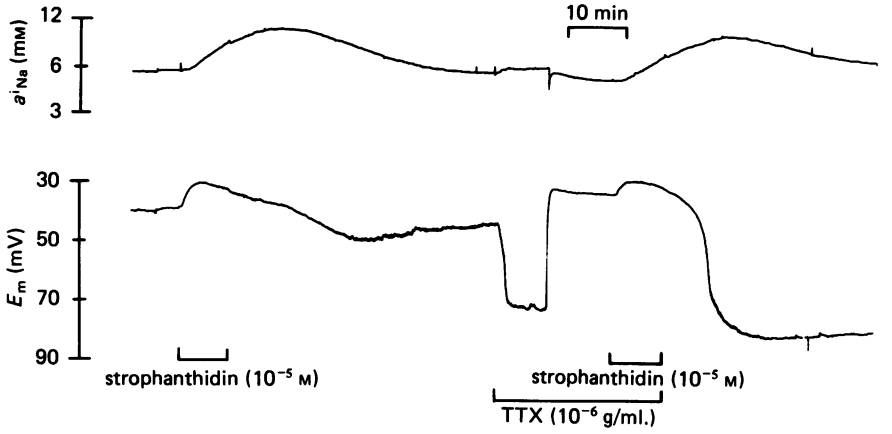

Fig. 7. The effect of 10^{-6} g TTX/ml. on the steady-state a^i_{Na} and on the rise of a^i_{Na} upon addition of 10^{-5} M-strophanthidin at a low membrane potential.

reduced in the presence of 10^{-6} g TTX/ml. by 45 %. The result indicates that TTX-sensitive Na influx was reduced by 40 % during the period in which the membrane was depolarized. This could have been produced by an increased inactivation of TTX-sensitive, voltage-dependent Na channels at low membrane potentials.

The ability of TTX to cause the membrane potential to switch to its normal polarized state is similar to the effects of TTX on canine Purkinje fibres (Gadsby & Cranefield, 1977). It would appear that closing the TTX-sensitive Na channels can decrease the Na permeability of the membrane enough to allow hyperpolarizing currents to 'switch' the membrane potential to a high level. However, as the membrane potential spontaneously depolarized subsequently, it suggests that the membrane was very near to the threshold for the 'switch' between the two polarized levels. The hyperpolarizing currents responsible for the initial switch may have been quite large if, after exposure to strophanthidin, the intracellular free Ca level was high. A high level of intracellular Ca could increase the K conductance of the cell membrane (Meech, 1974; Eckert & Lux, 1976; Isenberg, 1975). Later in the recovery from the first exposure to strophanthidin, when perhaps intracellular Ca had returned to normal and the K conductance had decreased, this may have been just sufficient to trigger the later spontaneous depolarization.

We have tried to investigate further the voltage dependence of the ability of TTX to decrease the Na influx by increasing the external potassium concentration ($[K]_o$), to depolarize the cell membrane. In one experiment the $[K]_o$ was increased from 6 to 12 mM. This increase in $[K]_o$ produced a depolarization of 12 mV. The depolarization produced by the addition of strophanthidin, however, is less at the higher $[K]_o$. The E_m in strophanthidin containing solutions was -58 mV in 12 mM-$[K]_o$ compared to -66 mV in 6 mM-$[K]_o$. Therefore there was a difference in the net depolarization of only 8 mV in the presence of 2×10^{-5} gTTX/ml. TTX reduced the rate of rise of a^i_{Na} in 6 mM-K by 49 % and in 12 mM-K by 44 %. These results are consistent with a larger effect of TTX on the net passive Na influx at the higher membrane potential. However, due to the varying depolarizations produced by strophanthidin in 6 and 12 mM-K the difference in the TTX effects were rather small. Larger changes in the $[K]_o$ were not used as high K con-

trations inhibit cardioactive steroid binding to the Na–K pump (e.g. see Schwartz, Lindenmeyer & Allen, 1975) and very low K concentrations produce unusually large depolarizations which may be due to an increased Na permeability.

The net Na influx may vary greatly with the membrane potential since both the relative Na permeability of the cell membrane and the driving force for Na inward movement would be affected. We have therefore attempted to estimate the influence of the membrane potential on the net Na influx by varying the $[K]_o$. We have described some of these experiments previously (Deitmer & Ellis, 1978*b*),

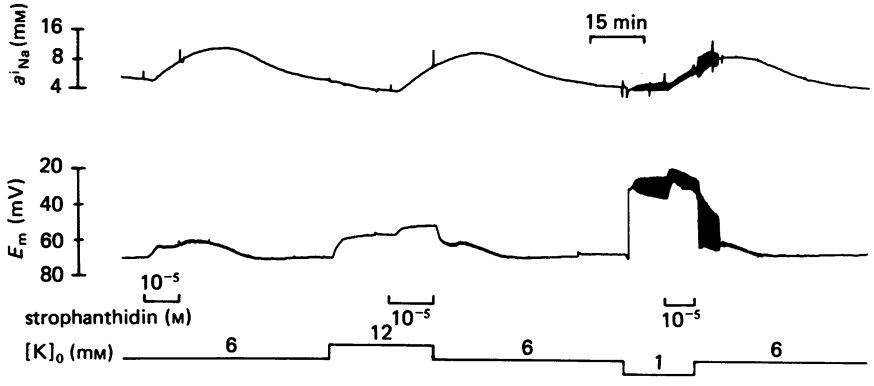

Fig. 8. The effects of different E_m (the changes being produced by alteration of the $[K]_o$) on the steady-state a^i_{Na} and on the rise of a^i_{Na} upon addition of 10^{-5} M-strophanthidin. The black areas during and shortly after the exposure to 1 mM-K_o were due to oscillations of the membrane potential and spontaneous action potentials, respectively.

where we also discussed some factors that could render this type of analysis inaccurate. One of the major problems is that binding of cardioactive steroids is affected by the $[K]_o$ (Schwartz *et al.* 1975). A significant effect might have been anticipated in these experiments when the $[K]_o$ was raised to 12 mM as this would tend to inhibit strophanthidin binding. However, the onset of the rise in intracellular Na produced by 10^{-5} M-strophanthidin was not delayed in 12 mM-$[K]_o$ when compared with that in 6 mM-$[K]_o$. A significant delay might have been expected if strophanthidin binding was the rate limiting step in our assessment of the rate of Na influx. An experiment of this type is shown in Fig. 8. The rate of rise of the intracellular Na activity in 10^{-5} M-strophanthidin was measured in 1, 6 and 12 mM-$[K]_o$. Changing the $[K]_o$ affects the steady-state intracellular Na activity by altering the rate of Na pumping (Ellis, 1977; Deitmer & Ellis, 1978*b*). Therefore care was taken to measure the rate of rise of a^i_{Na} *over the same range of* a^i_{Na} *levels*. The mean membrane potentials in these 10^{-5} M-strophanthidin-containing solutions were 64·1 mV in 6 mM-$[K]_o$, 54·3 mV in 12 mM-$[K]_o$ and 32·1 mV in 1 mM-$[K]_o$. The mean rates of rise of intracellular Na activity compared to that in 6 mM-$[K]_o$ (taken as 100 %) were 92·5 ± 8·0 % (± S.D., n = 4) and 90·7 ± 14·2 % (n = 5) in 12 and 1 mM-$[K]_o$ respectively. We have calculated the mean Na electrochemical gradients across the cell membrane at the times at which these rates were measured. If the driving force in 6 mM-$[K]_o$ is taken as being 100 % then this was reduced to 92 % in 12 mM-$[K]_o$ and to 74 %

in 1 mM-$[K]_o$. Thus the decrease in the Na driving force in 12 mM-$[K]_o$ agrees well with the observed decrease in the rate of rise of the intracellular Na activity (to 92 and 92·5 % respectively).

In 1 mM-$[K]_o$, however, the decrease in the rate of rise of intracellular Na is considerably less than that predicted from the reduction in the Na driving force (90·7 and 74 %, respectively). This deviation of the net passive Na influx and the change in Na electrochemical gradient could be accounted for by an increase in the Na permeability of the cell membrane in 1 mM-$[K]_o$. This effect is presumably even larger than is suggested by these measurements as the above results (with TTX) indicated some inactivation of the voltage- (and TTX-) sensitive Na channels at the low membrane potential, which contribute to the over-all Na permeability.

DISCUSSION

The present results show that the local anaesthetics procaine and lidocaine can produce decreases in the intracellular Na activity of quiescent sheep heart Purkinje fibres (for example, Figs. 1 and 2). It seems likely that these decreases of intracellular Na are due to a decrease in the net passive Na influx. This in turn briefly allows the Na–K pump to extrude more Na than leaks in, thus causing a reduction in the steady-state intracellular Na activity.

We have estimated the changes in the net passive Na influx by measuring the rate of rise of intracellular Na activity on inhibition of the Na–K pump with the cardioactive steroid strophanthidin. This rate of rise was 50 % reduced by procaine concentrations between 10^{-4} and 10^{-3} M. This effect could not have been due solely to interference by procaine with the binding of strophanthidin. When both drugs were applied to a preparation and then removed simultaneously, the rate of rise of intracellular Na initially increased. This suggests that procaine is washed off much more quickly than strophanthidin, and that procaine can have an effect independent of any possible interaction with the strophanthidin binding to the membrane. This is confirmed by the fact that the rate of rise of the intracellular Na activity in K-free solutions was also reduced by procaine (Fig. 1) despite the fact that the membrane potential (and therefore the driving force for inward movement of Na) was increased.

The ability of procaine to block the large depolarization in K-free solution adds further weight to the suggestion that the K-free depolarization is due to an increase in the relative permeability of Na to that of K (Carmeliet, 1961; Gadsby & Cranefield, 1977). Procaine also appears to decrease the membrane K permeability (Shanes *et al.* 1959; Taylor, 1959; Ochi & Hashimoto, 1978) which could explain the *depolarization* of the membrane with procaine concentrations above 10^{-3} M. Arnsdorf & Bigger (1972) attributed the hyperpolarization they observed in dog Purkinje fibres with low lidocaine concentrations (2×10^{-5} M) to an increased K conductance (see also Gliklick & Hoffman, 1978). These authors therefore suggest that the K permeability of cardiac tissue may be affected by local anaesthetics in a different way to that of other types of excitable cell.

Local anaesthetics have been used clinically for the treatment of various types of cardiac arrhythmia (Likoff, 1959; Frieden, 1959) including those induced by digitalis toxicity (Lown, Fakhro, Hood & Thorn, 1967) and myocardial infarction (Gianelly,

Von der Groeben, Spiwack & Harrison, 1967). The clinically effective doses are lower than those used in the present study, so our results suggest that only small effects on intracellular Na activity occur with therapeutic concentrations of local anaesthetics. Electrophysiological studies have shown that high concentrations of local anaesthetics decrease the overshoot and the rate of rise of the Purkinje fibre action potential (Weidmann, 1955; Davis & Temte, 1969; Bigger & Mandel, 1970). This 'stabilizing' effect of local anaesthetics was also apparent in the present experiments, for example, the inhibition of the spontaneous activity shown in Fig. 4.

The addition of TTX to the bathing solution produced decreases of intracellular Na activity with a similar time course to those occurring on addition of local anaesthetics. The effect of TTX on the steady-state intracellular Na activity was paralleled by its effect on the rate of rise of intracellular Na produced by the addition of 10^{-5} M-strophanthidin.

TTX has been widely used as a specific inhibitor of the voltage-dependent Na channels in a variety of excitable tissues (see Blankenship, 1976). Cardiac tissue has a rather low sensitivity to TTX inhibition (for example, Baer, Best & Reuter, 1976; Dudel *et al.* 1967). It is, however, assumed that TTX also acts by blocking similar Na channels in cardiac tissue. Our results suggest that the net passive Na influx is greatly reduced by TTX (the rate of rise of the intracellular Na activity was reduced by approximately 40 % in 10^{-6} g TTX/ml.). The membrane depolarization in these strophanthidin containing solutions was 4–9 mV, so TTX-sensitive channels may contribute even more to the net passive Na influx at the normal membrane potential, as these channels inactivate with membrane depolarization. Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, (1969) found that TTX (10^{-6} g/ml.) reduced the Na influx in squid axons by approximately 60 %. In our experiments higher concentrations of TTX did not produce larger effects on the intracellular Na and rate of rise in intracellular Na. This, however, was probably due to the fact that the TTX we used had a relatively high content of citrate buffer. The citrate would have chelated a significant amount of Ca in our experimental solutions when TTX concentrations much higher than 10^{-6} g/ml. were used.

In one experiment we were able to examine the effect of TTX on the rate of rise of the intracellular Na activity in the depolarized state and at the normal resting potential level. It seems somewhat surprising that the rate of rise of intracellular Na (in the absence of TTX) appeared to be slightly greater in the depolarized state, despite the reduction in the driving force for Na influx. It is possible that the injury caused by the micro-electrode impalement at the beginning of the experiment resulted in a transient increase in the Na influx. It seems unlikely, however, that such injury could cause a *specific* decrease in the TTX-sensitive Na influx observed at the low membrane potential. This experiment suggested that the TTX-sensitive Na influx was not completely inactivated at the membrane potential of -40 mV. The results of Weidmann (1955), however, indicate that the Na channels associated with the action potential in Purkinje fibres are fully inactivated at this membrane potential.

As there were differences in the membrane potential of up to 5 mV in the various TTX concentrations (in the presence of strophanthidin) in our experiments, this could of course significantly influence the TTX-sensitive Na influx (see also Cohen &

Strichartz, 1976). Our results concerning the TTX-sensitive Na permeability obtained by the measured rates of rise of intracellular Na activity in strophanthidin are therefore subject to an unknown error. Taking the rates of rise of internal Na at larger depolarizations in the absence and presence of TTX, this error should however be less than 20% and would only have a small effect on the 'dose–response curves' given for TTX (Fig. 6). Ideally, the membrane should have been voltage clamped during our measurements with TTX, but this technique has not yet been applied in combination with the measurement of intracellular ion activities in mammalian cardiac muscle.

In conclusion, our results show that intracellular Na activity can be decreased in the presence of local anaesthetics or TTX. The fall in intracellular Na is presumably brought about by the Na–K pump following a decrease in the passive Na influx. The larger effect of local anaesthetics in our experiments could have been due to the ability of local anaesthetics to affect both TTX-*sensitive* (voltage-dependent) and the TTX-*insensitive* Na pathways. If it is assumed that the TTX-sensitive component of the passive Na influx (as estimated by the addition of strophanthidin) reflects the contribution of voltage-dependent Na channels to the total passive Na influx, then our results indicate that at least 40% of the passive Na influx in quiescent Purkinje fibres occurs through the voltage-dependent Na channels, even at membrane potentials slightly lower than normal (due to the influence of strophanthidin).

We wish to thank Dr R. C. Thomas for his encouragement throughout the course of this work. We also thank him and Dr R. D. Vaughan-Jones for their comments on an early version of this manuscript. We are also grateful to the M.R.C. (grant to R. C. Thomas) and the Deutsche Forschungsgemeinschaft (grant to J.W.D., De 231/2) for financial support.

REFERENCES

ARNSDORF, M. F. & BIGGER, J. T. JR (1972). Effect of lidocaine hydrochloride on membrane conductance in mammalian cardiac Purkinje fibres. *J. clin. Invest.* **51**, 2252–2263.

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

BAKER, P. F., BLAUSTEIN, M. P., KEYNES, R. D., MANIL, J., SHAW, T. I. & STEINHARDT, R. A. (1969). The ouabain sensitive fluxes of sodium and potassium in squid giant axons. *J. Physiol.* **200**, 459–496.

BIGGER, J. T. & MANDEL, W. J. (1970). Effect of lidocaine on conduction in canine Purkinje fibres and at the ventricular muscle–Purkinje fibre junction. *J. Pharmac. exp. Ther.* **172**, 239–254.

BLANKENSHIP, J. E. (1976). Tetrodotoxin: from poison to powerful tool. *Perspect. Biol. Med.* **19**, 509–526.

CARMELIET, E. E. (1961). Chloride ions and the membrane potential of Purkinje fibres. *J. Physiol.* **156**, 375–388.

COHEN, I. S. & STRICHARTZ, G. R. (1977). On the voltage-dependent action of tetrodotoxin. *Biophys. J.* **17**, 275–279.

DAVIS, L. D. & TEMTE, J. V. (1969). Electrophysiological actions of lidocaine on canine ventricular muscle and Purkinje fibres. *Circulation Res.* **24**, 639–655.

DEITMER, J. W. & ELLIS, D. (1978*a*). Changes in the intracellular sodium activity of sheep heart Purkinje fibres produced by calcium and other divalent cations. *J. Physiol.* **277**, 437–453.

DEITMER, J. W. & ELLIS, D. (1978*b*). The intracellular sodium activity of sheep heart Purkinje fibres during inhibition and re-activation of the Na–K pump. *J. Physiol.* **284**, 241–259.

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

ECKERT, R. & LUX, H. D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of *Helix*. *J. Physiol.* **254**, 129–151.

ELLIS, D. (1977). The effect of external cations and ouabain on the sodium activity in sheep heart Purkinje fibres. *J. Physiol.* **273**, 211–240.

FRIEDEN, J. (1959). Antiarrhythmic drugs. VII. Lidocaine as an antiarrhythmic agent. *Am. Heart J.* **70**, 713–715.

GADSBY, D. C. & CRANEFIELD, P. F. (1977). Two levels of resting potential in cardiac Purkinje fibres. *J. gen. Physiol.* **70**, 725–746.

GIANELLY, R., VON DER GROEBEN, J. O., SPIWACK, A. P. & HARRISON, D. C. (1967). Effect of lidocaine on ventricular arrhythmias in patients with coronary heart disease. *New Engl. J. Med.* **277**, 1215–1219.

GLIKLICH, J. I. & HOFFMAN, B. F. (1978). Sites of action and active forms of lidocaine and some derivatives on cardiac Purkinje fibres. *Circulation Res.* **43**, 638–651.

HASTINGS, A. B., MCLEAN, F. C., EICHELBERGER, L., HALL, J. L. & DA COSTA, E. (1934). The ionisation of calcium, magnesium and strontium citrates. *J. biol. Chem.* **107**, 351–370.

HILLE, B. (1973). Potassium channels in myelinated nerve. Selective permeability to small cations. *J. gen. Physiol.* **61**, 669–686.

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

ISENBERG, G. (1975). Is potassium conductance of cardiac Purkinje fibres controlled by $[Ca^{2+}]_i$? *Nature, Lond.* **253**, 273–274.

LIKOFF, W. (1959). Cardiac arrhythmias complicating surgery. *Am. J. Cardiol.* **3**, 427–429.

LOWN, B., FAKHRO, A. M., HOOD, W. B. & THORN, G. W. (1967). The coronary care unit. *J. Am. med. Ass.* **199**, 188–198.

MEECH, R. W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol.* **237**, 259–277.

NARAHASHI, T., MOORE, J. W. & SCOTT, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. gen. Physiol.* **47**, 965–974.

NOBLE, D. (1962). A modification of the Hodgkin–Huxley equations applicable to Purkinje fibre action and pacemaker potentials. *J. Physiol.* **160**, 317–352.

OCHI, R. & HASHIMOTO, K. (1978). The effect of procaine on the passive electrical properties of guinea-pig ventricular muscle. *Pflügers Arch.* **378**, 1–7.

SCHWARTZ, A., LINDENMAYER, G. E. & ALLEN, J. C. (1975). The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects. *Pharmac. Rev.* **27**, 3–134.

SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H. & AMATNIEK, E. (1959). Anaesthetic and calcium action in the voltage clamped squid axon. *J. gen. Physiol.* **42**, 793–802.

STRICHARTZ, G. R. (1976). Molecular mechanisms of nerve block by local anaesthetics. *Anaesthesiology* **45**, 421.

TAYLOR, R. E. (1959). Effect of procaine on electrical properties of squid axon membrane. *Am. J. Physiol.* **196**, 1071–1078.

THOMAS, R. C. (1970). New design for sodium-sensitive glass micro-electrodes. *J. Physiol.* **210**, 82–83*P*.

THOMAS, R. C. (1978). *Ion-sensitive Intracellular Microelectrodes: How to Make and Use Them.* London: Academic Press.

WEIDMANN, S. (1955). Effect of calcium ion and local anaesthetics on electrical properties of Purkinje fibres. *J. Physiol.* **129**, 568–582.