Br. J. Pharmac. (1987), **92**, 77–86

The effects of external and internal application of disopyramide on the ionic currents of the squid giant axon

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1 The actions of the class I anti-arrythmic agent, disopyramide, on the ionic currents of the voltage-clamped squid axon have been investigated, by use of both extra-axonal and intra-axonal routes of application.

2 Extra-axonal application of 0.1 mM disopyramide produced no significant effects on the membrane currents. External disopyramide at 1.0 mM caused small, poorly reversible inhibition of both sodium and potassium currents. This block was use-dependent and was enhanced by use of test stimuli to more positive membrane potentials.

3 Intra-axonal application of 0.1 mM disopyramide caused a 40% reduction in the first-pulse sodium current (tonic block) and an additional use-dependent block. Analysis of first-pulse currents in terms of the Hodgkin-Huxley formalism indicated that the block resulted mainly from a reduction in the maximum available sodium conductance (g_{Na}); there were no effects on the voltage dependence of the steady-state activation and inactivation parameters, m_∞ and h_∞.

4 The use-dependent actions of disopyramide were investigated with a double voltage-clamp pulse protocol. The significant use-dependent effects of the drug were a further reduction in g_{Na} and an increase in the time constant of inactivation (τ_h).

5 Disopyramide appears to enter a blocking site in the sodium channel which is only readily accessible from the axoplasmic phase. Partition to the site depends on membrane voltage and on the state of the channel gates. Disopyramide binds at a significant rate to both open and inactivated forms of the sodium channel.

Introduction

There is a close relationship between the class I anti-arrhythmic agents and local anaesthetics. This is based on the observation that both types of drug inhibit the fast sodium current in cardiac cells and in nerve (Singh & Vaughan-Williams, 1971; Edwards *et al.*, 1976; Hille, 1978; Gintant & Hoffman, 1984). It has been suggested that common molecular mechanisms may be involved for both types of drug and in both tissues (Hondeghem & Katzung, 1977). The clinical anti-arrhythmic actions of disopyramide have in part been related to effects on the fast sodium current of cardiac cells (Danilo *et al.*, 1977). The relative simplicity of axonal geometry means that voltage-clamp data on the sodium current in nerve are considerably more accurate than corresponding data for cardiac cells. Accordingly it has often proved more satisfactory to use results of experiments on nerve to formulate hypotheses concerning the mechanisms of sodium current inhibition. The experiments presented here were designed to investigate the actions of disopyramide on the ionic currents of the squid giant axon.

It has been proposed that local anaesthetics and class I anti-arrhythmics may act by binding to a site within the sodium channel; it is further suggested that for cationic agents the site is only accessible from the intracellular aqueous phase (Hille, 1977; 1978; Hondeghem & Katzung, 1977). For a number of substances, e.g. lignocaine and derivatives, there is evidence that binding to this blocking site is a function of membrane voltage and of the state of the channel gates (Hille, 1977; Kendig *et al.*, 1979). One widely accepted view is that local anaesthetics bind very slowly, if at all, to the inactivated form of the sodium channel but that open channels that bind

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anaesthetic are more likely to transform to the inactivated state. Local anaesthetic binding therefore stabilizes the inactivated form of the channel (Hille, 1977). The inhibition of the cardiac sodium current caused by disopyramide is enhanced by positive membrane voltages and it has been suggested that disopyramide also interacts in some way with the inactivated form of the sodium channel (Danilo *et al.*, 1977; Hondeghem & Katzung, 1984).

The squid giant axon is well-suited to a detailed investigation of the foregoing suggestions, since drugs can be applied at known concentrations to either side of the membrane and accurate voltage-clamped records obtained. In the present study disopyramide has been applied intra- and extra-axonally to investigate the hypothesis of a binding site accessible to only one side of the membrane. Use-dependent inhibition of the sodium current does occur and this has been investigated with a double voltage-clamp pulse protocol (Cahalan, 1978; Elliott *et al.*, 1985). A preliminary account of part of this work has been given (Elliott & Hendry, 1984).

Methods

Giant axons were dissected from the mantles of freshly killed *Loligo forbesi*. The axons were then finely cleaned and were usually between 600 and 1000 μm in diameter. Both intact axons and axons in which the potassium currents had been suppressed by internal perfusion with solutions containing caesium fluoride, (CsF) were used. All experiments involving intra-axonal application of disopyramide were performed on CsF-perfused axons.

The external bathing solution for intact axons contained (concentrations in mM): NaCl 430, KCl 10, $CaCl_2$ 10, $MgCl_2$ 50, Trizma base 10. The pH was adjusted to 7.4 by the addition of HCl. This solution will be referred to as full-sodium artificial sea water (full-Na ASW). For CsF-perfused axons the external NaCl concentration was reduced to 107.5 mM and 322.5 mM choline chloride added (1/4-Na ASW). Sodium currents were suppressed where necessary (ie to obtain leakage currents) by addition of 0.3 μM tetrodotoxin (TTX). The internal perfusate contained (mM): CsF 345, sucrose 400, NaCl 5, HEPES 10. The pH was adjusted to 7.3 by addition of Cs_2CO_3.

Details of the chamber in which the axons were mounted, the electrodes and the means of introducing the external bathing solution and controlling its temperature have been described previously (Haydon *et al.*, 1980). Briefly, the internal electrode was of the 'piggy-back' type and consisted of a platinized platinum-iridium wire 75 μm in diameter mounted on a 100 μm glass capillary containing 0.5 M KCl and an electrically floating 50 μm platinum-iridium wire to reduce impedance. The external current-carrying electrodes were platinized platinum sheets consisting of a central collecting area and two flanking guard areas. Potential differences were measured between the internal 100 μm capillary and an external pipette containing artificial sea water. Electrical connections to these internal and external pipettes were through chloridized silver wires. External solutions were delivered through a heat exchanger at flow rates in excess of 10 ml min^{-1}. Internal perfusion was by a modification of the Tasaki technique, as described by Haydon & Kimura (1981). The perfusion capillary had an external diameter of ca. 450 μm. When changing the internal perfusate from a control to a test solution the axon was reperfused by at least two insertions of the capillary. The voltage-clamp and data acquisition procedures were as in Kimura & Meves (1979) and the numerical analysis of the sodium currents were as described by Haydon & Kimura (1981). The double pulse protocol employed to investigate use dependence was as in Elliott *et al.* (1985).

Compensation for ca. 95% of the series resistance was applied in all experiments. Consistent with this, it was shown that when the sodium current was reduced by 50% in tetrodotoxin (TTX) solution the shift in the current-voltage curve was ca. 1 mV. The experiments were carried out at 6 ± 1°C. Disopyramide phosphate was kindly donated by Cassenne Limited. It was established that addition of disopyramide to the aqueous solutions did not cause a significant alteration in pH.

Results

External application of disopyramide to intact axons

The resting potentials of the intact axons used were between −50 and −60 mV. The axons were voltage-clamped at −60 mV. Before the 15 ms test depolarization, a 50 ms pre-pulse to −80 mV was applied to remove fast sodium current inactivation. The test pulse for measurement of maximum inward sodium current (I_P) was always that which gave the maximum current under control conditions. This pulse was to a membrane potential of −10 or 0 mV.

The squid axon ionic currents were relatively insensitive to extra-axonal application of disopyramide. The class I anti-arrhythmic actions of disopyramide in canine cardiac Purkinje fibres occur at aqueous concentrations in the range of 0.01 to 0.1 mM (Danilo *et al.*, 1977). External application of 0.1 mM disopyramide to an intact squid axon for 30 min produced no significant effects on voltage-clamped sodium and potassium currents. External application at 1 mM produced small reductions in the sodium current. I_P was reduced by ca. 15% over 25 min

exposure. This effect was only partially reversible. The membrane potential eliciting maximum inward current and the voltage-dependence of steady-state sodium current inactivation (h_∞) were not affected by the drug.

External disopyramide at 1.0 mM induced a use-dependence in the ionic currents of intact axons which was only clear when highly depolarizing test pulses were used. An example of this behaviour is shown in Figure 1. The records show membrane current responses to 15 ms steps to a membrane potential of 120 mV. At this test voltage both sodium and potassium currents were outward. Figure 1a shows the current responses obtained in control conditions by applying three stimuli at a frequency of 10 Hz. The early peak (arrowed) represents the outward sodium current and the three control traces coincide at the peak. Figure 1b shows the data obtained after 20 min exposure to 1.0 mM external disopyramide. The three voltage pulses produced successively smaller sodium current responses. Reversal of this action is shown in Figure 1c. The records in Figure 1 also show that disopyramide caused a droop and enhanced use-dependence in the maintained outward (potassium) currents. This was a reproducible finding and the droop became more pronounced as the test pulse was made more positive.

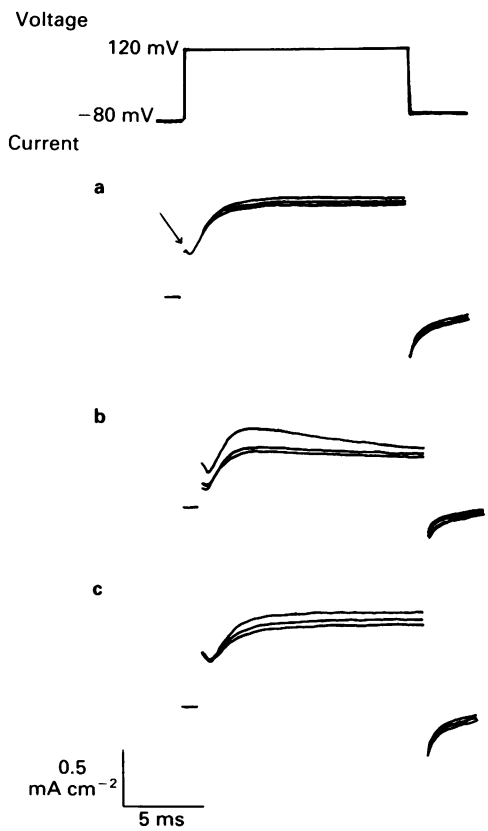

Figure 1 Use-dependence produced by 1.0 mM disopyramide applied externally to an intact squid axon in full-Na artificial sea water. The upper diagram shows the test voltage pulse used. These pulses were imposed in groups of three at 10 Hz. The lower diagrams show the superimposed membrane current responses to a burst of three stimuli in control conditions (a), in the presence of 1.0 mM external disopyramide (b) and after recovery (c). The peak of the early outward (sodium) current is indicated by an arrow in (a).

Disopyramide applied to CsF-perfused axons

CsF-perfused axons were voltage-clamped at −70 mV and a 50 ms pre-pulse to −90 mV was employed. The test depolarizing stimuli were of 15 ms duration. The final records in each experiment were obtained in the presence of TTX and these TTX-insensitive currents were subtracted prior to the quantitative analysis of sodium currents.

The analysis was performed as in earlier work (Haydon & Urban, 1983; Elliott *et al.*, 1985) according to equations derived from the relationships of Hodgkin & Huxley (1952). Thus effects on the sodium current may be separated into effects on the individual Hodgkin-Huxley parameters. Those considered were: the maximum membrane sodium conductance (g_{Na}), the steady-state activation and inactivation parameters (m_∞ and h_∞) and the time constants of activation and inactivation (τ_m and τ_h). The steady-state inactivation parameter was determined by applying 50 ms pre-pulses at various potentials followed by a constant test pulse chosen to give the maximum sodium current (I_P).

As for intact axons, 0.1 mM external disopyramide applied for 30 min had no significant effect on the sodium currents of CsF-perfused axons. Figure 2 shows the peak sodium current as a function of membrane potential for an axon before and during external application of 0.1 mM disopyramide. The potential which elicits the maximum inward current and the potential for zero current are unaffected by external application. None of the Hodgkin-Huxley parameters were significantly affected.

Disopyramide was considerably more effective in reducing the sodium current when applied inside the axon. Figure 3a shows the current-voltage relationships for a CsF-perfused axon in control conditions and in the presence of 0.1 mM internal disopyramide. Control data (open circles) are compared with test data obtained using a delay of 7 s between stimuli (closed circles). This delay was used to minimize accumulation of use-dependent block in the presence

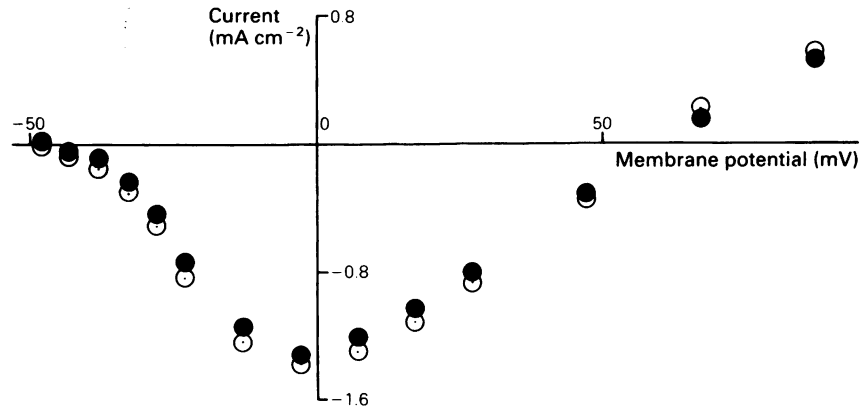

Figure 2 Membrane current-voltage relationships for the sodium current in a CsF-perfused axon in 1/4-Na artificial sea water, under control conditions (○) and in the presence of 0.1 mM external disopyramide (●). Inward current is represented by negative values. In this and subsequent plots against membrane potential, each data point has been offset by −3 mV on the voltage axis to compensate for the measured junction potential between 1/4 Na ASW and the perfusion fluid.

of disopyramide and data obtained in this manner will be referred to as first-pulse data. Figure 3a shows a significant first-pulse inhibition of sodium current by internal disopyramide. This was the result of a reduction in the maximum available sodium conductance ($\bar{g}_{Na}$). For three axons the mean reduction of g_{Na} (first pulse) by 0.1 mM disopyramide was to 0.62 of control values. There was no significant shift in the voltage dependence of activation and no effect on h_∞, τ_m or τ_h. Figure 3b shows the voltage-dependence of h_∞ for first-pulse currents under control conditions and in the presence of 0.1 mM internal disopyramide.

Internal application of the drug caused the sodium current to become strongly use-dependent. Stimulation at frequencies of 1–5 Hz caused an accumulation of block in the presence of internal disopyramide which was not present under control conditions. A quantitative analysis of this use-dependence was attempted with a double-pulse protocol. A full description of the protocol is given in Elliott *et al.* (1985) and the pulse sequence is illustrated in Figure 4a. The holding potential was −70 mV and the first voltage pulse (P1) was a depolarizing step of ΔV_1 mV which lasted t_1 ms. There was then a delay of d ms at the holding potential followed by a second depolarizing pulse (P2) of ΔV_2 mV lasting 15 ms. The first pulse was a conditioning step prior to the measurement of current elicited by P2.

Experiments were performed to measure the peak second-pulse current ($I_{P,2}$) as a function of t_1. ΔV_1 was set at 160 mV, ΔV_2 was 60 mV and d was 50 ms. Figure 4b shows the effect on $I_{P,2}$ of varying t_1 from 0 to 70 ms in control conditions and in the presence of 0.1 mM internal disopyramide. The line drawn through the test data represents a single exponential with a time constant of 26.9 ms. This relationship provides a good fit for $t_1 > 2$ ms. Similar results were obtained in 4 axons, giving a mean fitted time constant of 23.9 ms (range 20.3 to 26.9 ms). In all cases the form of the deviation from a simple exponential relationship at $t_1 < 2$ ms was as in Figure 4b.

A membrane current-voltage relationship for the second-pulse current was obtained (as in Elliott *et al.*, 1985) by varying ΔV_2 while holding the other double-pulse parameters constant. An example of this is shown in Figure 3a. These data (open triangles) were obtained in the presence of 0.1 mM disopyramide with t_1 set at 20 ms, d at 50 ms and ΔV_1 at 160 mV. Figure 3a therefore shows both first-pulse block and the additional effect of use-dependent block. In Hodgkin-Huxley terms the use-dependent inhibition of current was due to a further reduction in $\bar{g}_{Na}$. The mean reduction in g_{Na}, measured as the ratio of second-pulse to first-pulse values in the presence of 0.1 mM internal disopyramide was 0.68 (4 axons, range 0.58 to 0.78). The prior depolarizing pulse had no significant effect on m_∞ or τ_m whether or not disopyramide was present. Analysis of second-pulse currents showed that in the presence of disopyramide, τ_h was increased compared to first-pulse values. An example of this is shown in Figure 5 which presents τ_h data from the same axon as in Figure 3 and with the same double-pulse parameters. As mentioned previously, the presence of 0.1 mM internal disopyramide had very little effect on first-pulse τ_h values. In the absence of drug, the first-pulse and second-pulse τ_h values were not significantly

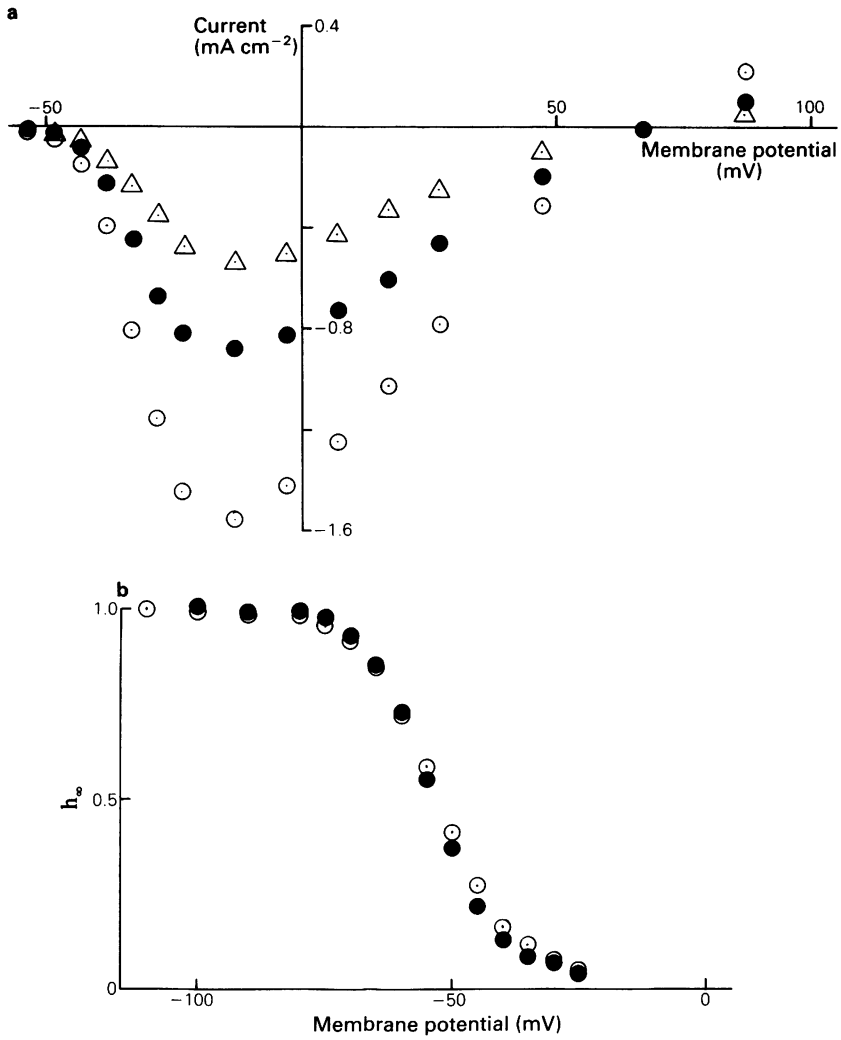

Figure 3 (a) Membrane current-voltage relationships for the sodium current in a CsF-perfused axon in 1/4-Na artificial sea water under control conditions and in the presence of 0.1 mM internal disopyramide. Control data (○) are compared with first-pulse data (7 s intervals between stimuli) obtained in the presence of disopyramide (●). The second-pulse data (△) were obtained in the presence of disopyramide using the double-pulse protocol illustrated in Figure 4a. The second-pulse data are a plot of $I_{P,2}$ as a function of the voltage during the second pulse ($V_{P,2}$). The holding potential was −70 mV, t_1 was 20 ms, d was 50 ms and ΔV_1 was 160 mV. In the absence of disopyramide there was no significant difference between first- and second-pulse currents. (b) The voltage-dependence of steady-state inactivation (h_∞) in first-pulse sodium currents in control conditions (○) and in the presence of 0.1 mM internal disopyramide (●). Same axon as in (a).

different. The mean increase in τ_h in the presence of internal disopyramide at a membrane potential of 0 mV, expressed as a ratio of second- to first-pulse results, was 1.20 (4 axons, range 1.15 to 1.23).

To investigate the effects of varying ΔV_1 on $I_{P,2}$, the holding potential was changed from −70 to −100 mV and ΔV_2 was increased from 60 to 90 mV to maintain the potential during P2 at −10 mV. Figure 6 shows data obtained in the presence of 0.1 mM internal disopyramide. The peak current produced by pulse 2 ($I_{P,2}$) is plotted as a function of the voltage during pulse 1 ($V_{P,1}$). The data indicate that $I_{P,2}$ had a maximum at

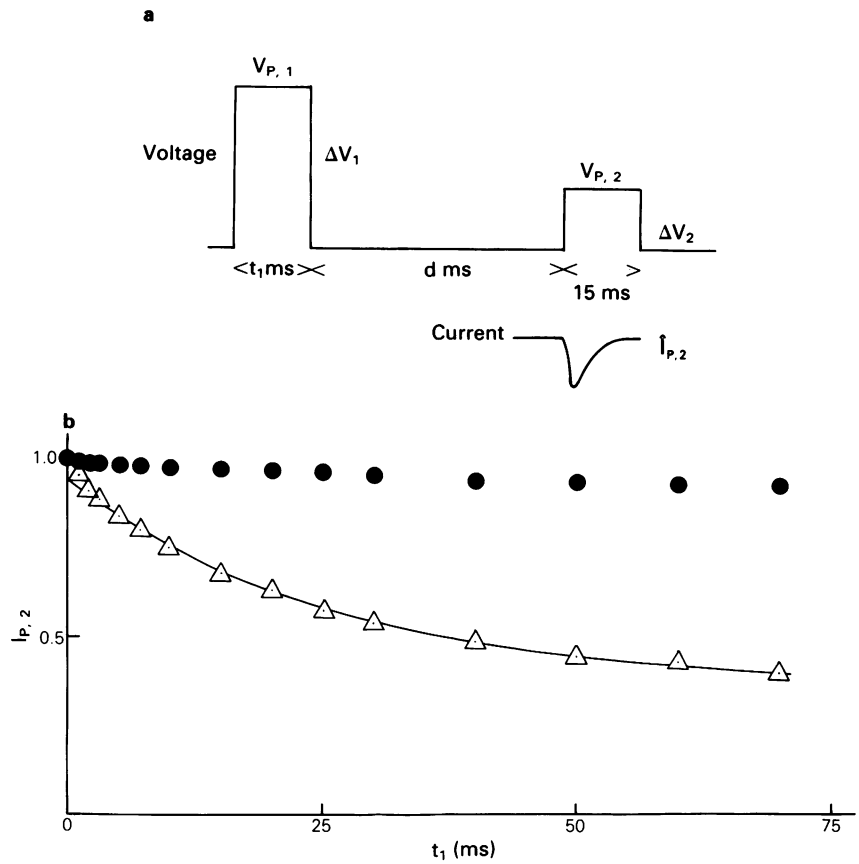

Figure 4 (a) A schematic diagram of the double-voltage-pulse protocol used to investigate use dependence. P1 is of magnitude ΔV_1 and duration t_1. P2 occurs after a delay (d) and is of magnitude ΔV_2 and duration 15 ms. The sodium current elicited by P2 was monitored and is termed I_2. The peak value of I_2 is designated $I_{P,2}$. (b) The effects of varying t_1 on the maximum inward sodium current produced by the second pulse ($I_{P,2}$) in a CsF-perfused axon in 1/4-Na artificial sea water, under control conditions (○) and in the presence of 0.1 mM internal disopyramide (△). $I_{P,2}$ is normalised and expressed as a fraction of its value for $t_1 = 0$ ms. ΔV_1 was 160 mV, ΔV_2 was 60 mV and d was 50 ms. The holding potential was −70 mV. The line shown is a single exponential of time constant 26.9 ms. Same axon as in Figure 3.

$V_{P,1} = -40$ mV. For $V_{P,1} > 0$ mV the values of $I_{P,2}$ clearly decrease as $V_{P,1}$ increases. This did not occur in control axons (Elliott *et al.*, 1985). The results obtained in 4 axons showed quantitative variability but all had the same general form.

Recovery from the drug-induced, use-dependent block took place over a period of seconds. Checks were performed to establish the accumulation of block during an experiment using 7 s intervals between test stimuli. One such check was performed during the experiment to obtain data shown in Figure 3. The value of $V_{P,2}$ was varied in steps from −50 to 90 mV with 7 s intervals between test stimuli. After a further 7 s the stimulus was repeated with $V_{P,2}$ set at −10 mV. The value of $I_{P,2}$ had declined by about 10% compared with the earlier result for −10 mV. Similar results were obtained from other checks and the data given here can be regarded as accurate to within about 10%. Delays between stimuli longer than 7 s were not used because the time taken to obtain the necessary data then became prohibitively long. The kinetics of recovery from use-dependent block were not investigated in detail. Increasing the delay between double-pulse stimuli from 50 ms to 2 s reduced the use-dependent block by ca. 50%.

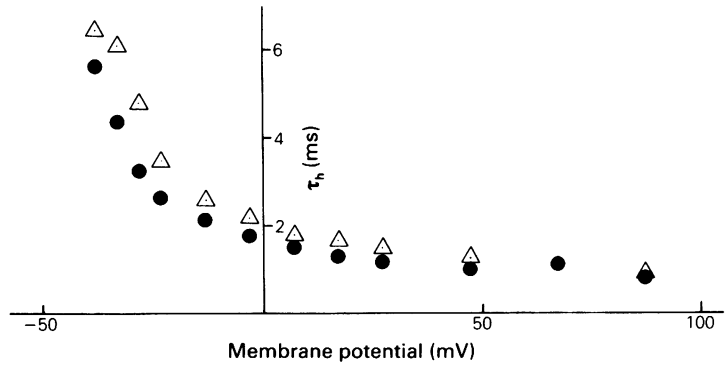

Figure 5 A comparison of τ_h values obtained by analysis of first-pulse (●) and second-pulse (△) currents in the presence of 0.1 mM external disopyramide from a CsF-perfused axon in 1/4-Na artificial sea water. The set parameters are as for Figure 3 and the currents analysed are those whose maxima are plotted in Figure 3. Same axon as in Figure 3.

Discussion

Asymmetrical membrane-sensitivity to disopyramide

Certain features of the results for intact axons suggested that disopyramide was acting on the axonal membrane from the intracellular aqueous phase. Externally applied disopyramide appeared inactive at 0.1 mM. This is unlikely to be the result of a barrier to its diffusion to the axonal membrane as tetrodotoxin at 0.3 μM reaches the membrane within a few minutes. The high concentration (~1 mM) of external disopyramide and the prolonged incubation times required to affect the ionic currents could be related to a requirement for the drug to cross the axonal membrane to reach its site of action, Disopyramide is predominantly in cationic form at pH 7.0–7.4 and the cell membrane presents a significant barrier to the diffusion of cations. The effects of 1.0 mM external disopyramide were enhanced by positive membrane potentials; this observation is consistent with a model in which cationic disopyramide is driven from the axoplasmic phase to blocking sites within the membrane by the transmembrane voltage gradient.

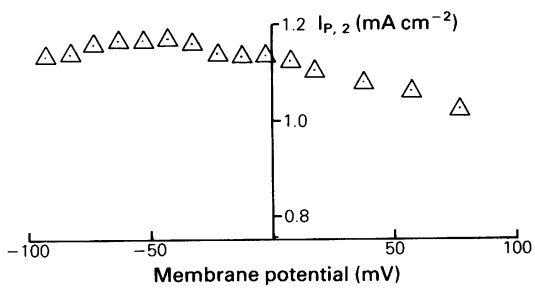

Figure 6 The relationship between the membrane voltage during the first pulse ($V_{P,1}$) and the maximum sodium current produced by the second pulse ($I_{P,2}$) in the presence of 0.1 mM internal disopyramide. The holding potential was −100 mV, d was 50 ms, ΔV_2 was 90 mV and t_1 was 25 ms. The axon was perfused with a solution containing CsF and the external solution was 1/4-Na artificial sea water.

These ideas of an asymmetry in the axonal membrane-sensitivity to disopyramide were supported by the experiments on CsF-perfused axons. Such axons were also insensitive to 0.1 mM external disopyramide but were significantly affected by internal application of the same concentration of drug. These actions of disopyramide are similar to those of cationic derivatives of local anaesthetics such as QX 222 and QX 314 (Strichartz, 1973; Hille, 1977; Cahalan, 1978) and to the actions of *n*-dodecyltrimethylammonium ions (C_{12}TMA) (Elliott *et al.*, 1985). These substances all appear to act at the internal surface of the axonal membrane and are thought to enter a blocking site within the sodium channel pore itself. The molecular basis of the actions of disopyramide may well be similar and this question will be discussed further below.

The insensitivity of the axon to external disopyramide at concentrations that are known to affect cardiac cells (Danilo *et al.*, 1977) raises some doubts as to the relevance of peripheral nerve experiments to the study of the pharmacological cardiac actions of disopyramide. It is of interest that the cationic substances QX 222 and QX 314, which are inactive when applied outside peripheral nerve axons, do inhibit cardiac sodium currents following simple extracellular

application (Strichartz, 1973; Gintant & Hoffman, 1984). It is possible that cardiac sodium channels are affected through a different site of action, or that the same site is involved in both cardiac and nerve cells but in cardiac tissue the site is directly accessible to large cations from the extracellular aqueous phase. Such hypotheses cannot be ruled out but appear unlikely in view of the very similar nature of the sodium current inhibition in the two types of cell. For example, cardiac sodium current block exhibits use- and voltage-dependence which closely resembles that seen in nerve and which suggests a similar mechanism of block via intracellular cations moving to an intrachannel site (Gintant & Hoffman, 1984; Hondeghem & Katzung, 1984). Another explanation for the different sensitivities of cardiac cells and nerve axons to the effects of external disopyramide is that the cardiac cell membrane is more permeable to amphipathic cations than the nerve membrane. If this is so, then disopyramide, QX 222 and QX 314 may enter the cardiac cell and then block sodium channels in a use- and voltage-dependent manner from the cytoplasmic phase. On this model the final molecular mode of action may be similar in both cardiac cells and peripheral nerve.

The molecular mode of action of disopyramide

Several studies of the actions of disopyramide on the cardiac action potential have been reported (Danilo *et al.*, 1977; Kus & Saszniuk, 1978; Kojima *et al.*, 1982; Yamada *et al.*, 1982). These studies have involved measurement of monophasic action potentials by intracellular electrodes. At concentrations of 0.01 to 0.1 mM disopyramide reduces the maximum rate of rise of the action potential ($\dot{V}_{max}$). Effects on action potential duration have also been reported and disopyramide shortens the plateau phase and lengthens the terminal phase (Kus & Saszniuk, 1978). The effects on $\dot{V}_{max}$ tend to be increased in cardiac cells depolarized by increased extracellular potassium concentrations (Danilo *et al.*, 1977; Kojima *et al.*, 1982). These actions of disopyramide are also use-dependent in that at stimulation rates above 1 Hz the reduction in $\dot{V}_{max}$ increases as the stimulation rate is increased (Kojima *et al.*, 1982). Precise quantitative interpretation of these results is difficult as they were not obtained from voltage-clamped cells. Nevertheless the phenomenon of dysopyramide-induced sodium current inhibition in cardiac cells appears similar to that seen in the experiments reported here using internal application to squid axons.

An explanation for the use- and voltage-dependent actions of intracellular amphipathic cations on the sodium current has been proposed by a number of authors (Strichartz, 1973; Hille, 1977; Hondeghem & Katzung, 1977; Kendig *et al.*, 1979). Widely known as the modulated receptor hypothesis, its major feature is that the cation enters a blocking site within the sodium channel which is only accessible from the intracellular aqueous phase. Binding to the site is facilitated when the channel is in the open state and occurs either very slowly or not at all when the channel is in the inactivated or closed states. The site is within the membrane and so the cation traverses part of the transmembrane voltage gradient when binding occurs. Accordingly the rates of binding and of dissociation are dependent on membrane voltage. The overall effect is that channel block occurs most readily in the presence of open channels at positive membrane voltages. Most of the results for disopyramide described here can be explained using a slightly modified version of this model.

The present data suggest a modification to the model to account for the observation that disopyramide appears to bind to both the inactivated and open channels. The results given in Figure 4b are consistent with binding of disopyramide to sodium channels which (at a membrane potential of 90 mV) are predominantly in the inactivated state. The observed deviation from a single exponential fit at $t_i < 2$ ms suggests that binding to the open form of the channel is faster than that to the inactivated channel. Nevertheless, internal disopyramide at 0.1 mM appears to bind to inactivated sodium channels with a time constant of about 25 ms. Similar results have been reported by Elliott *et al.* (1985) for the effects of *n*-dodecyltrimethylammonium cations applied inside the squid axon. Recent work concerning the actions of lignocaine on the sodium channel of rat ventricular cardiac cells also suggests a significant rate of binding to inactivated channels (Sanchez-Chapula *et al.* 1983).

One interesting question concerns the extent to which the sodium current inhibition caused by disopyramide can be relieved by increasingly negative voltage pre-pulses. Figure 3b shows that the steady-state voltage dependence of sodium current inactivation (h_∞) was not affected by internal disopyramide; in other words the block caused by disopyramide was not relieved by negative pre-pulses. This behaviour is different from that of lignocaine which is reported to affect the voltage-dependence of h_∞. A central feature of the modulated receptor hypothesis is that local anaesthetic binding stablizes the inactivated form of the sodium channel such that the voltage-dependence of h_∞ for bound channels is moved in the hyperpolarizing direction compared to that for unbound channels (Hille, 1977). It is difficult to obtain direct evidence concerning the inactivation process for bound channels but the experiments described here are not obviously consistent with the hypothesis that disopyramide stabilizes the inactivated state or causes any shift in the voltage-dependence of inactivation.

The present data do, however, indicate an effect of

disopyramide on the rate of inactivation. The data shown in Figure 5 demonstrate that second-pulse τ_h values were increased by disopyramide. Elliott *et al.* (1985) showed similar actions of *n*-decyl- and *n*-dodecyltrimethylammonium cations. Such effects are rather complex but if each channel which binds an amphipathic cation is thereby blocked (i.e. has a conductance of zero) it is difficult to explain the observed alteration in the rate of inactivation of the current elicited by the second pulse. This difficulty arises from the natural assumption that the channels giving rise to this current do not (on the above model) have bound cations and so might be expected to behave 'normally' in their voltage- and time-dependent gating. One hypothesis to explain such increases in second-pulse τ_h was proposed by Elliott *et al.* (1985). On this model the binding of an amphipathic cation does not immediately produce a channel conductance of zero. On the contrary, the bound channel can still move between resting, open and inactivated states with normal kinetics and the bound open state has a conductance equal to the unbound open state. The bound open state does, however, transform rapidly and reversibly into a blocked state. This blocked state cannot inactivate or enter the resting state directly but only through the bound open state. This model explains the increase in second-pulse τ_h as the channels which give rise to the second-pulse current may have bound cations and may enter a blocked state in which the channel gates are open but from which direct inactivation cannot occur.

The clinical actions of disopyramide are not all accounted for by its actions on the fast inward sodium current. It also affects the cardiac action potential duration and has anti-cholinergic properties (Danilo *et al.*, 1977; Heel *et al.*, 1978). This lack of specificity is evident in its actions on the squid giant axon. In Figure 1 the use-dependent actions on the transient sodium current were shown concurrent with a similar action on the delayed potassium current. The sensitivity of the two currents to disopyramide appear to be similar. Disopyramide may prove to exert rather non-selective blocking actions on a wide variety of ion channels.

We thank Professor D.A. Haydon for numerous helpful discussions. J.R.E. acknowledges financial support from the M.R.C. B.M.H. was an M.R.C. Training Fellow.

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(Received December 17, 1986.
Revised April 24, 1987.
Accepted May 13, 1987.)