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A kinetic analysis of the endplate ion channel blocking action of disopyramide and its optical isomers

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1 The effects of the antiarrhythmic agent disopyramide was studied on responses from voltage-clamped endplates at the neuromuscular junction of the garter snake.

2 Disopyramide reduced endplate current amplitude and decay time constant in a concentration- and voltage-dependent manner. Endplate current decays remained monophasic in the presence of the drug. These results were interpreted in terms of the drug blocking the open form of the acetylcholine receptor-ion channel complex.

3 Disopyramide produced a greater reduction of the amplitude of endplate currents than of miniature endplate currents. The reduction in miniature endplate current amplitude was not voltage-dependent. Analysis of endplate current driving functions showed that this was due to the rapid occurrence of channel block during the rising phase of the endplate current. The residual reduction, apart from that produced by channel block, is most probably due to receptor block.

4 Disopyramide had a voltage-dependent blocking rate constant of about 10^7 M^{-1} s^{-1} at -90 mV. The unblocking rate constant was estimated from the results of experiments using paired iontophoretically applied pulses of acetylcholine. This value was again voltage-dependent and approximately 1 s^{-1}.

5 The actions of the (+)- and (−)-stereoisomers of disopyramide on endplate current decay were identical, indicating that the channel binding site at the neuromuscular junction is not stereoselective.

Introduction

Recently it has been reported that the antiarrhythmic agent disopyramide is capable of producing ganglion and neuromuscular blockade, albeit in high concentrations (Byrne *et al.*, 1981; Healy *et al.*, 1981). The neuromuscular block produced by disopyramide appears to be mainly postjunctional in origin, the compound produces non-parallel rightwards shifts of dose-response curves to agonists and the twitch block cannot be relieved by anticholinesterase administration (Healy *et al.*, 1981; Jones & Marshall, 1987). On the basis of these results in contracting preparations, we chose to study the effects of the drug at voltage-clamped endplates (Jones & Marshall, 1987). These studies showed that disopyramide produced a concentration- and voltage-dependent reduction in endplate current time constant suggesting that the compound was acting by blocking the open form of the endplate receptor-ion channel complex.

Disopyramide is a non-quaternary compound possessing one tertiary amine nitrogen. The pK_a value of the amine nitrogen of disopyramide is 9.7, and thus it will be almost totally ionized at physiological pH levels. The substance has one asymmetric centre and hence two optical isomers. The clinically used form of disopyramide is the racemate and this form has been used in the previous studies performed at the neuromuscular junction. The work described here represents a study of the kinetics and voltage-dependence of the channel blocking action of disopyramide. In addition we have studied the possible stereospecificity of the action of the compound on the endplate ion channel by examining the actions of the two stereoisomers of disopyramide. Some of the results have been presented before in abstract form (Harvey, *et al.*, 1984; Jones & Marshall, 1984).

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Methods

Electrophysiological techniques

All experiments were carried out *in vitro* at room temperature (18–22°C) on twitch muscle fibres of the costocutaneous muscle of the garter snake, *Thamnophis sirtalis*, with the two-microelectrode voltage clamp technique. In experiments in which endplate currents (e.p.cs) were recorded, an approximately 20 mm length of motor nerve supplying the muscle was isolated. The muscles were mounted in physiological solution maintained at pH 7.1–7.2 containing (mM): NaCl 159, KCl 4.2, $CaCl_2$ 2 and HEPES buffer (N-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid) 1.0. To eliminate muscle contraction during experiments in which nerve-evoked e.p.cs were recorded, a cut muscle preparation was used as described previously (Fiekers *et al.*, 1983). In some experiments in which evoked endplate currents (e.p.cs) were too large in amplitude to allow adequate voltage clamping, the calcium concentration was lowered to 1.0–1.5 mM. No differences in e.p.c. decay rates were observed in the different levels of calcium. M.e.p.cs were recorded in uncut muscle preparations.

Microelectrodes were filled with 3 M KCl and had resistances of 3–8 MΩ. During evoked e.p.cs adequacy of voltage clamping was assessed from the voltage deviation, less than 1% of the driving force (holding potential-reversal potential) being regarded as adequate. The reversal potential was assumed to be −5 mV (Fiekers *et al.*, 1983). The motor nerve was stimulated at a frequency of 0.5 Hz with rectangular pulses of 0.05 ms duration and of strength greater than the threshold required to elicit e.p.cs.

To allow the calculation of channel unblocking rate constants, paired e.p.cs were evoked ionophoretically as described by Adams (1976). Paired e.p.cs were evoked from muscles paralysed with tetrodotoxin (10^{-7} M). Double-barrelled electrodes were used, each pulse of acetylcholine emanating from a separate barrel in order to avoid pulse interval-dependent changes in acetylcholine discharge (Adams, 1976). Both barrels had similar resistances within the range 100–150 MΩ, and were filled with 2.5 M acetylcholine chloride. The ionophoretic currents were adjusted to produce equal amplitude e.p.cs from each barrel.

Collection and analysis of e.p.c. and m.e.p.c. data.

E.p.cs and m.e.p.cs were passed through a low pass (d.c. – 5 kHz) filter and recorded on an FM tape recorder (Racal 4DS, d.c. – 5 kHz) for later analysis. Signals from the tape recorder were amplified and sampled every 40 μs by an analog to digital converter interface unit (Cambridge Electronic Design 502) and analysed by a laboratory minicomputer (PDP 11/23, DEC). Peak amplitude and decay characteristics of individual currents were measured by an e.p.c. analysis programme (Dempster, 1985), and subsequently each series of currents (10–20 for e.p.cs, 20–100 for m.e.p.cs) was aligned at the mid-point of the rising phase, averaged and re-analysed. Rise times were calculated from the averaged signal as the time from 10–90% of the peak current. At maximum gain the rise time of the voltage clamp was 0.07 ms (10–90% peak).

Driving function analysis

Endplate current driving functions are a measure of the rate of opening of endplate ion channels, cal-

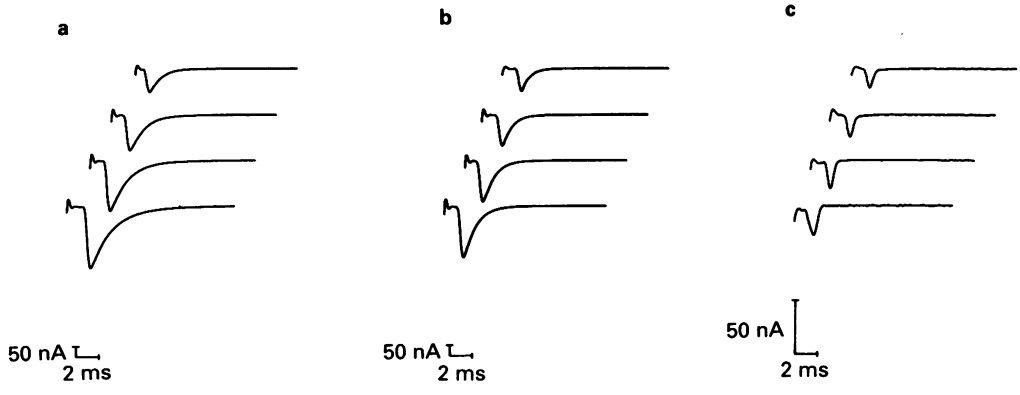

Figure 1 The effects of disopyramide on families of e.p.cs recorded at −50, −70, −90, and −110 mV (top to bottom), (a) control and disopyramide; (b) 5×10^{-5} M; (c) 5×10^{-4} M. Each e.p.c. is the digitized average of approximately 20 e.p.cs. Note the reduction of amplitude and increase of decay rates of e.p.cs recorded in the presence of disopyramide.

culated by computer deconvolution of digitized e.p.cs as described previously (Beam, 1976; Connors *et al.*, 1983; Henderson *et al.*, 1986).

$$W(t) = FFT^{-1}\left[\frac{FFT(\text{e.p.c.}(t))}{FFT(e^{-t/\tau_{ion}})}\right] \Big/ (V_h - V_r)$$

where W(t) is the driving function, e.p.c.(t) is a 512 point digitized average of 20 e.p.cs, $e^{-t/\tau_{ion}}$ is an exponential function representing the decay of the endplate ion channel current with τ_{ion} usually being estimated from the time constant of e.p.c. decay. W(t) has been scaled by the current driving force (V_h, voltage clamp holding potential; V_r, reversal potential).

By reversing the deconvolution calculation, simulated e.p.cs can be generated from the driving function with any chosen value of τ_{ion} (Henderson *et al.*, 1986).

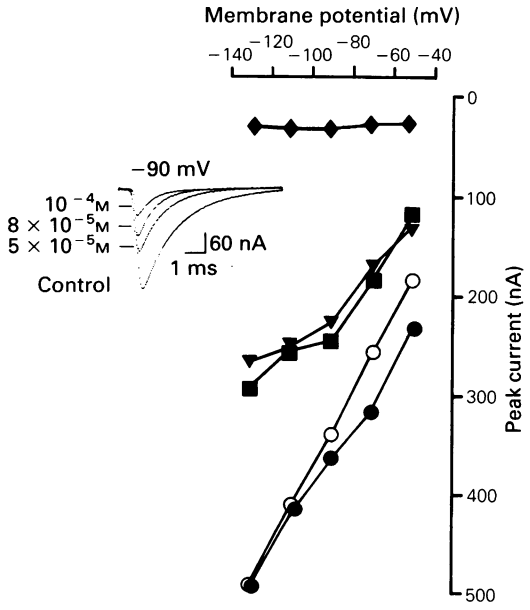

Figure 2 Effects of disopyramide (5×10^{-5}–5×10^{-4} M) on current-voltage relationships of e.p.cs in a representative cell. Control amplitudes are shown by (○), and amplitudes of e.p.cs recorded in disopyramide by (■) (8×10^{-5} M), (▼) (10^{-4} M) and (◆) (5×10^{-4} M). Values after washout are shown by (●). The inset (from Jones & Marshall, 1987) shows e.p.cs recorded at −90 mV in the absence and presence of 5×10^{-5} M, 8×10^{-5} M and 10^{-4} M disopyramide (bottom to top). Note the reduction in e.p.c. rise time.

Drug application

The drugs used were racemic disopyramide phosphate (Roussel or Searle), (+)- and (−)-disopyramide phosphate (Roussel), acetylcholine chloride and tetrodotoxin (Sigma). After a series of control measurements in normal physiological solution, the solution containing the disopyramide was introduced into the muscle chamber at an approximate rate of 5 ml min^{-1} for 5 min. After a further 10 min at equilibrium, measurements were made. In most experiments, three concentrations of drug were used at the same endplate. Only one endplate per muscle was used and only one drug per endplate. All results in the text and tables are expressed as mean ± standard error (mean ± s.e.).

Results

Effects of racemic disopyramide on e.p.c. and m.e.p.c. time course and amplitude

As reported previously for the snake cut costocutaneous muscle preparation (Fiekers *et al.*, 1983; Henderson *et al.*, 1986) e.p.cs and m.e.p.cs decayed as single exponential functions between 95% and 5% of peak amplitude at all holding potentials tested between −50 and −130 mV. That is,

$$I(t) = I(0)e^{-t/\tau}$$

where I(t) is the current at time t after the peak, I(0) is the peak current amplitude and τ is the decay time constant. Also, τ increased exponentially with hyperpolarization. Thus,

$$\tau(V_m) = \tau(0)e^{-Vm/H}$$

where V_m is the holding potential and H is the characteristic change in V_m required to produce an e-fold change in τ.

The major effect of disopyramide (5×10^{-5} to 5×10^{-4} M) was that it produced a marked concentration-dependent reduction of e.p.c. amplitude and shortened e.p.c. decays. The concentration-dependent effects of disopyramide on e.p.cs recorded over a range of holding potentials are shown in Figure 1.

Plots of holding potential versus peak current amplitude (Figure 2) show some non-linearity at negative membrane potentials. Thus the effect of the drug is slightly greater at hyperpolarized potentials. Two effects were observed on e.p.c. time course. Firstly τ was markedly reduced, but e.p.cs continued to decay as single exponential functions at all holding potentials. At the highest concentration (5×10^{-4} M, Figure 1c) the e.p.c. decay phase was almost as fast as the rising phase, and was less well fitted by an exponential curve, probably due to distortions

introduced by the transmitter concentration within the synaptic cleft not yet having decayed to zero. No evidence for more than one decay component was obtained. Secondly, the compound produced a reduction in the rise time of e.p.cs. This effect can be seen clearly in the inset to Figure 2. The effects of disopyramide (5×10^{-5} M) on e.p.c. amplitude at several holding potentials are shown in Table 1. Semilogarithmic plots of τ versus membrane potential (Figure 3) show that increasing concentrations of disopyramide not only reduce the absolute value of τ but also reduce the dependence of τ on membrane potential. Thus in five cells, 10^{-4} M disopyramide virtually abolished the voltage-dependence of τ, increasing the control H value of -80 ± 6 mV to -1058 ± 394 mV, while the highest concentration (5×10^{-4} M) reversed the voltage-dependence (H = 479 ± 244).

In addition to its effects on e.p.cs, disopyramide also shortened the decay time constant of m.e.p.cs, but had no measurable effect on rise time. Some reduction of m.e.p.c. amplitude was observed but the percentage reduction was not dependent upon membrane potential (Table 1). Thus a comparison of the percentage reduction of e.p.c. and m.e.p.c. amplitudes in 5×10^{-5} M disopyramide showed that the drug produced a $27 \pm 6\%$ reduction in e.p.c. amplitude at -90 mV but only a $6 \pm 1\%$ reduction in m.e.p.c. amplitude at the same holding potential.

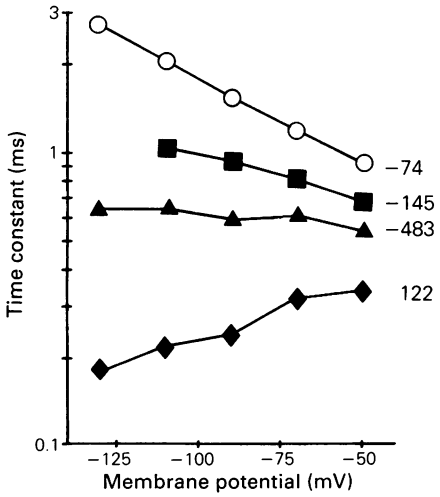

Figure 3 Effects of disopyramide (5×10^{-5} M–5×10^{-4} M) on e.p.c. decay time constants. Results are expressed as semilogarithmic plots of decay time constant (τ) versus membrane potential, recorded from a representative cell. Control time constants are shown by (○) and those recorded in disopyramide by (■) (5×10^{-5} M), (▲) (10^{-4} M) and (◆) (5×10^{-4} M). H values representing the voltage-dependence (mV) are shown associated with each plot.

Table 1 The effects of membrane potential on the reduction of e.p.c. and m.e.p.c. amplitude by disopyramide ($5 = 10^{-5}$ M)

Membrane potential (mV)	*e.p.cs* (% control) ($n = 7$)	*m.e.p.cs* (% control) ($n = 9$)
−50	80 ± 15	86 ± 9
−70	78 ± 13	97 ± 9
−90	73 ± 16	93 ± 5
−110	67 ± 6	91 ± 5
−130	55 ± 6	88 ± 5

Results are mean ± s.e. and are expressed as percentage of control e.p.c. and m.e.p.c. amplitudes recorded from the same cells.

How much do receptor block and effects on quantal content contribute to the effects of disopyramide?

The observation that disopyramide reduced peak e.p.c. amplitude more than it did peak m.e.p.c. amplitude suggested that the substance might be affecting quantal release of transmitter. Quantal content calculated by the direct method (i.e. the ratio of peak e.p.c. over peak m.e.p.c. amplitude) indicated a decrease in quantal release. E.p.cs, however, have a more prolonged rising phase than m.e.p.cs, since the 200–300 quanta in the e.p.c. are not released synchronously, compared with the single quantum in the m.e.p.c. We therefore considered that the rapid channel block produced by the compound might cause block of a substantial number of channels during the e.p.c. rising phase and thereby reduce its peak amplitude to a greater extent than that of the m.e.p.c. We investigated this by using a deconvolution calculation (see methods) which when applied to an e.p.c. produces the e.p.c. driving function, a measure of the time course of channel opening which is dependent only on the rate of transmitter release and receptor binding. Therefore the ion channel blocking effects of disopyramide which occur only after the channel has opened should not affect the size or time course of the e.p.c. driving function. We derived driving functions from averages of approximately 20 e.p.cs both in control and in the presence of 10^{-4} M disopyramide, a concentration that causes an approximately 40% reduction in amplitude and shortens decay time constants to around 0.6 ms. At this concentration the e.p.c. decay time constant, τ, could be used as a good approximation to the channel closure time constant, τ_{ion}, required for calculating the driving function. Simulated e.p.cs were then generated from each driving function by reversing the deconvolution calculation using the same value of τ for both the control and drug. The simulated e.p.cs have in effect had the open

ion channel blocking effect of the drug subtracted, allowing us to observe any effects of the drug on quantal content or on receptors, or forms of ion channel block which do not require the channel to open first before block can occur. Simulated e.p.cs in the presence of 10^{-4} M disopyramide were approximately 20% smaller in amplitude than control e.p.cs (Figure 4). This percentage depression of e.p.cs was similar to that shown for m.e.p.cs at the same holding potential, suggesting that reduction of quantal content does not play a role in the action of disopyramide on e.p.cs.

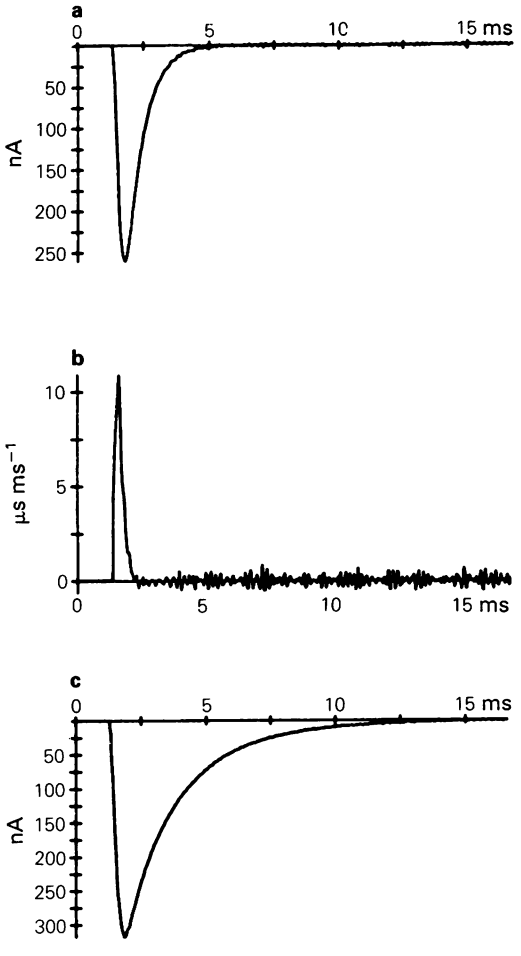

Figure 4 Recorded and simulated e.p.cs in the presence of disopyramide, and their associated driving function. The e.p.c. in (a) is a digitized average of 20 e.p.cs recorded at −110 mV in 10^{-4} M disopyramide. The amplitude of the averaged e.p.c. is 61% of the amplitude of the control e.p.c. at −100 mV from the same endplate. Deconvolution of the e.p.c. ($\tau = 0.68$ ms) shown in (a) produced the driving function shown in (b). This driving function was then reconvoluted with an exponential decay function with the same decay time constant as for the control e.p.c. ($\tau = 2.30$ ms), to create the simulated e.p.c. shown in (c). The simulated e.p.c. represents the remaining effects of disopyramide on the e.p.c. after the channel block effect has been subtracted. The amplitude of this simulated e.p.c. is 83% of the amplitude of the actual control e.p.c.

Calculation of rate constants for the endplate channel blocking action of disopyramide

The results were interpreted in terms of the sequential model of channel block (Adams, 1975; Ruff, 1977; Adler *et al.*, 1978):

$$\mathrm{ACh} + \mathrm{R} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{AChR} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \mathrm{AChR^*} + \mathrm{D} \underset{\mathrm{F}}{\overset{\mathrm{G}}{\rightleftharpoons}} \mathrm{AChR^*\,D}$$

closed open blocked

Where ACh is acetylcholine, R is the receptor, AChR is the ACh bound closed state of the receptor-channel, and AChR* is open state of the receptor-channel and D is the channel blocking drug. k_1, β and G are the forward rate constants and k_2, α and F are the backward rate constants. The actions of D are therefore determined by G, the blocking rate constant and F the unblocking rate constant. K_B, the dissociation constant for the channel blocker is F/G.

If the blocking drug produces, like disopyramide, fast single exponential decays, it is likely that F will be very small and can therefore be ignored in the calculation of G. It is possible to calculate G from

$$\mathrm{GD} = 1/\tau_{\mathrm{drug}} - 1/\tau_{\mathrm{control}} \text{ (Adams, 1976).}$$

Values of G over a range of holding potentials are shown in Table 2. At the highest concentration of disopyramide used (5×10^{-4} M), G was smaller than at lower concentrations. We considered this apparent reduction in G to be another consequence of the prolonged period of transmitter release which is

Table 2 Calculated values of blocking rate constant (G) in disopyramide (10^{-4} M and 5×10^{-4} M) at several membrane potentials

Membrane potential (mV)	*G* ($\times 10^6$ M^{-1} s^{-1})	
	10^{-4} M	5×10^{-4} M
−50	7.1 ± 0.6	5.7 ± 1.1
−70	7.8 ± 0.5	6.1 ± 1.0
−90	9.7 ± 0.9	7.0 ± 0.9
−110	10.3 ± 0.7	7.9 ± 1.7
−130	12.7 ± 1.1	10.2 ± 2.1

Results are expressed as mean ± s.e. ($n = 4$–9).

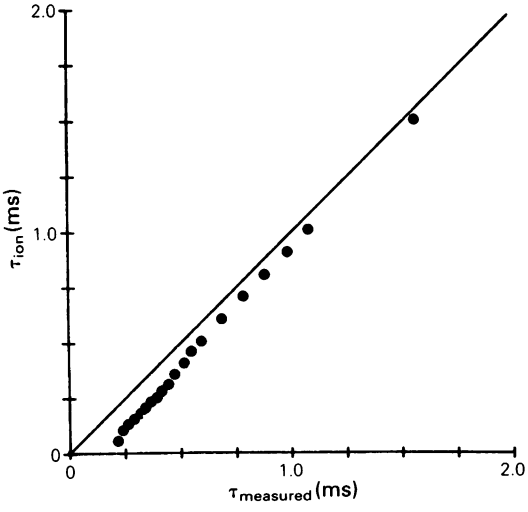

Figure 5 Errors introduced into the measurement of time constants by non synchronous transmitter release. Using a driving function derived from a control e.p.c., a family of simulated e.p.cs were generated from a range of exponential decay functions representing ion channel open times from 0.15 to 2 ms. The figure shows the measured decay time constant, $\tau_{measured}$, of the simulated e.p.c., plotted versus the ion channel open time, τ_{ion}, used to generate it. It can be seen that, particularly at small τ_{ion} values (i.e. rapid channel block) $\tau_{measured}$ markedly overestimates τ_{ion}. For comparison the solid line indicates the $\tau_{measured} = \tau_{ion}$ line that would result if release had been instantaneous.

characteristic of the e.p.c. The e.p.c. can decay no faster than the time course of transmitter release which causes the channels to open. Therefore at the highest drug concentration used, when GD may be of the same order as, or greater than, the rate of decay of transmitter release, the measured e.p.c. time constant, $\tau_{measured}$, may greatly overestimate the decay time constant of the ion channels, τ_{ion}. To investigate this possibility, a family of simulated e.p.cs was generated from a single control driving function, using values of τ_{ion} varying from 0.05 ms to 2.0 ms. Figure 5 shows $\tau_{measured}$, obtained by fitting a single exponential curve to the simulated e.p.c. decay phase, plotted versus the τ_{ion} value from which it was generated. As previously reported (Henderson *et al.*, 1986) $\tau_{measured}$ can be a considerable overestimate of τ_{ion} when the τ value is small. For example, when τ_{ion} is 0.20 ms, $\tau_{measured}$ is 0.32 ms, a 60% overestimate which is sufficient to account for the reduction in G at a concentration of 5×10^{-4} M compared to the lower concentration shown in Table 2.

Due to the above uncertainties the highest concentration of disopyramide was omitted from calculations

Table 3 Calculated value of unblocking rate (F), blocking rate constant (G from Table 2) and dissociation constant (K_B) for disopyramide (10^{-4} M) at two membrane potentials

	Membrane potential		*Voltage dependance*
	−90 mV	−100 mV	(mv)
F (s^{-1})	1.1 ± 0.2	0.9 ± 0.2	125
G (M^{-1} s^{-1})	9.7 ± 0.9	10.3 ± 0.7	−138
K_B (M)	1.1×10^{-7}	9×10^{-8}	93

The voltage-dependence values for each rate constant are also shown.

of the voltage-dependence of G. At the lower concentrations the change in membrane potential required to produce an e-fold change in G was −469 mV at 5×10^{-5} M and −138 mV at 10^{-4} M disopyramide. Because of the absence of any discernible slow phase of decay of e.p.cs it was not possible to calculate F directly from the e.p.c. data. Accordingly F was calculated from the results of recording pairs of ionophoretically evoked e.p.cs in the presence of disopyramide (Figure 6). F was calculated from the reciprocal of the slope of the semilogarithmic plot of the reduction in amplitude of the second e.p.c. versus

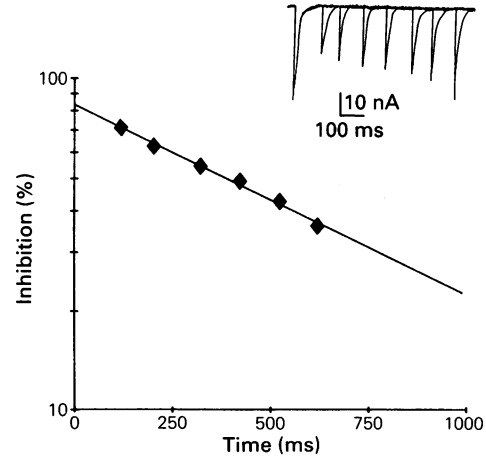

Figure 6 Effect of 10^{-4} M disopyramide on paired responses to inophoretically-applied acetylcholine. The first pulse is shown at the beginning of the sweep. The second pulse was applied at variable intervals after the first. Holding potential −90 mV. The graph shows the relative percentage inhibition of the test pulse produced by the prepulse on a similogarithmic scale as a function of time. The time constant estimated from the slope of the fitted line was approximately 700 ms.

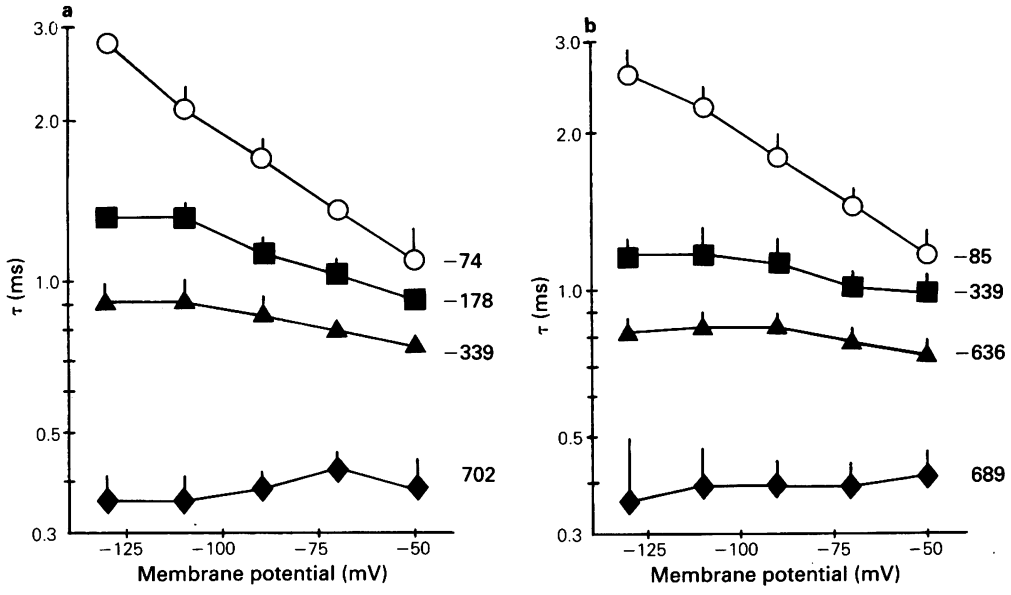

Figure 7 The effects of the (+)- and (−)-stereoisomers of disopyramide on e.p.c. decay time constants. Results are expressed as in Figure 3: (a) shows the effects of (+)-disopyramide and (b) the effects of (−)-disopyramide. In both (a) and (b), control is shown by (○), disopyramide 5×10^{-5} M by (■), 10^{-4} M by (▲) and 5×10^{-4} M by (◆). H values (mV) are shown to the right of each plot. Each point represents the mean of 5–10 experiments in (a) and 3–8 experiments in (b); vertical lines show s.e.

the time of separation from the first e.p.c. F values and their associated voltage-dependence values at two holding potentials are shown in Table 3. Also shown in Table 3 are dissociation constant values calculated from the F values and G values from Table 2. F and G were found to exhibit opposite voltage-dependencies. Hence the dissociation constant K_B was also found to be voltage-dependent (Table 3).

The effects of (+)- and (−)-disopyramide on e.p.cs

Both (+)- and (−)-stereoisomers of disoypyramide reduced e.p.c. amplitude and time course in the same concentration-range. As with racemic disopyramide, both stereoisomers produced rapid e.p.c. decay phases which could be fitted by a single exponential function. A comparison of the effects of the (+)- and (−)-isomers of disopyramide on e.p.c. decay time constants is shown in Figure 7. The effects of the two isomers are virtually superimposable.

Discussion

Our results indicate that the previously reported non-competitive anticholinesterase-resistant neuromuscular block produced by the antiarrhythmic agent disopyramide in isolated skeletal muscle preparations is most probably due to blockade of the open form of the endplate receptor-ion channel complex. Evidence for this is the concentration- and voltage-dependent shortening of the decay phase of e.p.cs and m.e.p.cs. In addition to the action of the compound on the ion channel, it is probable that the observed non-voltage dependent reduction in amplitude of m.e.p.cs is due to receptor block. Although at first sight it appears as if the compound is reducing quantal content, i.e. e.p.c. amplitude is affected much more than m.e.p.c. amplitude, we believe that this is due to channel block occurring during the rising phase of the e.p.c. This is because of the temporal dispersion of evoked transmitter release, a conclusion confirmed by the lack of effect of the drug on m.e.p.cs which are the result of virtually instantaneous and simultaneous receptor activation by the contents of one quantum of acetylcholine. By using the method of driving function analysis and simulation of e.p.cs from driving functions we were able to correct for the effects of channel block on e.p.c. amplitude. When this was done we found that disopyramide (10^{-4} M) reduced both e.p.c. and m.e.p.c. amplitude by around 20%. This finding is consistent with a postjunctional receptor blocking action of the compound. Thus it is not necessary to

invoke a reduction of quantal content as a possible contributory factor to the reduction of e.p.c. amplitude.

The type of change in e.p.cs produced by disopyramide was typical of compounds which have relatively slow unblocking rate constants such as tubocurarine (Colquhoun *et al.*, 1979), atropine (Adler *et al.*, 1978) and the local anaesthetic QX314 (Beam, 1976). All these compounds produce fast single exponential decays which contrast with the double exponential decays shown with compounds with faster unblocking rate constants. This prediction is confirmed by the observed F value of approximately 1 s^{-1}, a value similar to those for the above compounds. Both F and G, the blocking rate constant, were voltage-dependent, F decreasing and G increasing with membrane hyperpolarization. This result indicates that the compound is acting as a charged molecule. This is consistent with the pK_a value of 9.7, which results in 98% of the compound being in ionized form at pH 7.2. The G value of around $10^7 M^{-1} s^{-1}$ measured by -90 mV in the presence of disopyramide is similar to that of many other voltage-dependent drugs at the endplate ion channel (Colquhoun, 1981).

Recently we showed that four diastereoisomers of the antibiotic substance, chloramphenicol, showed no stereoselectivity for the endplate channel of the snake (Henderson *et al.*, 1986). One limitation to the interpretation of this result was that stereospecificity decreases with compounds of low affinity (Lehmann, 1980), and the chloramphenicols had dissociation constants for the channel in the region of 1 mM. The stereoisomers of disopyramide afforded an opportunity to test further the stereoselectivity of channel block by using compounds of considerably higher affinity (equilibrium dissociation constants around 0.1 μM). Not surprisingly, in view of the large number of chemically dissimilar compounds that have been demonstrated to cause endplate ion channel block (Pennefather & Quastel, 1980; Lambert *et al.*, 1983), no evidence of stereoselectivity was obtained. The lack of stereoselectivity at the endplate channel contrasts sharply with the stereoselectivity on the cardiac action potential (Mirro *et al.*, 1981) and at cardiac muscarinic receptors (Mirro *et al.*, 1980).

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