The activation gate of the sodium channel controls blockade and deblockade by disopyramide in rabbit Purkinje fibres

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- 1 The effect of disopyramide on the maximum upstroke velocity ($\dot{V}_{max}$) and the sodium current of rabbit cardiac Purkinje fibres was studied with the two-microelectrode voltage-clamp technique.
- 2 In the absence of stimulation the drug did not cause block at membrane potentials ranging from -100 to -65 mV. Use-dependent block of $\dot{V}_{max}$ was most pronounced at -75 mV. At hyperpolarized membrane potentials development of use-dependent block was faster than at depolarized membrane potentials. The time course of development of use-dependent block was not significantly influenced by the duration of the depolarizing pulse. These results strongly suggest that disopyramide predominantly blocks activated sodium channels.
- 3 The relative decrease of the sodium current at the beginning of a 2 s depolarizing clamp to -45 mV was almost the same as at the end, implying a rapid blockade of activated sodium channels. The Hill plots were linear with slopes ranging from 0.98 to 1.08 indicating a first order reaction; the dissociation constant for activated channels was $70 \mu\text{M}$.
- 4 Recovery of $\dot{V}_{max}$ from use-dependent block during rest was strongly voltage-dependent, the time constant of recovery increasing upon hyperpolarization. When the fraction of charged molecules was reduced by changing the pH of the external solution, the voltage-dependence of recovery was decreased. In contrast, recovery of $\dot{V}_{max}$ for a change in holding potential from -80 to -95 mV was very fast during repetitive stimulation.
- 5 It is concluded that disopyramide blocks the sodium channel during activation and is trapped in the channel when the activation gate closes.

Introduction

The modulated receptor hypothesis (Hille, 1977b; Hondeghem & Katzung, 1977) postulates a variable-affinity channel binding site and a modification of inactivation gate kinetics in drug complexed channels. Therapeutically useful antiarrhythmic drugs are assumed to have a low affinity for rested sodium channels but to bind preferentially when the membrane is depolarized. Therefore these agents show a pronounced use-dependent block of sodium channels whenever the diastolic interval during repetitive stimulation is too short for complete recovery from block. Since blocked channels have their inactivation kinetics shifted in the hyperpolarized direction and it has been shown for several drugs that dissociation of the drug from the binding site occurs more slowly in inactivated than in rested channels (Hondeghem & Katzung, 1984), recovery should be voltage-dependent, i.e. the rate of recovery should increase with hyperpolarization.

In contrast, the guarded receptor hypothesis (Starmer *et al.*, 1984) assumes use-dependent block as the result of transient access to a binding site of constant affinity, controlled by the channel gate conformation. For lipid soluble agents, receptor access is postulated as being controlled by only the activation gate, whereas for hydrophilic drugs both activation and inactivation gates are required in the open configuration. Drug molecules bound to the binding site in the channel may become trapped by closure of the channel gates. The first evidence for gate-trapping was provided by Strichartz (1973) and by Yeh (1979). As regards the recovery time course, the gate trapping hypothesis predicts acceleration or slowing of recovery with hyperpolarization, depending on whether the drug is trapped by the inactivation or the activation gate. In the case of the activation gate trapping, recovery is slowed as the membrane is hyperpolarized because the probability of channel

opening is decreased. Quaternary compounds and those with a low deprotonation rate, will only be able to leave the channel when both gates are in the open configuration.

According to those two hypotheses the determining factor for recovery is either the drug's dissociation from the binding site or the channel gating mechanism. In order to differentiate between those two alternatives the study of voltage sensitivity of recovery from block may be a discriminating test.

Recent results obtained in squid axons with QX-314 and QX-222 (Yeh & Tanguy, 1985) and with disopyramide and analogues (Yeh & Ten Eick, 1987) indicate a slowing of recovery as the membrane is hyperpolarized and give additional support for the activation gate trapping model. In cardiac muscle, disopyramide shows similar potential dependence of the time constant of recovery from use-dependent block, when the membrane potential is changed by using variable potassium concentrations in the perfusion solution (Gruber *et al.*, 1986).

Therefore, the present investigation was done to test this hypothesis under voltage-clamp conditions in rabbit Purkinje fibres, in an attempt to differentiate between the activation gate trapping and the modulated receptor hypothesis for the action of a sodium channel blocking drug in cardiac tissue.

Methods

The experiments were performed on rabbit Purkinje fibres, dissected from both ventricles. The fibres were shortened to less than 1 mm by crushing them with a wire grid on the bottom of the perfusion chamber.

The composition of the modified Tyrode solution was (mM): NaCl 137, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaHCO₃ 24, glucose 11. The fibres were superfused with this solution, which was gassed with 95% O₂-5% CO₂, at a rate of 3 ml min⁻¹ at a constant temperature of 37°C. For experiments performed at a pH of 8.0, the concentration of HCO₃⁻ was increased and the CO₂ content of the gas mixture was reduced to 3%. Cs 20 mM was added to block the inward rectifying potassium channel; this allowed continuous clamping at very negative potentials without causing potassium depletion. Disopyramide (Mead Johnson) was applied in concentrations ranging from 10 to 300 µM.

The two-microelectrode technique was used to measure currents under voltage-clamp conditions, or to impose different degrees and durations of depolarizing pulses. Sodium channel activity was measured directly by recording the slowly inactivating sodium current (Carmeliet, 1987a) or indirectly by measuring the maximum rate of rise of an action potential ($\dot{V}_{max}$). The current-passing electrode penetrated

the middle of the fibre, whereas the potential-measuring electrode was positioned between the first one and the end of the segment. Both electrodes were filled with 3 M KCl. This combination of voltage-clamp methods and measurement of $\dot{V}_{max}$ in unclamped fibres was chosen, because it is difficult to apply a well controlled voltage-clamp during periods of peak sodium current flow. Thus, the voltage-clamp allows the application of 'conditioning' protocols which permit testing of hypotheses concerning the importance of the state of voltage-dependent channels for drug binding, and $\dot{V}_{max}$ provides a good measure of sodium channel availability during periods of high sodium current density.

The membrane potential was amplified and led to the input of the voltage-clamp amplifier which also received rectangular command pulses. Transmembrane current was measured by an operational amplifier circuit that held the bath potential at virtual ground. Signals were displayed on an oscilloscope or a pen recorder, or were stored on magnetic tape.

For statistical analysis mean values $\pm$ s.e. are given.

Results

Figure 1a,b illustrates the existence of a pronounced use-dependent block of $\dot{V}_{max}$ by disopyramide (10 µM). The clamp protocol consisted of 50 ms clamp periods at -95 mV (holding potential) interrupted by 10 ms to -45 mV. Between the two clamp periods the preparation was stimulated to measure $\dot{V}_{max}$. $\dot{V}_{max}$, for the first pulse applied after a rest period of at least 3 min, was identical with the control value in the absence of the drug, indicating the absence of a tonic block. Repetition of the depolarizing clamp steps resulted in a gradual decline of $\dot{V}_{max}$, which could be described as a mono-exponential decay (Figure 1b). In the absence of drug such a decrease was not seen (Figure 1a). The existence of use-dependence implies extra-block occurring during activity and insufficient recovery during the rest period between stimuli. It also means that the extra block during one depolarization does not reach equilibrium concentration. The question to be answered is whether disopyramide binds preferentially to the activated or inactivated state.

Absence of inactivation block

In published studies (Kus & Sasyniuk, 1978; Kojima *et al.*, 1982) disopyramide was described as an inactivation blocker. In the present experiments the possibility of preferential binding to the inactivated state was tested in two ways, either by prolonging

Figure 1 Development of use-dependent block for depolarizing pulses of different duration. After a depolarizing pulse and measuring $\dot{V}_{max}$ the fibres were held at a membrane potential of -45 mV for either 10 ms (a,b) or 500 ms (c,d) before clamping back to the holding potential of -95 mV for 50 ms (see inset: period is 60 ms for a and b and 550 ms for c and d). Original recordings of $\dot{V}_{max}$ are shown under control conditions (a,c) and during exposure to disopyramide ($10 \mu\text{M}$) (b,d). The time course of development of use-dependent block is largely independent of the duration of the clamp step to -45 mV.

the duration of the depolarizing step (Figure 1c,d) or by changing the holding potential preceding the test depolarization (Figure 2). As shown in Figure 1, prolongation of the depolarizing step from 10 ms to 500 ms had no effect on the amplitude of the block in steady-state. The time course of the block development was slightly faster with the long depolarizations, but a certain degree of $\dot{V}_{max}$ decay was also seen under control conditions and is due to the existence of a slow phase in the recovery from inactivation (Saikawa & Carmeliet, 1982). The fact that prolongation of the depolarization did not increase block means that inactivation beyond a duration of 10 ms had no effect on the block. A block of inactivated channels reaching steady-state in less than 10 ms can also be excluded because of the existence of use-dependency. The effect of different degrees of inactivation was further studied by varying the membrane holding potential between -100 mV and -65 mV and imposing a series of voltage-clamp depolarizations of the type shown in Figure 2 (inset) at a frequency of 1000 min^{-1} . Between each series and before changing the holding potential the preparation was rested for at least 3 min. Under control conditions $\dot{V}_{max}$ values at the start and the end of a

series of depolarizations did not differ (Figure 2). The same values were also obtained in the presence of $10 \mu\text{M}$ disopyramide when only one single stimulus was applied. Therefore, it is evident that disopyramide did not cause any block in the absence of stimulation, although inactivation was clearly present under control conditions.

Use-dependent block and membrane potential

The results of Figure 2 further demonstrate that the steady-state use-dependent block obtained at the end of a series of depolarizing clamps was dependent on the holding membrane potential, although it is not a simple function of inactivation. In absolute terms the block was greatest at -75 mV, and gradually decreased at hyper- or depolarized levels (see also Figure 5a,b for an illustration of a greater block at a holding potential of -80 mV compared to -95 mV). In relative terms the depression of $\dot{V}_{max}$ was $9.7 \pm 2.6\%$ at -100 mV and this depression increased as the membrane potential became less negative, until a constant level of about 57% reduction was reached at -70 mV.

Figure 2 Block with or without stimulation as a function of membrane potential. From a distinct holding potential (E_H) between -100 to -60 mV a depolarizing pulse was applied and $\dot{V}_{max}$ measured. Following this pulse the fibre was kept at -45 mV for 10 ms before switching back to a certain holding potential for 50 ms. This protocol was repeated until steady-state block was obtained. Stimulation was then stopped and a rest period of at least 3 min was interpolated until complete disappearance of the block. Mean values of $\dot{V}_{max}$ for 5 experiments are given, vertical lines indicate s.e. Control is indicated by (○). Disopyramide ($10 \mu\text{M}$) did not cause tonic block (absence of stimulation) at any tested holding potential (Δ), whereas use-dependent block ($\bullet$) was most pronounced at membrane potentials of -75 mV, declining when the membrane was hyper- or depolarized during diastolic intervals.

The time course of the development of use-dependent block can be described by one exponential and a constant. The time constant was shorter for hyperpolarizing and longer for depolarizing holding potentials. The mean values (ms) for three experiments are given in Table 1. Expressed in rates

Figure 3 Slow sodium current in the absence and presence of disopyramide (10 , 30 and $300 \mu\text{M}$). From a holding potential of -100 mV a 2 s depolarizing clamp to -45 mV was applied. The control current is the total tetrodotoxin-sensitive current measured after exposure to $60 \mu\text{M}$ (see Carmeliet, 1987a,b for further details). Characteristic current traces recorded in the presence of 10 , 30 and $300 \mu\text{M}$ disopyramide are shown. For these concentrations the current was 81%, 60% and 16% of control at the beginning and 83%, 59% and 13% at the end of the depolarizing pulse. The holding current in this experiment was -7 nA.

and taking the number of depolarizations (activations) into account these values changed from 0.239 pulse^{-1} at -100 mV to 0.025 pulse^{-1} at -65 mV.

Activation block

Since the preceding experiments have shown that disopyramide neither exerts a resting nor an inactivation block, we decided to measure the blocking of the sodium channels during activation directly. Since it is difficult to control voltage during the initial peak sodium current flow, the present analysis is limited to the slow component of the tetrodotoxin(TTX)-sensitive sodium current. This

Table 1 Time constants for development of use-dependent block (τ) at different membrane potentials

	Membrane potential (mV)							
	-100	-95	-90	-85	-80	-75	-70	-65
τ (ms)	251 ± 10.2	310 ± 26.1	376 ± 81.5	450 ± 47.7	691 ± 91.6	817 ± 24.6	1528 ± 38.6	2379 ± 99.3

Values are means $\pm$ s.e., $n = 3$.

Figure 4 Time course of relative block during a depolarizing pulse. Data were taken from the same experiment as shown in Figure 3. The ratio of the current in the presence of 10, 30 and 300 μM disopyramide to control current is illustrated. The onset of block was so fast that no reliable time constants for the time course of development of block during the depolarizing pulse could be determined. The results indicate that disopyramide rapidly blocks activated sodium channels.

was studied by applying a voltage-clamp depolarization of 2 s from a holding potential of -100 mV to -45 mV ($n = 4$). The same method was used to measure the time course of TTX-binding (Carmeliet, 1987b) and of transcaïnide (Carmeliet, 1988a) to activated sodium channels. At any drug concentration tested between 10 and 300 μM , the initial slow sodium current was reduced to the same extent as the current at the end of the 2 s depolarizing pulse (Figures 3 and 4). The ratio of the current in the presence of disopyramide to the current in control Tyrode solution showed no obvious decay of relative block during a depolarizing pulse, indicating that no additional blockade of sodium channels could be detected during the depolarization. Since disopyramide did not cause resting block (Figures 1 and 2) this signifies that all or most of the blocking of sodium channels occurred during the initial ms of the depolarizing pulse (Figure 4). Taking those results into account, disopyramide can be classified as a fast activation blocker. The Hill plots ($n = 4$) of the data were linear with slopes ranging from 0.98 to 1.08, suggestive of a first order reaction between the drug and the binding site. The dissociation constant determined for activated sodium channels was 70 μM .

At first sight the finding of steady-state block which developed during the initial 10–15 ms of a clamp to -45 mV seems against the existence of a

use-dependent block as illustrated in Figures 1 and 2. However one should take into account the fact that block is estimated in one case by measuring the slow Na current and in the other case by measuring $\dot{V}_{\text{max}}$, an estimate of the fast Na current. The time allowed for development of activation block of the fast Na current is very short, i.e. only part of the action potential upstroke. In the case of the slow Na current the time for development of block is much longer, and amounts to 10 to 15 ms, i.e. the time during which the clamp is out of control.

Recovery from use-dependent block

Since the block exerted by disopyramide at a given frequency was dependent on membrane potential (Figure 2) and disappeared in the absence of stimulation it is possible to distinguish between two types of recovery: (1) recovery can be studied by changing the membrane holding potential or (2) it can be estimated by lowering the frequency.

Fast recovery from block: activation deblockade

Figure 5a and b first confirms the occurrence of a greater block at a holding potential of -80 mV compared to -95 mV (see also Figure 2). In the absence of drug (a) an abrupt decrease of $\dot{V}_{\text{max}}$ was seen when the membrane potential was changed from -95 mV to -80 mV . This fall in $\dot{V}_{\text{max}}$ is due to inactivation. In the presence of drug (b) and continuous stimulation, a certain degree of block had already developed at -95 mV (time course not shown). When the holding potential was changed to -80 mV , an instantaneous decrease, due to inactivation, was followed by a pronounced time-dependent decrease, due to extra-block.

Recovery from the extra block, when the reverse change in holding potential was applied, is seen in Figure 5d. (Figure 5c illustrates the change in $\dot{V}_{\text{max}}$ due to a difference in inactivation, when the same step in potential is applied in the absence of drug.) $\dot{V}_{\text{max}}$ reached the steady-state level of the use-dependent block at -95 mV within a few pulses. The time constants determined from two experiments were 194 and 185 ms. This result indicates a fast deblockade of sodium channels during activation.

Slow recovery from block at rest

For estimation of the time constant of recovery during rest, repetitive stimulation was interrupted for variable periods at holding potentials in the range -95 to -70 mV . The time course of recovery of $\dot{V}_{\text{max}}$ can be described by one exponential and a constant and shows a strong voltage-dependence, in that recovery was markedly slowed at hyperpolarized levels. The time constant of recovery increased from $11.2 \pm 1.0\text{ s}$ ($n = 5$) at a

Figure 5 Fast recovery from block by changing the holding potential during continuous stimulation. From the holding potential of -95 (a and b) or -80 mV (c and d) a depolarizing pulse was applied every 60 ms and $\dot{V}_{max}$ measured. Before clamping back to one of these holding potentials the fibre was held at -45 mV up to 10 ms. Original recordings of $\dot{V}_{max}$ are shown under control conditions (a,c) and after exposure to $10 \mu\text{M}$ disopyramide (b,d). Under control conditions most of the change in $\dot{V}_{max}$ was abrupt; the small, slow changes are due to a process of slow inactivation. When, in the presence of disopyramide, the holding potential was switched from -95 to -80 mV, an abrupt change in $\dot{V}_{max}$ was followed by a gradual decrease to a new steady-state, demonstrating that use-dependent block is more pronounced at -80 than at -95 mV. On returning to the holding potential of -95 mV this extra block disappeared within a few pulses, indicating that sodium channels in their open state facilitate the escape of the drug from the binding site.

membrane potential of -75 mV to 15.5 ± 1.6 s ($n = 9$) at -80 mV, 21.9 ± 1.8 s ($n = 7$) at -85 mV, 45.7 ± 6.7 s ($n = 7$) at -90 mV and finally to 70.8 ± 8.0 s ($n = 4$) at -95 mV. When the membrane was depolarized from -75 to -70 mV ($n = 6$), the time constant of recovery did not decrease further (Figure 6). The semilogarithmic plot of the time constants of recovery as a function of the membrane potential gives a straight line between -75 and -95 mV ($r = 0.9885$, $P < 0.01$) (Figure 7). As demonstrated in one experiment the voltage-dependence of recovery was unaffected by prolongation of the depolarizing pulse from 10 to 500 ms. At a membrane potential of -80 mV the time constant was 20 s and 85 s at a holding potential of -95 mV. A more developed inactivation apparently has no effect on this slow recovery phenomenon.

Assuming that the charged disopyramide molecules are responsible for the slowed recovery of $\dot{V}_{max}$ at hyperpolarized membrane potentials, the pH

of the external solution was increased to 8.0 in order to reduce the fraction of charged molecules. At all membrane potentials tested, the amplitude of use-dependent block at pH 8 was slightly more pronounced than at pH 7.4; its time course could be fitted as a mono-exponential. As expected, recovery from use-dependent block was accelerated, the relative change being dependent on the membrane potential. Between -80 and -70 mV time constants were practically constant (9.7 ± 0.7 s at -80 mV, $n = 6$; 9.6 ± 1.1 s at -75 mV, $n = 5$ and 9.04 ± 0.5 s at -70 mV, $n = 4$). Upon hyperpolarization to -85 mV the time constant increased to 12.2 ± 1.6 s ($n = 6$), to 18.1 ± 2.6 s ($n = 5$) at -90 mV and to 28.3 ± 3.0 s ($n = 3$) at -95 mV (Figure 6). The time constant of recovery, semilogarithmically plotted as a function of the membrane potential, followed a linear relation between -80 and -95 mV ($r = 1.007$, $P < 0.01$). On changing the pH of the external solution from 7.4 to 8.0 a shift of that correlation to the

Figure 6 Time course of recovery as a function of the membrane potential and pH. From holding potentials (E_H) of between -70 and -95 mV depolarizing clamp pulses of 10 ms to -45 mV were applied and repeated every 60 ms until steady-state block was obtained. Disopyramide concentration was $10 \mu\text{M}$. The time constants for recovery of $\dot{V}_{\text{max}}$ (τ) from use-dependent block were estimated by interruption of the repetitive stimulation for varying periods of time. ($\circ$) Indicate mean values of the time constants from 4–9 experiments performed at pH 7.4. The time constant is markedly increased when the membrane is hyperpolarized. Therefore, disopyramide can be considered an activation gate trapped agent. This strong voltage-dependence of the time constant of recovery is somewhat reduced and is shifted to the hyperpolarizing direction by 5 mV when the fraction of charged disopyramide molecules is decreased by raising the pH of the bathing solution to pH 8.0, as shown by ($\bullet$) ($n = 4$ –6). Mean values are shown with vertical lines indicating s.e.

hyperpolarizing direction occurred, and additionally the slope of the straight line decreased somewhat (Figure 7).

Discussion

Blockade of open channels

In the present experiments evidence for blockade of open channels by disopyramide has been provided by demonstrating the existence of use-dependent block and the absence of inactivation block.

The conclusion that disopyramide does not bind preferentially to inactivated channels is based on two arguments: (1) disopyramide ($10 \mu\text{M}$) did not shift the instantaneous inactivation curve, obtained by applying a depolarizing pulse after a rest period of sufficient duration to ensure complete recovery of $\dot{V}_{\text{max}}$

Figure 7 Correlation between time constant of recovery and membrane holding potential. The semi-logarithmic plot of the time constants as a function of the membrane potential gives a straight line in the range -95 to -75 mV at a pH of 7.4 ($\circ$, $r = 0.9885$, $P < 0.01$) and for membrane potentials ranging from -95 to -80 mV at a pH of 8.0 ($\bullet$, $r = 1.007$, $P < 0.01$).

from the effect of the drug. This indicates that disopyramide is no inactivation blocker at that concentration. Misinterpretation of the results due to the non-linear relationship between $\dot{V}_{\text{max}}$ and the sodium current (Strichartz & Cohen, 1978; Cohen *et al.*, 1984) is improbable. Fozzard *et al.* (1987) recently pointed out that, at least in single canine cardiac Purkinje cells, overestimation of sodium channel availability by $\dot{V}_{\text{max}}$ is less than found for rabbit Purkinje fibres (Cohen *et al.*, 1984). Therefore, we conclude from our results that disopyramide does not block closed sodium channels. (2) A similar conclusion can be reached from experiments in which the duration of a repetitive depolarization was prolonged from 10 ms to 500 ms without causing any important change of the use-dependent block. This conflicts with the data of Kojima *et al.* (1982), based on the increased effect of disopyramide in preparations bathed in low K concentrations when combined with low rates of stimulation (0.1 Hz). They interpreted their results to be due to a promoted inactivation block, caused by a lengthening of the action potential duration in low potassium solution. They did not take into account the marked hyperpolarization, which is accompanied by slow recovery.

Since disopyramide does not affect rested or inactivated sodium channels, the only possibility left is a block of activated sodium channels. A direct measurement of the time constant for binding to activated sodium channels, using the slowly inactivating

current, failed; the blockade of the sodium current was so fast that a steady-state level was already obtained during the period when the clamp was out of control, which is in the range of 10 ms. However, the existence of use-dependent blockade and its change with holding potential offers an indirect way to estimate τ_d , the binding constant for the change in bound drug at the depolarized level. Use-dependent blockade followed an exponential time course and can be characterized by λ , the rate constant for uptake of drug, expressed in pulse^{-1} , since the blockade associated with the n^{th} pulse can be described recursively in terms of blockade acquired during the previous pulse. The rate constant for uptake of the drug decreased for positive potentials (see Table 1). Using the formula

$$\lambda = \frac{t_d}{\tau_d} + \frac{t_r}{\tau_r}$$

(Starmer *et al.*, 1986; equation 6) τ_d can be calculated. The repolarizing interval t_r is defined by the clamp protocol, while recovery time constant τ_r and uptake rate λ were determined experimentally. The mean receptor access time t_d was taken as 1 ms. Then, for membrane potentials between -95 mV and -75 mV, a τ_d was calculated, which varied between 5 and 15 ms. These values should only be taken as an estimation of the order of magnitude.

Assuming this estimation to be valid, it is understandable why the $\dot{V}_{\text{max}}$ value of the first action potential following a long period of rest is not affected by the presence of disopyramide (Figure 2). The time between start of the upstroke and time at which $\dot{V}_{\text{max}}$ is measured does not allow sufficient channels to be blocked. On the other hand it also becomes understandable why most of the block was already present when the slow Na current was measured directly (Figures 3 and 4): a quantitative measurement, without interference of escape artefacts, was only possible after 10–15 ms.

Recovery from block

Block by antiarrhythmic drugs is generally assumed to be relieved by hyperpolarization (Hondeghe & Katzung, 1977; 1984), as has been demonstrated in the case of lidocaine (Bean *et al.*, 1983) and quinidine (Weld *et al.*, 1982). However our results show a completely different behaviour for disopyramide: (1) When recovery measured during rest and time constants were compared at different membrane potentials, recovery was found to be markedly slowed at hyperpolarized levels. (2) When recovery was measured during a pulse train by changing the holding potential from -80 to -95 mV, recovery was fast (in the order of 0.1–0.2 s instead of tens of seconds). Following the quasi-instantaneous recovery from

inactivation of the non-blocked channels, $\dot{V}_{\text{max}}$ increased in an exponential way and reached steady-state within a few depolarizing pulses. Not only blockade, but also deblockade is apparently facilitated by opening of the sodium channels. Such results support the activation gate trapping hypothesis (Yeh & Tanguy, 1985; Starmer *et al.*, 1986). The activation gate trapping hypothesis suggests that hydrophilic charged agents bind to the channel during the depolarizing phase and are trapped within the channel by the activation gate. The trapped molecules can only escape from the channel when the channel is open. This can explain slower recovery at hyperpolarized membrane potentials, since the probability of channel random openings decreases with hyperpolarization. Voltage-dependent recovery kinetics of disopyramide (present paper) and its congener penticainide (Gruber & Carmeliet, 1987; Carmeliet, 1988b) are comparable to that of a quaternary compound or 9-aminoacridine, a molecule which deprotonates very slowly (Yeh & Tanguy, 1985). The slow time constant of recovery is not only a function of the membrane potential (Starmer *et al.*, 1986), but also of the pK_a of the drug and the pH of the external solution (Hille, 1977a; Schwarz *et al.*, 1977). Our results support these assumptions in that recovery of $\dot{V}_{\text{max}}$ is accelerated when the pH of the external solution is changed from 7.4 to 8.0, since the fraction of charged molecules will be reduced. However, the acceleration of the time course of recovery is notably greater at membrane potentials more negative than -80 mV. This indicates that, for disopyramide, unblocking predominantly occurs via the hydrophilic pathway as suggested for quaternary drugs.

According to the size-solubility hypothesis (Courtney, 1980) large tertiary compounds, thus having a small unblocking rate via the hydrophobic pathway, or tertiary compounds with high pK_a s, thus providing larger protonation rates and/or smaller deprotonation rates, may behave like permanently charged molecules. Starmer & Courtney (1986) assume a higher pK_a value of the drug in the channel than in free solution. They argue that local potential fields near the drug binding site may influence the protonation-deprotonation reactions and that the relation of the protonation site to the proton access path may influence the rates, too. As regards the size of the molecule, disopyramide resembles quinidine, and its pK_a of 8.36 is even lower than that of quinidine which has a pK_a of 10.0 (besides one of 5.4). Quinidine, however, does not show voltage-dependence of recovery like disopyramide (unpublished observation). Weld *et al.* (1982) even found that the rate constant for dissociation of quinidine from quinidine-blocked channels becomes larger as the transmembrane voltage

becomes more negative; so quinidine behaves in the opposite way to disopyramide in respect to voltage-dependent recovery from use-dependent block of $\dot{V}_{max}$. These findings lead us to conclude that other determining factors may be important, such as stereochemical properties of a molecule.

If activation block and activation trapping were the only factors determining use-dependent block, this block should be most pronounced when applying depolarizations from very negative holding potentials: the number of available Na channels for activation is greater and recovery rate is slower. The use-dependent block as a function of holding potential (Figure 2), however, was quite different and showed a maximum block for intermediate potentials. We suggest that a voltage-shift to negative potentials of blocked inactivated channels may be responsible for this behaviour. Such a voltage shift results in more block at less negative potentials. The peculiar voltage relationship illustrated in Figure 2 could thus be explained as follows: (1) at very negative potentials the block is solely determined by activation trapping, (2) at intermediate potentials the

block is greater because it consists of rested channels, in which the drug is trapped, and inactivated blocked channels. (3) At less negative potentials the block is again smaller because activation and therefore the possibility of the drug entering and blocking a channel is reduced.

From the therapeutical point of view slowed recovery from use-dependent block at membrane potentials more negative than -75 mV may not be advantageous. However, this is partly compensated for by the smaller amount of use-dependent block at these potentials. Furthermore it should be realized that changes in block (increase or decrease) induced by a change in membrane potential are rather fast phenomena when the preparation is stimulated.

In conclusion, disopyramide is thought to be an activation blocker and an activation gate trapped agent.

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References

- BEAN, B.P., COHEN, C.M. & TSIEN, R.W. (1983). Lidocaine block of cardiac sodium channels. *J. Gen. Physiol.*, **81**, 613–642.
- CARMELIET, E. (1987a). Slow inactivation of the sodium current in rabbit cardiac Purkinje fibres. *Pflugers Arch.*, **408**, 18–26.
- CARMELIET, E. (1987b). Voltage-dependent block by tetrodotoxin of the sodium channel in rabbit cardiac Purkinje fibres. *Biophys. J.*, **51**, 109–114.
- CARMELIET, E. (1988a). Slowly developing activation block of cardiac sodium channels by a lidocaine analog, trans-cainide. *J. Cardiovasc. Pharmacol.*, **12**, 110–115.
- CARMELIET, E. (1988b). Activation block and trapping of penticainide, a disopyramide analogue, in the Na^+ channel of rabbit cardiac Purkinje fibres. *Circ. Res.*, **63**, 50–60.
- COHEN, C.J., BEAN, B.P. & TSIEN, R.W. (1984). Maximal upstroke velocity as an index of available sodium conductance. *Circ. Res.*, **54**, 636–651.
- COURTNEY, K.R. (1980). Structure-activity relations for frequency-dependent sodium channel block in nerve by local anesthetics. *J. Pharmacol. Exp. Ther.*, **213**, 114–119.
- FOZZARD, H.A., HANCK, D.A. & SHEETS, M.F. (1987). Non-linear relationship of maximal upstroke velocity to the sodium current in single canine cardiac Purkinje cells. *J. Physiol.*, **382**, 103P.
- GRUBER, R. & CARMELIET, E. (1987). The antiarrhythmic agents disopyramide and penticainide act as activation blockers of the Na channel. *Arch. Int. Pharmacodyn. Ther.*, **287**, 332.
- GRUBER, R., ZILBERSZAC, A. & HEISTRACHER, P. (1986). Voltage- and pH-dependent recovery of $\dot{V}_{max}$ from the action of disopyramide in guinea-pig heart muscle fibres. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, R51.
- HILLE, B. (1977a). The pH-dependent rate of action of local anesthetics on the node of Ranvier. *J. Gen. Physiol.*, **69**, 475–496.
- HILLE, B. (1977b). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.*, **69**, 497–515.
- HONDEGHEM, L.M. & KATZUNG, B.G. (1977). Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochem. Biophys. Acta*, **472**, 373–398.
- HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: The modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 387–423.
- KOJIMA, M., BAN, T. & SADA, H. (1982). Effects of disopyramide on the maximum rate of rise of the action potential ($\dot{V}_{max}$) in guinea pig papillary muscles. *Jpn. J. Pharmacol.*, **32**, 91–102.
- KUS, T. & SASYNIUK, B. (1978). Disopyramide phosphate: is it just another quinidine? *Can. J. Physiol. Pharmacol.*, **56**, 326–331.
- SAIKAWA, T. & CARMELIET, E. (1982). Slow recovery of the maximal rate of rise ($\dot{V}_{max}$) of the action potential in sheep cardiac Purkinje fibres. *Pflugers Arch.*, **394**, 90–93.
- SCHWARZ, W., PALADE, PhT. & HILLE, B. (1977). Local anesthetics. Effect of pH on use-dependent block of sodium channels in frog muscles. *Biophys. J.*, **20**, 343–368.
- STARMER, C.F. & COURTNEY, K.R. (1986). Modeling ion channel blockade at guarded binding sites: application of tertiary drugs. *Am. J. Physiol.*, **251** (Heart Circ. Physiol.), **20**, H848–H856.

- STARMER, C.F., GRANT, A.O. & STRAUSS, H.C. (1984). Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. *Biophys. J.*, **46**, 15–27.
- STARMER, C.F., YEH, J.Z. & TANGUY, J. (1986). A quantitative description of QX 222 blockade of sodium channels in squid axons. *Biophys. J.*, **49**, 913–920.
- STRICHARTZ, G.R. (1973). The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.*, **62**, 37–57.
- STRICHARTZ, G. & COHEN, I. (1978). $\dot{V}_{max}$ as a measure of G_{Na} in nerve and cardiac membranes. *Biophys. J.*, **23**, 153–156.
- WELD, F.M., COROMILAS, J., ROTTMAN, J.N. & BIGGER, Jr. J.Th. (1982). Mechanisms of quinidine-induced depression of maximum upstroke velocity in ovine cardiac Purkinje fibres. *Circ. Res.*, **50**, 369–376.
- YEH, J.Z. (1979). Dynamics of 9-aminoacridine block of sodium channels in squid axons. *J. Gen. Physiol.*, **73**, 1–21.
- YEH, J.Z. & TANGUY, J. (1985). Na channel activation gate modulates slow recovery from use-dependent block by local anesthetics in squid giant axons. *Biophys. J.*, **47**, 685–694.
- YEH, J.Z. & TEN EICK, R.E. (1987). Molecular and structural basis of resting and use-dependent block of sodium current defined rising disopyramide analogues. *Biophys. J.*, **51**, 123–135.

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