Br. J. Pharmacol. (1988), **93**, 311–318

Quinidine blocks cardiac sodium channels during opening and slow inactivation in guinea-pig papillary muscle

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1 In order to quantify the time- and voltage-dependent block of sodium channels by quinidine, we voltage clamped guinea-pig papillary muscles and measured the maximum upstroke velocity ($\dot{V}_{max}$) of the cardiac action potential.

2 Quinidine reduces $\dot{V}_{max}$ presumably by blocking cardiac sodium channels. In therapeutic concentrations, quinidine causes a small amount of tonic block. Upon depolarization of the cardiac cell membrane, a use-dependent block develops.

3 A slow component of use-dependent block has time- and voltage-dependence similar to that of slow inactivation, develops for the duration of the depolarization or until a steady state is reached.

4 In addition, closely associated with the action potential upstroke, a fraction of the channels blocks very quickly. This represents block of activated or open channels.

5 Near the normal resting potential, channels recover from block with a time constant of 3 to 8 s. At more negative membrane potentials recovery from block occurs slightly faster, while at more positive potentials recovery from block proceeds somewhat more slowly.

6 In terms of the modulated receptor hypothesis, quinidine has a low affinity for the rested state, avidly blocks open sodium channels, but does not bind significantly to inactivated channels. In addition, quinidine blocks channels as they exhibit slow inactivation.

Introduction

Quinidine is a frequently used oral antiarrhythmic agent (Hondeghem & Mason, 1987). It belongs to class Ia (Vaughan Williams, 1985) and is most effective against atrial and ventricular arrhythmias and is believed to act, at least to a large extent, by blocking sodium channels (West & Amory, 1960). The magnitude of the block of sodium channels by quinidine is strongly frequency-dependent (Johnson & McKinnon, 1957) and the drug also shifts the inactivation curve to more negative potentials (Weidman, 1955). In 1977, Hille (1977) and Hondeghem & Katzung (1977) proposed that the affinity of local anaesthetic type antiarrhythmic agents for the sodium channel receptor is modulated by the primary states (rested, open and inactivated; see Figure 1) of the channel, which are in turn controlled by transmembrane potential (Hodgkin & Huxley, 1952). In general, clinically useful antiarrhythmic agents have a low affinity (mM to M range) for the rested state, while they have a higher affinity (μM range) for the open or inactivated states (Hondeghem & Katzung, 1984). Thus, during each depolarization sodium channels become blocked, while between depolarizations sodium channels recover from block.

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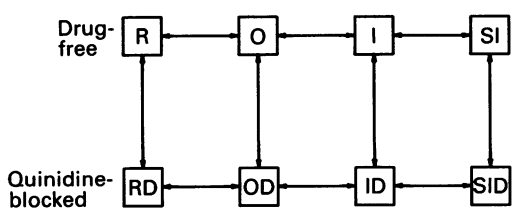

Figure 1 Modulated receptor pools with the addition of slow inactivation (SI). R: rested, O: open, I: inactivated state, RD, OD, ID and SID are the respective drug-associated states.

Hondeghem & Katzung (1977) derived a set of differential equations for the modulated receptor hypothesis and estimated from available experimental results from various laboratories that quinidine had a high affinity for open sodium channels (μM range) but had a much lower affinity for rested and inactivated channels. The estimated rate constants could approximate the action of quinidine over a wide range of experimental conditions (Hondeghem & Katzung, 1980). However, experiments to quantitate specifically the modulated receptor affinities have not yet been designed.

More importantly, a few experimental observations have been made that cannot be accounted for in terms of the modulated receptor hypothesis. First, during long depolarizations quinidine reduced the sodium current availability in a time- and voltage-dependent manner (Weld *et al.*, 1982; Hondeghem & Matsubara, 1984). This was somewhat surprising, because it had been found that the block of inactivated channels by lidocaine (Bean *et al.*, 1983; Sanchez-Chapula, 1985; Matsubara *et al.*, 1987) did not depend upon membrane potential over the -40 to $+40$ mV range. Second, in voltage-clamped cardiac myocytes no use-dependent reduction of the sodium current had been found in the presence of quinidine (Lee *et al.*, 1981).

The present experiments were undertaken to (i) characterize the relative contributions of block of rested, open and inactivated channels by quinidine; (ii) elucidate the mechanism for the time- and voltage-dependent block during prolonged depolarizations; (iii) explore the mechanism for lack of use-dependent block, and (iv) estimate the rate constants of binding for the various states of the sodium channel.

Methods

Guinea-pigs (200–450 g) were killed by cervical dislocation. Papillary muscles that had a diameter of 0.3 to 0.8 mm and a length of at least 3 mm were dissected from the right ventricle and mounted into a three compartment sucrose gap (New & Trautwein, 1972). Less than 1 mm of the tip of the papillary muscle protruded in the active chamber where measurements were made in a buffered salt solution (36°C) equilibrated with 100% oxygen and having the following composition (in mM): NaCl 143, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1.1, D-glucose 5 and HEPES 5 (pH 7.4). The middle chamber was perfused by isotonic sucrose, enriched with 40 μM calcium chloride and 5 mM glucose. The posterior chamber was perfused with the above salt solution in which all NaCl was replaced with KCl.

Whenever therapeutic concentrations (3.7 to 10 μM) of quinidine (Hoffman, 1957) were added to the bath, the drug was allowed to equilibrate for at least 40 min before experimental determinations were made. All results presented were done at the low therapeutic concentration of 3.7 μM, except for the experiment shown in Figure 3 which was done at the high therapeutic concentration of 10 μM. Membrane potential was measured using conventional micro-electrodes filled with 3 M KCl and a high impedance voltage follower with negative capacitance adjustment. The stimulation or voltage clamp current was applied via a large silver chloride electrode to the posterior chamber and through the segment in the sucrose gap into the anterior chamber, where a current to voltage transducer clamped the bath to ground (New & Trautwein, 1972). The command voltages were generated by a micro-processor based stimulator.

During pulse protocols, the membrane potential was voltage clamped at all times except during the stimulus and the 5 ms following the stimulus, when the action potential upstroke was allowed to run freely. The membrane's potential could be clamped to within 2 mV of the command potential in 1 to 2 ms. The action potential upstrokes were elicited by brief current pulses of 1 ms duration. The intensity of the current pulses was adjusted in order to maintain a constant latency between the beginning of the stimulus and the maximum rate of rise of the cardiac upstroke, $\dot{V}_{max}$ (Walton & Fozzard, 1979). $\dot{V}_{max}$ was measured using a differentiator and peak detector circuit (Hondeghem & Cotner, 1978). All experimental procedures were separated by 20 s rest periods at the resting membrane potential. The time course of block development during depolarizing pulses (-40 to $+40$ mV range) and recovery from block following repolarization (-70, -85 or -100 mV) was defined using a least square error non-linear exponential fitting routine. All $\dot{V}_{max}$ values were normalized to the value of $\dot{V}_{max}$ observed at a slow stimulation rate under controlled conditions. Paired comparisons between data points were done by use of Student's t test. Differences were considered significant if the P value was less than 0.05. All results are expressed as mean $\pm$ s.d. Least square error searches for the modulated receptor affinities were done as described in detail by Moyer (1985).

Results

Tonic and use-dependent effects of quinidine

The reduction of $\dot{V}_{max}$ by quinidine consists of two components: tonic and use-dependent (Hondeghem & Katzung, 1984). The tonic component is the reduction of $\dot{V}_{max}$ that persists even after a long rest period. At the resting potential (-85 mV) therapeutic concentrations of quinidine (< 10 μM) induce a small tonic reduction of $\dot{V}_{max}$ (to 0.89 ± 0.03) and this small

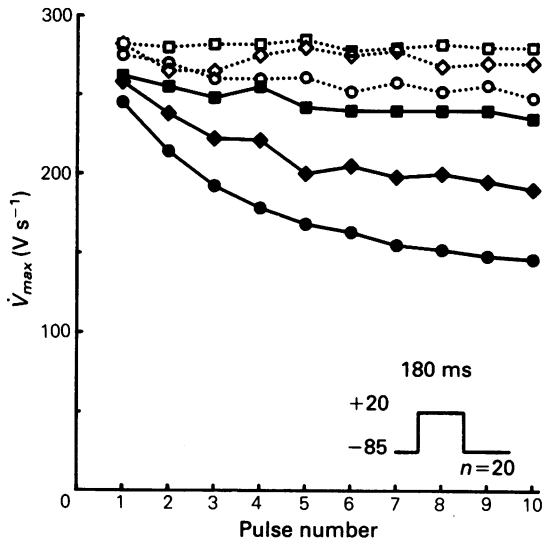

Figure 2 Tonic and use-dependent reduction of $\dot{V}_{max}$ by quinidine in a typical experiment. The use-dependent effects increase as the cycle length is shortened from 1,000 (□, ■) to 400 (◇, ◆) and 300 (○, ●) ms. Trains of 10 pulses applied after a 20 s rest period are shown for a typical experiment (similar results were obtained in 5 additional experiments). Control: broken lines and open symbols: quinidine (3.7 μM) solid lines and closed symbols.

reduction is significant ($P < 0.01$, $n = 7$). In two experiments, hyperpolarization to −100 mV attenuated tonic block, while depolarization to −70 mV augmented tonic block.

The use-dependent component consists of the additional reduction of $\dot{V}_{max}$ that develops upon application of depolarizing pulses. Under control conditions, application of a train of 180 ms depolarizations to +20 mV from a holding potential of −85 mV (inset Figure 2) at cycle lengths of 400 and 1000 ms results in little or no change of $\dot{V}_{max}$. Only at short cycle lengths (<300 ms) is there a small (10 ± 1.8%, $P < 0.01$, $n = 8$) reduction of $\dot{V}_{max}$ which probably reflects accumulation of slow inactivation (Clarkson *et al.*, 1984). In the presence of quinidine (3.7 μM), $\dot{V}_{max}$ declines on a beat basis until a steady state is reached. In the present experiments, the steady state reduction of $\dot{V}_{max}$ was frequently not reached within the application of 20 pulses (Heistracher, 1971). It is clear from Figure 2 that the reduction of $\dot{V}_{max}$ was frequency-dependent and was most marked at short cycle lengths (Johnson & McKinnon, 1957). At a cycle length of 300 ms, the use-dependent block developed in 10 depolarizations, was large (43 ± 6%, $P < 0.01$, $n = 6$). Upon the termination of the pulse train at a holding potential of −85 mV, $\dot{V}_{max}$ recovered with a time constant between 3 and 8 s (Weld *et al.*, 1982).

Block of channels during slow inactivation

In order to characterize the development of block during a depolarizing pulse, we interrupted the conditioning pulse at various times and following a fixed 100 ms recovery period at −100 mV applied a test pulse (inset Figure 3). A 100 ms recovery period was long enough for full recovery of the drug-free channels but still short enough so that a substantial reduction of $\dot{V}_{max}$ persisted (with a time constant of recovery from block of 3 to 8 s, less than 5% of $\dot{V}_{max}$ is expected to recover in this 100 ms interval). Therefore, the $\dot{V}_{max}$ of the test pulse is an indicator of the time course of block development during the conditioning pulse.

Under control conditions, for a pulse amplitude (to +20 mV) and a duration typical of the cardiac action potential (320 ms) $\dot{V}_{max}$ of the test pulse declines little (7 ± 4%; $n = 6$). The small decline of $\dot{V}_{max}$ observed probably results from incomplete reactivation and slow inactivation (Clarkson *et al.*, 1984). In the presence of quinidine (3.7 μM) $\dot{V}_{max}$ declined by 11 ± 5%, and this slightly larger reduction of $\dot{V}_{max}$ was significantly larger than in the control ($P < 0.05$). Although these results might indicate that quinidine blocks sodium channels while inactivated, careful

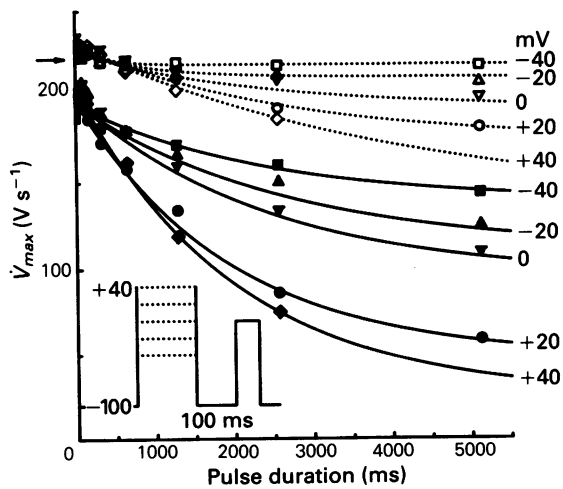

Figure 3 Reduction of $\dot{V}_{max}$ as a function of depolarizing potential and pulse duration (see Figure 2 legend). In the presence of quinidine (10 μM; closed symbols and solid lines) $\dot{V}_{max}$ declined with prolongation of the conditioning pulse duration, but the reduction was markedly dependent upon membrane potential. The average of 6 similar experiments at 3.7 μM are summarized in the text. Control: broken lines and open symbols.

examination of the block development at various membrane potentials and for various durations suggests that the reduction of $\dot{V}_{max}$ by quinidine might be related to slow inactivation.

In order to determine the voltage-dependence of block development during prolonged depolarizations, we clamped the membrane potential of the conditioning pulse to various levels between −40 and +40 mV for various durations starting from 5 ms and doubling until a duration of 5120 ms was reached (Figure 3). When clamping to +40 mV, the longest pulse was limited to 2560 ms (previous experiments showed that longer pulses requiring this current level might damage the preparation).

Under control conditions following prolonged depolarizations, there is a progressive reduction of $\dot{V}_{max}$ and this reduction is more marked at more positive membrane potentials. This time- and voltage-dependent reduction of $\dot{V}_{max}$ was previously described as slow inactivation (Saikawa & Carmeliet, 1982; Clarkson *et al.*, 1984). In the presence of quinidine, this voltage-dependent reduction of $\dot{V}_{max}$ is more marked. It is interesting to note that the voltage-dependence and the time course of the reduction of $\dot{V}_{max}$ by quinidine is similar to that of slow inactivation under control conditions. Similar results were obtained in six experiments. When clamping to the plateau range of the action potential (+20 mV) no significant difference exists between the time constants of development of slow inactivation in control (1888 ± 817 ms) and those in the presence of quinidine (2882 ± 1361; $n = 6$, $P > 0.05$). However, quinidine significantly increases the amplitude of slow inactivation from 35 ± 18% to 86 ± 29% ($P < 0.05$).

Block of open channels by quinidine

Extrapolation of the reduction of $\dot{V}_{max}$ by slow inactivation in the presence of quinidine (Figure 3), indicates that a small amount of block appears to develop instantaneously; i.e., it cannot be accounted for by tonic block (arrow on ordinate scale) or block during slow inactivation alone (extrapolation to time zero on the ordinate scale). It is during this early phase of the depolarization that the sodium channels are open. This suggests that channels become blocked while they are open, and that open channel block occurs with fast kinetics.

In order to gain further evidence for the presence of open channel block, the effect was examined by using pulse patterns that maximize the fraction of time channels spend in the open state, i.e. using very short depolarizing pulses (to keep inactivation time to a strict minimum) with an interpulse interval just long enough for sodium channels to recover from inactivation. In six experiments, under control conditions, depolarizations to +20 mV for 5 ms alternating with 30 ms repolarizations to −100 mV resulted in only small (<10%) reductions of $\dot{V}_{max}$. However, when repeating this pulse pattern in the presence of quinidine (3.7 μM; see Figure 4), $\dot{V}_{max}$ declined to about 50% in 20 pulses, and half of this reduction was reached in about five depolarizing pulses. More importantly, the reduction of $\dot{V}_{max}$ by a fast pulse train exceeded the reduction that could be obtained if channels were kept depolarized for an equivalent time period; e.g., compare the effect of eight 5 ms depolarizations in Figure 4 with that of a single 40 ms depolarization. This demonstrates that frequent opening of the channels induces a reduction of $\dot{V}_{max}$ that cannot be accounted for by block of channels in the I and SI states alone. Similar but more pronounced effects were observed in the presence of 10 μM quinidine ($n = 1$).

Recovery from block induced during slow inactivation

In four experiments we measured recovery from block after a pulse train and after a single long depolarization. Both methods of measuring recovery were characterized by a faster and a slower phase. After a pulse train the fast component had a time constant of about 20 to 30 ms (see initial 100 ms in Figure 5) and probably represents reactivation of drug-free channels or use-dependent unblock (Snyders & Hondeghem, 1987), while the slower component had a time

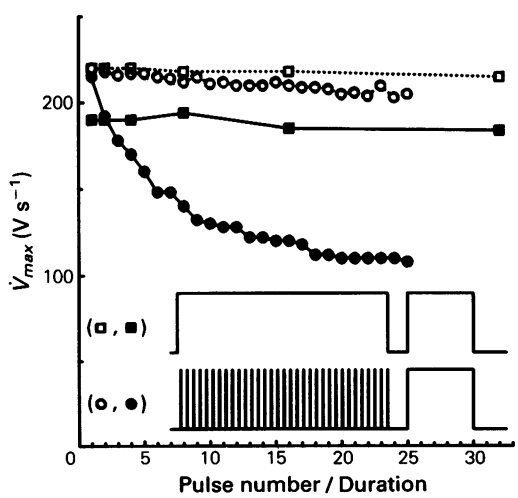

Figure 4 Reduction of $\dot{V}_{max}$ by a train of short (5 ms) pulses applied at a short (35 ms) cycle length. The pulse train was preceded by a 20 s rest period. Control (○, □); quinidine (3.7 μM; ●, ■). The abscissa scale represents the pulse number for the short pulses (○, ●), or the duration (in ms) of a single long pulse can be obtained by multiplying the number on the abscissa scale by 5 (□, ■).

constant between 3 and 8 s (at −85 mV), and represents recovery from quinidine block. Following a single long pulse, the fast component had a time constant of 0.5 to 2 s, typical for recovery from slow inactivation (Clarkson *et al.*, 1984), while the slower component had again a time constant of 3 to 8 s. Moreover, as for block developed by a pulse train, recovery from block after a single long depolarizing pulse was slightly slowed by depolarization to −70 mV ($n = 3$) and occurred somewhat faster upon hyperpolarization to −100 mV ($n = 1$). Thus, in the present experiments we could not resolve a clear difference between recovery from block by quinidine following a pulse train or a single long pulse.

Discussion

Our results clearly demonstrate that quinidine blocks cardiac sodium channels in a use-dependent manner; the more frequently the channels are depolarized per unit time, the greater the block. Moreover, the block associated with depolarization consists of two components: an early, virtually instantaneous phase which develops within the first few ms of membrane depolarization followed by a slower phase of block development having a time constant around two seconds. Since the rapid phase occurs in close association with channel opening and the slow phase exhibits time- and voltage-dependence typical of slow inactivation, we refer to these two phases as open channel block (O to OD transition in Figure 1) and block of channels during slow inactivation (SI to SID transition). We realize that 'open' channel block could actually be block of closed channels shortly before or immediately after opening rather than during opening. Similarly, block during slow inactivation could be binding of quinidine to a conformation of the channel receptor that is not slow inactivation itself, but just closely associated in voltage and time with slow inactivation. Unequivocal answers to the latter molecular questions will require more microscopic techniques (e.g., single channel experiments); nevertheless, our experiments clearly demonstrate that there exists a fast component of block closely associated with channel opening, and a slower phase of block that is closely associated with slow inactivation of the sodium channel.

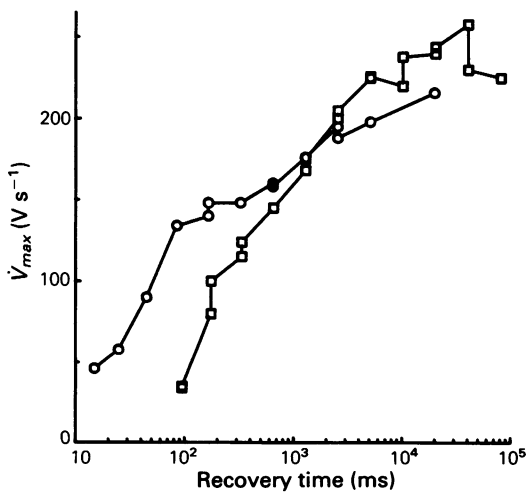

Figure 5 Comparison of recovery from block by quinidine after a pulse train (180 ms + 20 mV, 20 pulses; ○) and a single pulse (5 s, + 20 mV; □). The slow phase of recovery of $\dot{V}_{max}$ represents unblocking of quinidine and was similar for both pulse protocols. The early phase was faster after a pulse train (reactivation from inactivation) than after a single long pulse (reactivation from slow inactivation).

In contrast, when channels are maintained in the rested state, only a small amount of tonic block is present. Since tonic block results not only from block of rested channels but also from block of the other channel states (Hondeghem & Katzung, 1984), this indicates that quinidine must have a very low affinity for channels in the rested state. Indeed, after a long rest period any block of open channels occurring during the upstroke of the first action potential but before $\dot{V}_{max}$ will appear as tonic block. Similarly, any trapping of sodium channels via the I state (probably not very important for quinidine) or the SI state will present itself as tonic block.

Affinity for inactivated channels either must be very low or binding must occur very slowly. Indeed, most if not all of the block that develops during a depolarizing pulse can be accounted for by block of channels in the O and SI states. Actually, for a single brief depolarization where there is little or no slow inactivation, there is little or no development of block by quinidine (see, for example, Figure 4). At cold temperatures, where slow inactivation is minimal, quinidine was found to block mostly open channels, while causing no further block during prolonged depolarizations (Colatsky, 1982).

It was not possible to stimulate the long depolarizations with the modulated receptor hypothesis (Hondeghem & Katzung, 1977), since slow inactivation is not incorporated in the drug-free model. For stimulation of 62 experimental points consisting of short depolarization only, the least square error determination (normalized MSE = 0.004) indicated a low affinity for the rested and inactivated states (K_{dR} and K_{dI} both exceeding 10 mM) and a high affinity for the open state ($K_{dO} = 70\ \mu M$), while the voltage shift was estimated at 32 mV (Moyer, 1985). These results are similar to the earlier affinity estimates for the

modulated receptor affinities (Hondeghem & Katzung, 1977). In addition, under conditions where slow inactivation develops, quinidine appears to reduce markedly $\dot{V}_{max}$. Although numerous mechanisms could account for the present results, the two most simple schemes are that quinidine promotes slow inactivation (I to SI in Figure 1) or that quinidine avidly binds to channels during slow inactivation (SI to SID). Our observations cannot conclusively discriminate between these two possibilities. However, we favour the latter because the reduction of $\dot{V}_{max}$ by a long depolarizing pulse in the presence of quinidine recovers during diastole with a time constant that is similar to that for recovery from quinidine block induced by a pulse train, but slower than the recovery from slow inactivation. Also, over the -70 to -100 mV range the time constant of recovery of $\dot{V}_{max}$ following a long depolarization exhibits only a moderate voltage-dependence, which is similar to the shallow voltage-dependence for recovery from quinidine block after a pulse train (Weld *et al.*, 1982). In contrast, recovery from slow inactivation exhibits a much more marked voltage-dependence over the -70 to -100 mV range (Clarkson *et al.*, 1984). Conclusive statements will, however, require additional experiments combined with more detailed analyses.

The possibility was considered that both use-dependent block observed during the fast pulse train (Figure 4) and the fast component of block development might result from a drug-induced slowing of reactivation of drug-free channels instead of channel block (Clarkson *et al.*, 1984). However, reactivation of drug-free channels remained fast in the presence of quinidine (Figure 5). This hypothesis also was tested by comparing the reduction of $\dot{V}_{max}$ produced by a single conditioning pulse to $+20$ mV with that produced by a train of 5 ms conditioning pulses of equal total duration at $+20$ mV (Figure 4). If the rapid component of $\dot{V}_{max}$ reduction was due primarily to a slowing of reactivation of drug-free channels, a single short conditioning pulse to $+20$ mV that produces only a minimal amount of block during slow inactivation should produce a similar level of block as a train of short pulses having an equal total duration. However, this was not observed. Conditioning pulses of duration between 10–150 ms produced substantially less block than that produced by a train of 5 ms pulses of equal total duration at $+20$ mV (Figure 4). These data suggest that the rapid component of $\dot{V}_{max}$ depression observed during a two-pulse protocol (Figure 3) is due to rapid block of open channels and not to an effect of quinidine on the recovery time course of drug-free channels.

In a previous voltage-clamp study in which sodium current was measured directly, it was demonstrated that quinidine does not cause use-dependent block (Lee *et al.*, 1981). Discrepancies between results obtained from $\dot{V}_{max}$ versus I_{Na} measurements have been ascribed to non-linear behaviour of $\dot{V}_{max}$ as an estimator of sodium conductance (Cohen *et al.*, 1984). Although at 15°C there exists some non-linearity between I_{Na} and $\dot{V}_{max}$ it is relatively small (Fozzard *et al.*, 1986) and this non-linearity further declines with increased temperature (Fozzard, personal communication). Moreover, when comparing the results obtained by both techniques, the similarities are vastly more impressive than the differences (Grant *et al.*, 1980). More importantly, as long as a monotonic relationship exists between $\dot{V}_{max}$ and I_{Na} results obtained using $\dot{V}_{max}$ should at least be qualitatively correct. Lack of open channel block may instead result from differences in experimental condition. Specifically, in their voltage clamp experiments (Lee *et al.*, 1981) the authors clamped to rather negative potentials (more negative than -20 mV). In nerve it has been clearly demonstrated that block of open channels by local anaesthetics is strongly voltage-dependent (Yeh & Narahashi, 1977; Cahalan, 1978). Actually, when pulses are applied to relatively negative potentials, but positive enough to open sodium channels, unblocking may occur while the channels are open (Strichartz, 1973). In contrast, as the potential during the open state is made more positive, block progressively increases (Yeh & Narahashi, 1977; Cahalan, 1978). Preliminary results from our laboratory have shown that a similar voltage-dependence of open channel block by quinidine can be defined when measuring I_{Na} in voltage clamped isolated ventricular myocytes of the guinea-pig (Clarkson; unpublished observation). In the present experiments, the cardiac action potential usually runs freely to about $+30$ mV, so that open channel block would be expected to be prominent.

The extent of block by quinidine is a balance between block development during depolarization (O to OD and SI to SID) and the recovery from block during diastole. The latter is slightly voltage-dependent: recovery is somewhat faster at more negative potentials (Hondeghem & Katzung, 1980; Weld *et al.*, 1982). More importantly, as the diastolic potential is made more negative, open channel unblocking (OD to O in Figure 1) becomes more prominent (Snyders & Hondeghem, 1987). Thus, for a given pulse train as diastolic potential is made more negative, there will be more recovery from block and less use-dependent block will accumulate. However, at more positive holding potentials, tonic block (for quinidine mostly through accumulation of block during slow inactivation) will increase. As a consequence, fewer sodium channels are available for use-dependent block. In the extreme case when most channels are blocked by tonic block (at very high concentrations of quinidine especially at depolarized potentials), little or no use-dependent block may be observed (Lee *et al.*, 1981).

The relative contributions of open channel block and block during slow inactivation by quinidine will depend upon the voltage-time profile. Thus, at the onset of frequent short action potentials (e.g., atrial tachycardia) open channel block may be most important. In contrast, during prolonged depolarization (e.g., in ischaemia) block of channels in the SI state may be more important. Since slow inactivation is minimal in normal cardiac tissue and at normal heart rates (Clarkson *et al.*, 1984), agents that block channels in the SI state have an excellent mechanism to block selectively sodium channels under arrhythmogenic conditions.

Conclusion

The reduction of $\dot{V}_{max}$ by quinidine consists of tonic block (reduction of $\dot{V}_{max}$ of first pulse after a long rest period; see arrow in Figure 3) and use-dependent block (which develops upon depolarizing the cells). Use-dependent block has two components: block of open channels (developing during the upstroke of the action potential) and block of sodium channels during slow inactivation. The latter would be most important in tachycardias and in depolarized cardiac tissue.

Part of this work was done in the Department of Pharmacology University of California, San Francisco. The authors wish to thank Dr Bert Katzung for his numerous helpful suggestions and Mr Cotner for the design and construction of the electronics without which these experiments would not have been possible. The authors are also indebted to Mrs Mimi Klein and Ms Leah Himmelberg for their editorial assistance. This work was supported by grants HL 36020 and HL 21672 from the National Institutes of Health.

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(Received May 28, 1987.
Revised August 17, 1987.
Accepted August 20, 1987.)