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VOLTAGE- AND TIME-DEPENDENT DEPRESSION OF MAXIMUM RATE OF DEPOLARIZATION OF GUINEA-PIG VENTRICULAR ACTION POTENTIALS BY TWO STEROIDAL ANTIARRHYTHMIC DRUGS, CCI 22277 AND ORG 6001

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1 The voltage- and time-dependence of the depression of the maximum rate of depolarization ($\dot{V}_{max}$) by two steroidal anti-arrhythmic drugs, CCI 22277 and Org 6001 were studied in guinea-pig ventricle.

2 At normal resting potentials CCI 22277 (2 μM and 4 μM) produced very little depression of $\dot{V}_{max}$ at very low driving rates (resting block) but trains of stimuli at interstimulus intervals (ISI) of less than 10,000 ms led to an exponential decline in $\dot{V}_{max}$ to a new plateau over 100–200 beats.

3 This 'rate-dependent block' (RDB) increased with rate over the range ISI = 4800 to ISI = 200 ms.

4 Org 6001 30 μM and 60 μM produced a similar degree of RDB over the same range of frequencies but the new plateau level of $\dot{V}_{max}$ was reached much more rapidly (20–30 beats) and there was a moderate degree of depression of $\dot{V}_{max}$ in the resting tissue.

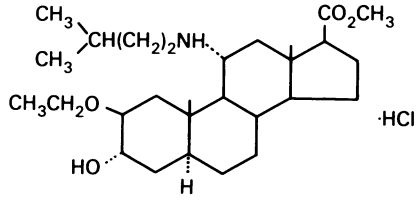
5 Recovery from RDB in the presence of both drugs was an exponential process with time constants (τre) of 80.4 ± 7.4 s for CCI 22277 and 4.6 ± 0.5 s for Org 6001.

6 Both drugs shifted the steady-state inactivation curve, relating $\dot{V}_{max}$ to resting membrane potential, in the hyperpolarizing direction, implying selective depression of depolarized cells.

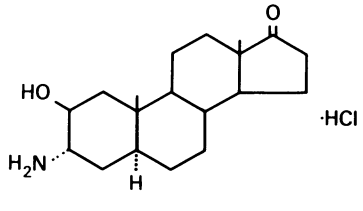
Introduction

It has been known for many years that the ability of many class I antiarrhythmic drugs (Vaughan Williams, 1980) to decrease the maximum rate of rise ($\dot{V}_{max}$) of cardiac action potentials is potentiated by either increasing stimulation frequency (Johnson & McKinnon, 1957; Heistracher, 1971) or by manoeuvres which reduce the resting membrane potential (Weidmann, 1955; Chen, Gettes & Katzung, 1975). Recent work on the effects of voltage and rate on class I activity has led to the proposal of a kinetic model for the interaction of these drugs with the sodium channel (Hondeghem & Katzung, 1977; 1980; Weld, Coromilas, Rottman & Bigger, 1982).

Org 6001 and CCI 22277 are both steroidal antiarrhythmic drugs (Figure 1), structurally unrelated to any other class I drugs, and it was therefore of interest to investigate whether either or both of them exhibited the time- and voltage-dependence observed with other drugs of this class. Org 6001 has considerable anti-arrhythmic potency in a number of animal models of arrhythmia (for review see Marshall & Winslow, 1981) and has been demonstrated *in vitro*, to cause a dose-dependent reduction of $\dot{V}_{max}$ without altering the resting membrane potential (Salako, Vaughan Williams & Wittig, 1976; Kane, 1980). CCI 22277 was developed more recently and shown to be more potent than Org 6001 at suppressing aconitine-



CCI 22277



Org 6001

Figure 1 Molecular structure of CCI 22277 and Org 6001.

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induced arrhythmias in the rat (Dodds, Dolamore, Sawyer, Straughan & Twissel, 1982). *In vitro* studies in this laboratory have confirmed it to be a predominantly class I drug with possibly some class IV activity at high concentrations (Campbell & Vaughan Williams, 1982). In addition, its class I potency was shown to be significantly enhanced by increasing the driving rate from 100/min to 200/min.

Methods

Guinea-pigs of either sex weighing 300–600 g were stunned and their hearts quickly removed. Strips were cut from the right ventricular free wall and pinned to the base of a bath (volume 8 ml) with their endocardial aspect uppermost. The tissue was superfused at approximately 12 ml/min with modified Locke solution gassed with 95% O_2 and 5% CO_2 and maintained at 36.5–37.0°C. The composition of the Locke solution was as follows (mM): NaCl 125, KCl 5.6, $CaCl_2$ 1.8, $NaHCO_3$ 25, glucose 11, and the pH was 7.5. Preparations were allowed to equilibrate for 1–2 h unstimulated. They were then driven at 1 Hz for 30–60 min during which time a stable impalement was obtained. A further period of 15–20 min without stimulation (to allow for 'sealing-in' of the microelectrode) preceded the recording of control values. The driving stimuli were provided from bipolar platinum electrodes, producing square waves of 2 ms duration (2 × threshold). Cumulative doses of drug were then added to the superfusate and at least 20 min exposure time allowed at each concentration before taking further readings. During this time, the preparation was not stimulated and remained quiescent. Action potentials were recorded by conventional 'floating' glass micro-electrodes filled with 3 M KCl coupled to a high input impedance d.c. amplifier with variable capacity compensation. The upstroke of the action potential was electronically differentiated to give the maximum rate of potential ($\dot{V}_{max}$), and this signal was fed to a sample-hold peak detector (modified after Hondeghem & Cotner, 1978). The output of this instrument was simultaneously displayed on a storage oscilloscope and also recorded on FM tape from which it would later be replayed at slow speed onto an X-Y plotter for analysis. To eliminate variation between cells within a preparation each experiment reported was performed during continuous impalement of a single cell.

To study rate-dependent effects, the preparations were driven by trains of stimuli at various rates and of sufficient duration to achieve a stable level of effect. Rest periods were interposed between trains of stimuli to ensure full recovery from rate-dependent effects. The kinetics of this recovery were studied by applying single extra-stimuli at varying intervals after the end of a train.

The voltage-dependence of the effects of the drugs was investigated by depolarizing the membrane potential as steps by the addition of small aliquots (0.1–0.2 ml) of 1 M KCl to the superfusate until the cell became inexcitable. In these experiments, the initial concentration of KCl was 2.8 mM rather than the usual 5.6 mM and the stimulus voltage was raised to 4 × threshold.

Where applicable, results have been given as means ± s.e.mean and the significance of differences between means estimated by Student's paired *t* test.

Org 6001 was kindly supplied by Organon Laboratories Ltd, and CCI 22277 by Glaxo Group Research Ltd who also provided the values for log P for the two drugs (calculated by the method of Rekker & de Kort, 1979).

Results

The results were obtained from a total of 26 cells, each from a separate preparation. The baseline characteristics (in control solution) of those cells

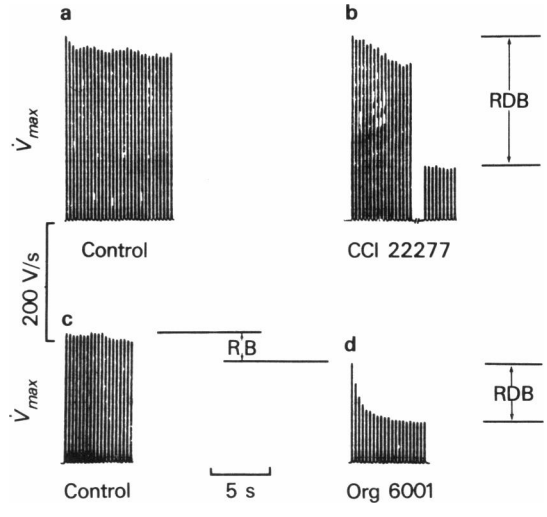

Figure 2 The effect on $\dot{V}_{max}$ of a train of action potentials in previously quiescent tissue in control solution and in the presence of CCI 22277 2 μM (b) and Org 6001 30 μM (d). The interstimulus interval in all cases is 300 ms. The spikes represent $\dot{V}_{max}$ of successive action potentials. There is minor rate-dependent depression (RDB) of $\dot{V}_{max}$ during the trains in control solution and marked depression of similar magnitude in the presence of either drug. RDB develops quickly in the case of Org 6001 but very slowly with CCI 22277 and hence only the first 17 and last 9 beats of a train of 200 beats are illustrated. There is also significant resting depression of $\dot{V}_{max}$ (RB) in the case of Org 6001 but not CCI 22277.

exposed to CCI 22277 ($n = 12$) were: resting membrane potential = -86.5 ± 0.2 mV; $\dot{V}_{max} = 286 \pm 12.1$ V/s; action potential amplitude = 125.3 ± 0.2 mV; action potential duration to 90% repolarization (APD_{90}) = 182 ± 3 ms.

The corresponding data for the cells exposed to Org 6001 ($n = 14$) were: resting potential = -86.7 ± 0.3 mV; $\dot{V}_{max} = 266.9 \pm 10.8$ V/s; amplitude = 125.0 ± 0.2 mV; $APD_{90} = 180 \pm 2$ ms. There are no significant differences in any of these parameters between control groups. Neither drug produced any effect on resting membrane potential.

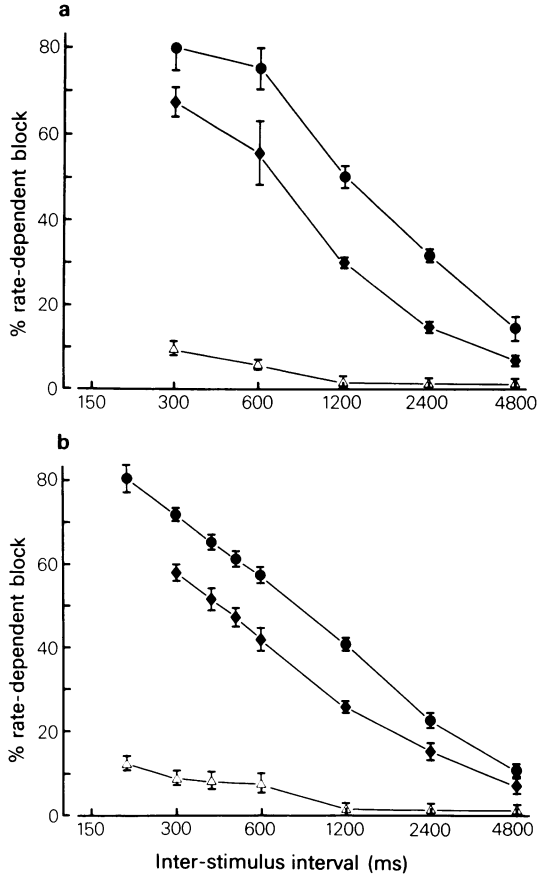

Figure 3 Relationship between the log of the interstimulus interval and the % rate-dependent reduction of $\dot{V}_{max}$ at two concentrations of CCI 22277 (a) and Org 60001 (b) (a) CCI 22277 4 μM (●), 2 μM (◆); control (△). (b) Org 6001 60 μM (●), 30 μM (◆); control (△). The points are means ($n = 4$ for CCI 22277 and $n = 7$ for Org 6001); vertical lines show s.e.mean.

Steady-state and rate-dependent depression of $\dot{V}_{max}$

CCI 22277. Even in the high concentrations used (2 μM and 4 μM), CCI 22277 produced very little depression of the $\dot{V}_{max}$ of the first action potential of a train of impulses after a prolonged period of quiescence (Figure 2). This steady-state or 'resting block' was expressed as a percentage decrease from the control value. It varied from 0 to 9. 4% at a concentration of 2 μM (mean $2.4 \pm 2.4\%$) and from 5–13% at 4 μM (mean $9.7 \pm 1.5\%$).

Continued, repetitive stimulation, however, produced a slow fall of $\dot{V}_{max}$ to a new equilibrium level which persisted indefinitely as long as the rate remained constant. The difference between this new plateau level and the $\dot{V}_{max}$ of the first beat of the train was expressed as a percentage decrease, and used as the measure of this rate-dependent block (RDB).

The value of this rate-dependent block increased markedly with increasing stimulation frequency over a range of interstimulus intervals (ISI) from 4800 ms to 300 ms (Figure 3), and fell to zero at ISI = 10 s. In six preparations exposed to a concentration of 2 μM CCI 22277, RDB rose from $6.5 \pm 0.8\%$ at ISI of 4800 ms to $68.0 \pm 3.6\%$ at ISI = 300 ms. The corresponding values for the 4 μM concentration were $14.5 \pm 3.0\%$ and $80.0 \pm 5.0\%$.

Org 6001. This compound produced a considerably greater resting block than CCI 22277 in concentrations which gave comparable values for RDB (Figures 2 and 3). Thus, resting block was $16.6 \pm 1.9\%$ at 30 μM and $39.4 \pm 3.3\%$ at 60 μM Org 6001 and once again, rate-dependent block rose sharply as the interstimulus interval decreased from 4800 ms to 200 ms. In the presence of 30 μM Org 6001, RDB was $7.6 \pm 1.4\%$ at ISI = 4800 ms and 57.7 ± 1.9 at ISI = 300 ms and at 60 μM Org 6001 these figures were $10.5 \pm 1.1\%$ and $71.6 \pm 1.2\%$ respectively (mean of 7 experiments).

Kinetics of onset of rate-dependent block

While CCI 22277 and Org 6001, in the concentrations used, produced very similar degrees of rate-dependent depression of $\dot{V}_{max}$ (Figure 3), it can be seen from Figure 2 that the rate at which $\dot{V}_{max}$ approached a new plateau level during a train of stimuli, differed markedly between the two drugs. Org 6001 required far fewer action potentials, at any given rate, to achieve a plateau level of depression of $\dot{V}_{max}$ than did CCI 22277. In both cases, however, the time course of onset of rate-dependent block was well fitted by a single exponential and estimates of the rate of onset were made from regression analysis of the first 16 action potentials of a train in the case of CCI 22277 and the first 10 in the case of Org 6001 (see

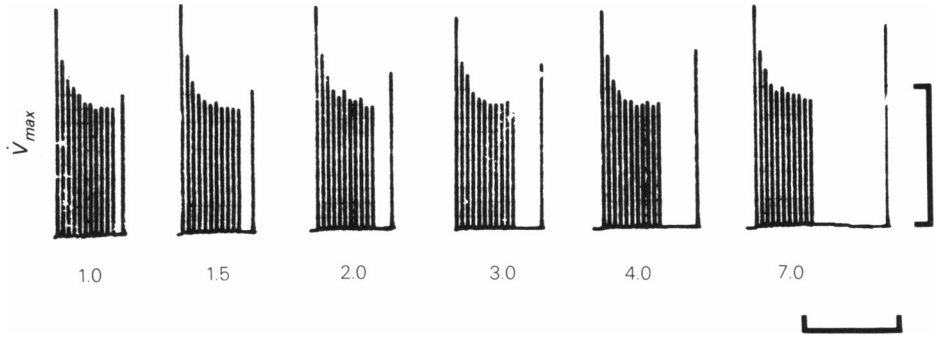

Figure 4 The method of estimating rate of recovery from rate-dependent block (RDB). The drug in this case is Org 6001 30 μM and the interstimulus interval of the trains is 600 ms. Numbers below each train represent the time (s) between the last beat of the train and the single extra-stimulus. At least 1 min rest was allowed between trains. Vertical calibration: 100 V/s. Horizontal calibration: 10 s.

Courtney, 1976). In six experiments at 2 μM CCI 22277, this rate was 0.014 ± 0.0009 AP^{-1} (AP = action potential) at ISI = 300 ms. The corresponding figure for 30 μM Org 6001 in six preparations was 0.079 ± 0.004 AP^{-1}.

Rate of recovery from rate-dependent block

The rate at which $\dot{V}_{max}$ recovered towards the initial resting block level at the end of a train of stimuli was studied by adding a single extra-stimulus at varying intervals after a series of trains of stimuli at a constant frequency (see Figure 4). The time-course of this process was also found to be well fitted by a single exponential which, in both drugs, was independent of the stimulation frequency of the train used to produce the RDB and of drug concentration. Once again the two drugs gave very different results. Recovery from RDB induced by CCI 22277 was very slow, with a time constant (τre) of 80.4 ± 7.4 s ($n = 5$) compared to 4.6 ± 0.5 s for Org 6001 ($n = 10$).

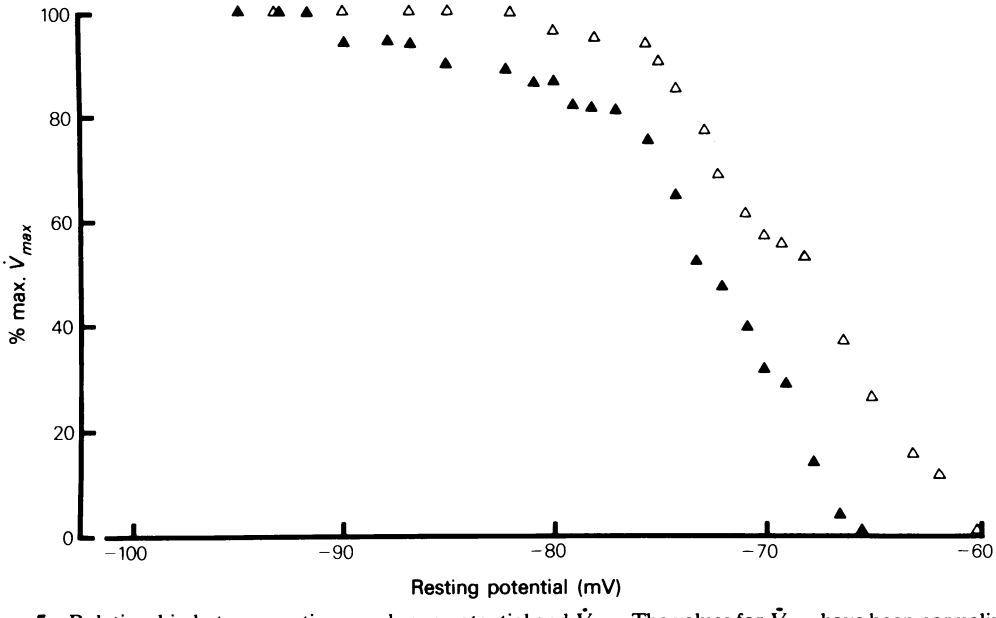

Figure 5 Relationship between resting membrane potential and $\dot{V}_{max}$. The values for $\dot{V}_{max}$ have been normalized by plotting as % of maximum $\dot{V}_{max}$ in control solution which, in this case, was 241 V/s. All recordings were from the same cell, in control solution (△) then in the presence of CCI 22277 2 μM (▲) which shifts the relationship in the hyperpolarizing direction.

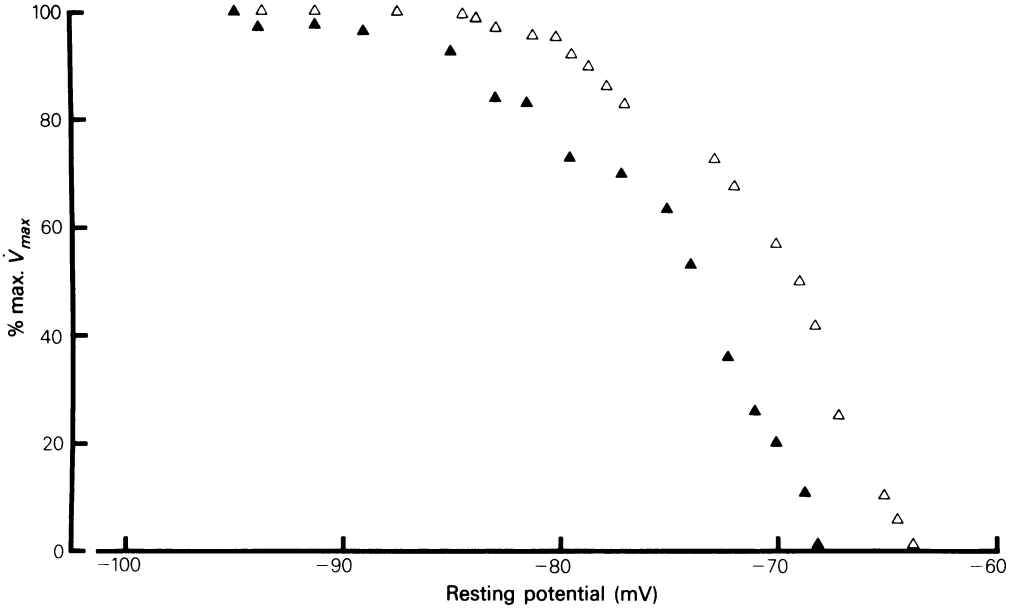

Figure 6 Relationship between resting membrane potential and $\dot{V}_{max}$; effect of Org 6001 30 μM. Plotted as in Figure 5 (Max. $\dot{V}_{max}$ in control solution = 253 V/s). Control (△); Org 6001 (▲). Again, the curve is shifted in the hyperpolarizing direction in the presence of drug.

Voltage-dependence of depression of $\dot{V}_{max}$

CCI 22277. A number of antiarrhythmic drugs including lignocaine (Chen *et al.*, 1975; Chen & Gettes, 1976) and procainamide (Sada, Kojima & Ban, 1979) have been shown to shift the steady-state inactivation ('h-infinity') curve, relating membrane potential to $\dot{V}_{max}$ in the hyperpolarizing direction. Figure 5 shows the data from one experiment with CCI 22277 in which membrane potential was varied in steps by the addition of small aliquots of 1 M KCl to the superfusate. This was done first in the absence of drug, then after washing off the excess KCl, repeated in the presence of 2 μM CCI 22277 while maintaining continuous impalement of the same cell. Intervals of at least 3 min between stimuli ensured that the effects of depolarization were being observed in the absence of any time-dependent effects of the drug.

It can be seen that in control conditions, in this cell, inexcitability (presumably due to inactivation of the sodium channels) occurred at a membrane potential of −61 mV and that $\dot{V}_{max}$ was reduced by 50% at −68.5 mV. CCI 22277 shifted this normalized curve in the hyperpolarizing direction so that inexcitability now occurred at −66 mV and 50% reduction of $\dot{V}_{max}$ at −64.8 mV. Two further such experiments gave qualitatively similar results though the voltage shifts in each case were larger, being 6.5 mV and 10 mV measured at the point of 50% reduction of $\dot{V}_{max}$.

In the range of resting potentials less negative than −80 mV, the curve relating $\dot{V}_{max}$ to resting potential is so steep that these small voltage shifts represent very large increases in the degree of resting depression of $\dot{V}_{max}$ by CCI 22277 (resting block), as the membrane is progressively depolarized. Thus in the experiment illustrated, resting block, which had been 0% at −95 mV rose to 13% at −80 mV, 25% at −70 mV and 35% at −67 mV. In the other two experiments the corresponding values were also 0% at −95 mV increasing to 39% and 12% at −80 mV, and 65% and 40% at −70 mV.

Org 6001. As seen in Figure 6, Org 6001 also produced a shift in the steady-state inactivation curve in the same direction and of similar magnitude to those produced by CCI 22277. In the experiment illustrated, Org 6001 30 μM has shifted the potential at which inexcitability occurred by 5 mV and the potential at which $\dot{V}_{max}$ was reduced by 50% is shifted 4.5 mV in the hyperpolarizing direction. This latter shift was 4.3 mV and 4.5 mV in two further preparations. Once again, these apparently small shifts in the inactivation curves, represent relatively large increases in resting block on depolarization. In the three experiments, resting block was 16%, 18.2% and 21.3% at −90 mV, 31%, 26% and 28% respectively at −80 mV and 50%, 45% and 40% at −70 mV.

Discussion

This study demonstrates that Org 6001 and CCI 22277 suppress the maximum rate of depolarization of guinea-pig ventricular action potentials in a manner that is both rate-dependent and voltage-dependent.

At interstimulus intervals (ISI) longer than 10 s, CCI 22277 had very little effect on $\dot{V}_{max}$ (−2.4% at 2 μM and −9.7% at 4 μM) whereas Org 6001 produced moderate depression of $\dot{V}_{max}$ even in the absence of stimulation (−16.6% at 30 μM and −39.4% at 60 μM). As ISI was reduced, both drugs exhibited progressively increasing reduction of $\dot{V}_{max}$ or rate-dependent block (RDB), which in the doses used was of comparable magnitude in the two drugs (Figure 3). However, when the kinetics of onset and offset of this RDB were studied, major differences between the drugs became apparent. While the final amounts of RDB were similar, the rate at which it was achieved and the time taken to recover from it were both much faster for Org 6001 than for CCI 22277.

Rate-dependence was first observed with quinidine by Johnson & McKinnon (1957) and has since been demonstrated in nerve and myocardium for a large number of class I drugs (Heistracher, 1971; Strichartz, 1973; Sada & Ban, 1980; 1981a, b; Courtney, 1975; 1979; 1980a, b, c; 1981). These workers and others also found that these drugs markedly prolonged the time constant of recovery from inactivation of the sodium channels, which is normally <300 ms (Gettes & Reuter, 1974; Brown, Lee & Powell, 1981). Attempts to explain these phenomena led to the proposal by Hondeghem & Katzung (1977, 1980), of a modulated receptor model for the interaction of class I drugs with the sodium channel. In this model, the sodium channel is assumed to exist in one of the three possible states, resting, inactivated and active, as proposed by Hodgkin & Huxley (1952). The drugs can bind to (and block) any of these three states but the affinity is greater in the active and/or inactivated states than in the resting state. This automatically produces the increase in the degree of block seen on repeated stimulation. Further, although the ultimate amount of RDB produced at a given rate depends largely on the affinity of the drug for the receptor, the rate at which the RDB develops or is removed may be limited by access to the receptor which is thought to be either from inside the cell or from the membrane phase. Ease of access and hence rapid onset and offset of block may be favoured by small molecular weight and lipophilicity. Courtney (1979, 1980c, 1981) has shown good correlation between molecular weight and rates of onset and offset of RDB for a number of class I drugs, and, by taking lipid solubility, estimated as the log of the *n*-octanol:water partition coefficient (log P), and pKa into account, has derived a regression equation which improves this correlation further. The degree of resting block, on the other hand, seemed to correlate much better with lipophilicity than with molecular weight. Sada & Ban (1981a, b) have published very similar findings for beta-adrenoceptor blocking drugs with class I activity.

The data obtained from the present study allow further insight into the structure-activity relations of class I antiarrhythmic drugs. CCI 22277 has a molecular weight of 463.7 whereas none of the drugs studied by Courtney (1979, 1980a, 1981) or Sada & Ban (1981b) had molecular weights above 350. In fact the rate of onset of RDB with 2 μM CCI 22277 ($0.014\ AP^{-1}$) was considerably slower than those reported at the same stimulation frequency (ISI = 300 ms) by Courtney (1980a) for nine antiarrhythmic and local anaesthetic drugs (range $0.04\ AP^{-1}$ to $0.60\ AP^{-1}$). These same nine drugs and 12 drugs studied by Sada & Ban gave time constants for recovery from RDB (τre) of from <0.5 s to approximately 10 s. Substituting the values of the molecular weight, pKa (9.5) and log P (7.19) of CCI 22277 into Courtney's (1981) regression equation gives a predicted τre of approximately 55 s which is in reasonable agreement with the figure actually obtained (80.4 s) and provides further evidence of the importance of molecular weight in limiting access to and removal from the sodium channel receptor. It is of interest to note at this point that studies in this laboratory on flecainide acetate, which has a molecular weight of 408, place it in an intermediate position, with a rate of onset of RDB of $0.028\ AP^{-1}$ and a τre of 15.5 s (Campbell & Vaughan Williams, unpublished).

Org 6001 is a considerably smaller molecule than CCI 22277 (molecular weight 305.4, pKa 8.0, log P 3.93) and therefore one would expect it to exhibit a faster onset and offset of RDB, as indeed was found. The rate of onset at ISI = 300 ms was $0.079\ AP^{-1}$ and τre was 4.6 s. This latter figure approximates reasonably with the value of 1.5 s predicted from the regression equation.

When one considers the ability of Org 6001 or CCI 22277 to produce depression of $\dot{V}_{max}$ in unstimulated myocardium (resting block) however, there is no such agreement with predictions. Previous studies have shown better correlation between resting block and lipophilicity than molecular weight (Courtney, 1980b; Sada & Ban, 1981a, b) and it has been concluded that resting block represents the ability of the drug to gain access to the receptors of closed sodium channels via the lipid membrane phase. Thus CCI 22277 (log P = 7.19) would be expected to produce more resting block than Org 6001 (log P = 3.93) in concentrations of similar potency in stimulated tissue. In fact, while Org 6001 produced

moderate resting block at 30 μM and 60 μM, CCI 22277 produced very little at 2 μM or 4 μM. One might argue that this is due to the higher pKa of CCI 22277 so that relatively more of the compound would be in the lipid-insoluble, cationic form at pH 7.5. This difference can be 'corrected for' by estimating the log of the distribution coefficient (log Q = log P − log $(1 + 10^{pKa - pH})$, (see Courtney, 1980c). This however produces a log Q for CCI 22277 of 5.19 at pH 7.5 which is still almost two log units greater than that for Org 6001 (3.31). It seems likely, therefore, that the large size of the CCI 22277 molecule does significantly impair its access to the sodium channel despite the very high lipid solubility of the drug.

Finally, both CCI 22277 and Org 6001 shifted the relation between $\dot{V}_{max}$ and resting potential in the hyperpolarizing direction as has been reported for many other class I drugs including lignocaine (Chen *et al.*, 1975), procainamide (Sada *et al.*, 1979) disopyramide (Kus & Sasyniuk, 1978) and mexiletine (Hohnloser, Weirich & Antoni, 1982). This has been interpreted as indicating a greater affinity of the drug for the inactivated than for the resting channels (Hondeghem & Katzung, 1977) or alternatively, that binding is directly dependent on membrane potential rather than on the functional state of the channels, (Weld *et al.*, 1982). Whatever the mechanism however, it implies a relatively selective depression of $\dot{V}_{max}$ and hence of conduction, in depolarized myocardium. Furthermore this effect is independent of rate. As partial depolarization is a well-known feature of myocardial ischaemia (see Janse & Kléber, 1981, for review), such a selective effect, already demonstrated for lignocaine (Kupersmith, Antman & Hoffman, 1975; El-Sherif, Scherlag & Lazzara, 1977), could well be of considerable clinical relevance.

Finally, it should be noted that any effect on refractoriness due to this shift in the $\dot{V}_{max}$-membrane potential relationship would only be minor, since membrane voltage is changing very rapidly with time during the terminal phase of the action potential.

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