Br. J. Pharmac. (1982), **76**, 337–345

ELECTROPHYSIOLOGICAL AND OTHER EFFECTS ON RABBIT HEARTS OF CCI 22277, A NEW STEROIDAL ANTIARRHYTHMIC DRUG

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1 CCI 22277 (methyl 2β-ethoxy-2α-hydroxy-11α-(3-methylbutylamino)-5α-androstane-17β-carboxylate hydrochloride) is an aminosteroid with antiarrhythmic properties in animal models.

2 It slowed significantly conduction velocity and spontaneous frequency.

3 CCI 22277 was a local anaesthetic on frog nerve, 62 times more potent than procaine. It greatly reduced the maximum rate of depolarization (MRD) in atria, Purkinje cells and ventricles, and reduced the overshoot potential. The effects were frequency-dependent and it was concluded that the drug probably delayed recovery from inactivation of fast inward current.

4 CCI 22277 had no anti-adrenergic actions, nor did it prolong action potential duration (APD) in any cardiac tissues.

5 The drug was negatively inotropic, and depressed the positive inotropic responses of atria to increased extracellular calcium.

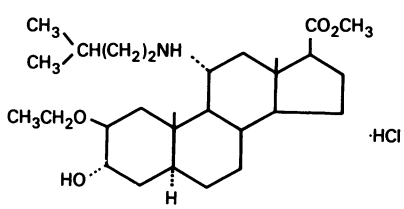
6 At high concentrations and at rapid pacing frequency, the action potential plateau disappeared and APD became very short.

7 It was concluded that CCI is primarily a class I antiarrhythmic drug at low concentration, with additional class IV action at higher concentrations.

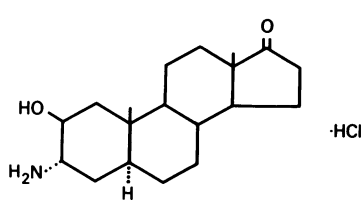
Introduction

Over the past decade a remarkable number of new antiarrhythmic drugs has been made available to the cardiologist, but none of them has proved outstandingly superior to the others. Recently, several reports have been published of the antiarrhythmic properties of Org 6001 (Marshall & Parratt, 1975; Vargäftig, Sugrue, Buckett & van Riezen, 1975; Verdouw, Schamhardt, Remme & De Jong, 1978), which was characterized by its effects on intracellular action potentials (Salako, Vaughan Williams & Wittig, 1976) as a class I antiarrhythmic drug (Vaughan Williams, 1975; 1980). It was of particular interest because its steroidal structure set it apart from all other class I drugs.

We now describe studies on a second steroidal antiarrhythmic drug, methyl 2β-ethoxy-3α-hydroxy-11α-(3-methylbutylamino)-5α-androstane-17β-carboxylate hydrochloride, (CCI 22277, Figure 1), which has been shown in preliminary animal studies to have greater antiarrhythmic potency both orally and intravenously than Org 6001 (Dodds, Dolamore, Sawyer, Straughan & Twissell, 1982).



CCI 22277



Org 6001

Figure 1 Structural formulae of CCI 22277 and Org 6001.

0007-1188/82/060337-09 $01.00

Methods

Local anaesthesia

Frog sciatic nerves were stripped of their sheaths and placed in a three-compartment chamber at room temperature. They were stimulated in moist air at one end (rectangular supramaximal pulses, 1 ms in duration) and action potentials were recorded from the other end. The segment of nerve in the central chamber was bathed in frog Ringer containing various concentrations of procaine or of CCI 22277. The Ringer solution contained (mM): NaCl 120, KCl 1.88, $CaCl_2$ 1.08, $NaHCO_3$ 2.38 and Tris buffer at pH 7.5, 10 ml/l. The height of the fastest wave of the action potential was measured before and after exposure for 30 min to each concentration of drug.

Pithed rats

Male Wistar rats weighing 330–390 g were anaesthetized by intraperitoneal injection of sodium pentobarbitone (60 mg/kg) and pithed. They were ventilated artifically and the right common carotid artery and left external jugular vein cannulated. Blood pressure was recorded with a mecury manometer and the electro-cardiogram recorded from needle electrodes.

Isolated atria

Rabbits weighing 1000–1400 g were stunned and their hearts rapidly removed. The atria were dissected from the heart and suspended vertically in an organ bath containing modified Locke solution at 32°C bubbled with 95% O_2 and 5% CO_2. Solutions were changed by introducing fresh fluid from below, and removing it by surface suction, so that the preparation was undisturbed. The atria were allowed to beat spontaneously, or were driven by rectangular pulses (2 × threshold, 2 ms duration) delivered by a pair of platinum electrodes. The upper end of the preparation was connected to a force transducer (Dynamometer UF1) and contractions were displayed on a chart recorder (MR2, Devices). Unless otherwise specified, the Locke solution had the following composition (mM): NaCL 125, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 25, glucose 11; ph 7.4.

Intracellular recordings

Atria The method used was that described by Vaughan Williams, (1958) and Szekeres & Vaughan Williams (1962). Single fibres were penetrated from the endocardial surface of rabbit isolated atria suspended horizontally in a bath through which Locke solution was recirculated at 32°C by an external oxygenator. During the recording of intracellular potentials, the atria were paced at a constant frequency 15–20% above the spontaneous rate by stimuli delivered to the left atrium. Contractions were measured as already described and intracellular potentials were recorded with glass micropipettes filled with 3 M KCl. Drug concentrations were changed after one hour's exposure to each.

Ventricles Papillary muscles 8–15 mm long and 0.8–1.5 mm in diameter were dissected from the right ventricles of 1000–2000 g rabbits and pinned to the base of the bath. They were driven at a basic cycle length (BCL) of 600 ms unless otherwise stated. To estimate the effective refractory period (ERP) a premature stimulus S_2 was introduced after every eighth basic drive stimulus, S_1. The ERP was defined as the longest S_1 S_2 interval that failed to provoke a premature action potential.

Purkinje fibres The interventricular septa of rabbit hearts were dissected out and pinned to the base of the bath with their right ventricular aspect uppermost. The preparation was driven by bipolar electrodes placed on the proximal right bundle branch (BCL 600 ms) and ERP was determined as for papillary muscles. Action potentials were recorded from the terminal Purkinje fibres at the base of the anterior papillary muscle.

All data obtained during the intracellular studies were displayed on a storage oscilloscope (Tektronix 5103N) and recorded by an F.M. tape recorder (Racal, Store Four) for later analysis by computer programme as previously described (Vaughan Williams, 1977).

Data have been presented as means ± standard errors (s.e.) and the significance of differences has been estimated by Student's *t* test or paired *t* test.

Drugs used were CCI 22277 (donated by Glaxo Group Research Ltd.), procaine HCl (B.D.H.), pentobarbitone sodium (May and Baker), isoprenaline sulphate (Burroughs Wellcome), (−)-phenylephrine HCl (Sigma) and Tris (Sigma).

Results

Local anaesthesia

The concentration of CCI 22277 required to reduce the amplitude of the nerve action potential by 50% was measured in 12 experiments and found to be 62 times less than the concentration of procaine required to produce the same response. The effect of CCI 22277, unlike that of procaine, was only partially reversible even after wash-off periods of up to 3.5 h.

Electrical threshold, conduction velocity and maximum follow frequency in rabbit isolated atria

CCI 22277 had no effect on the electrical threshold of isolated rabbit atria in the concentrations used (up to 0.25 μM). There was a trend, which failed to reach statistical significance (NS), for maximum follow frequency to fall with increasing doses and a small but significant fall in conduction velocity of 12%. These results are presented in Table 1.

Spontaneous frequency and contractions of rabbit atria

CCI 22277, in the experiments presented in Table 1, caused a small, but not statistically significant, reduction of spontaneous frequency. This was, however, dose-related, as was a moderate and statistically significant negative inotropic effect (−23.6% at 0.25 μM). In a second series of experiments (Figure 5) CCI 22277 at a concentration of 1 μM produced an acute and statistically significant fall in both spontaneous frequency and contractions.

Atrial intracellular potentials

The effects of CCI 22277 on atrial intracellular potentials are summarized in Table 2. The drug caused a small reduction of resting potential (significant only at 0.125 μM), reversed on wash-out. It also produced a significant and concentration-dependent decrease of action potential amplitude (APA) reaching −6.8 mV at 0.25 μM. Thus, the 'overshoot' potential was reduced by 3.6 mV. By far the most striking effect of the drug was on the maximum rate of depolarization (MRD) which was reduced by 25.4% at 0.0625 μM and by 47.5% at 0.25 μM. These changes were only partially reversed on wash-out. There were no significant effects on the times from

Table 1 Effects of CCI 22277 on the electrical threshold, maximum follow frequency, conduction velocity, spontaneous frequency and peak developed tension of isolated rabbit atria

Concentration of CCI 22277 (μM)	*Electrical threshold* (V)	*Maximum follow frequency* (Hz)	*Conduction velocity* (m/s)	*Spontaneous frequency* (Hz)	*Peak developed tension* (g)
Control	4.6 ± 0.4	8.5 ± 0.5	0.51 ± 0.02	1.7 ± 0.25	0.72 ± 0.02
0.0625	4.6 ± 0.4	8.5 ± 0.4	0.50 ± 0.02	1.6 ± 0.2	0.72 ± 0.02
0.125	4.7 ± 0.5	8.0 ± 0.3	0.47 ± 0.02	1.6 ± 0.2	0.63 ± 0.02
(*P* value)**			(<0.05)		(<0.05)
0.25	4.8 ± 0.6	7.6 ± 0.2	0.45 ± 0.01	1.5 ± 0.2	0.55 ± 0.02
(*P* value)			(<0.05)		(<0.001)
Washout	4.7 ± 0.6	7.5 ± 0.3	0.45 ± 0.01	1.5 ± 0.2	0.54 ± 0.01
(*P* value)			(<0.05)		(<0.0001)

Pooled results from 8 preparations, given as means ± s.e.; exposure time at each concentration = 1 h.
****P** values are for significance of differences from control values and are only given where $P < 0.05$.

Table 2 Effects of CCI 22277 on the intracellular action potentials of isolated rabbit atria

Concentration of CCI 22277 (μM)	*No. of fibres impaled*	*Resting membrane potential* (mV)	*Action potential amplitude* (mV)	*Maximum rate of depolarization* (V/s)	*Action potential duration* (APD) APD_{20} (ms)	APD_{50} (ms)	APD_{90} (ms)
Control	60	−72.8 ± 1.3	94.9 ± 1.2	117.1 ± 6.6	25.6 ± 0.9	51.0 ± 1.0	92.1 ± 1.6
0.0625	67	−71.0 ± 1.3	91.5 ± 1.1	87.3 ± 5.3	25.1 ± 0.6	49.4 ± 0.8	89.5 ± 1.4
(P value)**				(<0.02)			
0.125	71	−68.2 ± 0.9	90.0 ± 1.3	67.0 ± 4.4	24.6 ± 0.6	48.9 ± 0.7	87.0 ± 0.9
(P value)		(<0.05)		(<0.0001)			
0.25	59	−69.6 ± 1.2	88.1 ± 1.2	61.5 ± 5.1	24.9 ± 0.7	48.3 ± 0.7	87.8 ± 1.5
(P value)			(<0.01)	(<0.0001)			
Washout	74	−71.8 ± 0.9	90.5 ± 1.9	64.2 ± 5.0	25.5 ± 0.5	48.1 ± 0.7	87.2 ± 1.2
(P value)				(<0.0001)			

Means ± s.e. from 8 experiments; exposure time at each concentration = 1 h.
****P** values are for significance of differences from controls.

action potential peak to 20% (APD_{20}), 50% (APD_{50}) or 90% (APD_{90}) repolarization.

Ventricular potentials

Ventricular muscle proved to be less sensitive to the class I effects of CCI 22277 than atrial muscle. Two separate series of experiments (5 preparations in each series), involving exposure to a total of six different drug concentrations, were undertaken. The results are presented in Figure 2. Whereas a concentration of 0.25 μM produced a 47.5% reduction of MRD in atrial fibres, twenty times this concentration (5 μM) was required to produce a comparable reduction of MRD (− 53%) in ventricular muscle. A small decrease in RP, and a more marked reduction of APA and overshoot, were observed in the ventricle, as in the atrium. The higher dose ranges of CCI 22277 used in the ventricle significantly shortened APD_{50} and APD_{90}. Unlike the class I effects, which were partially reversed on wash-out, the APD continued to shorten during the recovery period. Refractoriness, measured as ERP, decreased with increasing drug concentrations. This is not surprising in view of the close relationship between ERP and APD. When the data were corrected for the shortened APD, by dividing ERP by APD_{90}, a small but not statistically significant increase in the ERP/APD_{90} ratio became apparent at higher concentrations.

In a preliminary experiment (not included in the pooled results), the ventricular preparation became inexcitable in a concentration of 5 μM CCI 22277. It was noted that, before this, the 'plateau' was intermittently absent, and the very short action potentials excited virtually no contractions. Subsequently an 'alternans' pattern developed (Figure 3). The stimulation frequency was increased (from 1 to 1.7 Hz) and the alternans pattern disappeared within 20 s. All the action potentials were then of the narrow type. When the rate was slowed again (to 0.33 Hz) the action potential duration and the contractions returned to normal. The electrical threshold, conduction velocity, RP and MRD remained unaltered throughout, in spite of these changes in APD and contraction. Similar effects were reproduced in two subsequent experiments, but on those occasions higher concentrations of the drug (10 μM and 25 μM) were required.

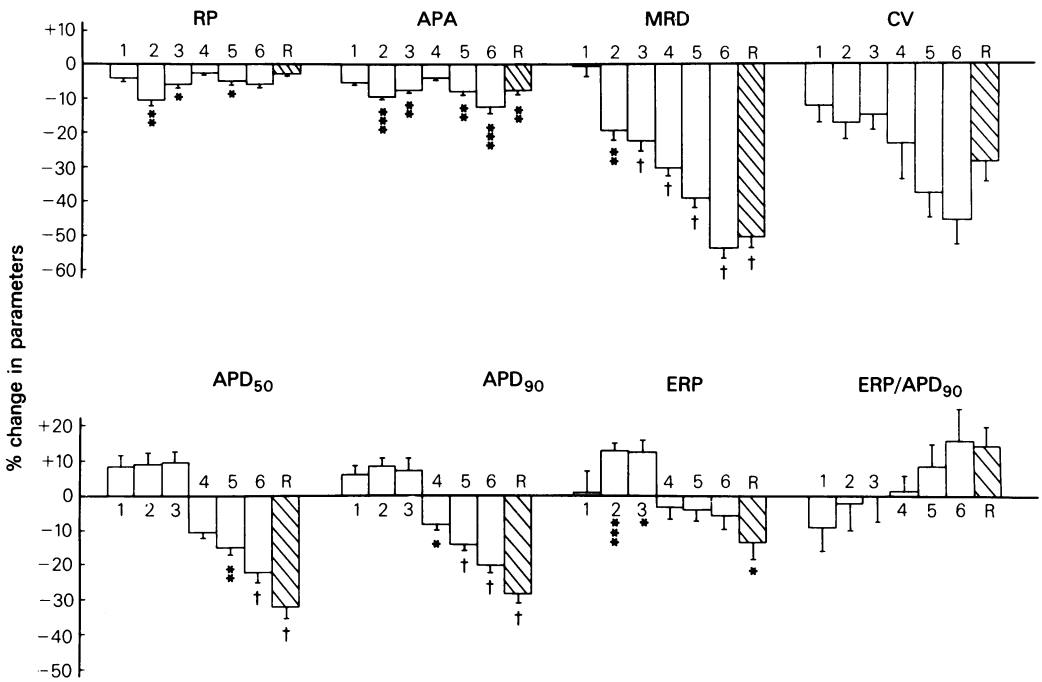


Figure 2 Effects of increasing concentrations of CCI 22277 on rabbit ventricular action potentials, expressed as % change from control values of the parameters measured: (1) 0.0625 μM; (2) 0.25 μM; (3) 0.5 μM; (4) 1.0 μM; (5) 2.5 μM; (6) 5.0 μM; (R) 'recovery' after 1 h of washing with drug-free solution. RP, resting potential; APA, action potential amplitude; MRD, maximum rate of depolarization; CV, conduction velocity; $APD_{50, 90}$, action potential duration to 50 and 90% repolarization; ERP, effective refractory period. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $\dagger P<0.0001$ (significance of difference from control).

Table 3 Influence of basic cycle length (BCL) on the class I effects of CCI 22277 on rabbit papillary muscle fibres

Concentration of CCI 22277 (μM)	*BCL* (ms)	*No. of* fibres	*Overshoot potential* (mV)	*Maximum rate of depolarization* (V/s)
Control	600	50	22.8 ± 1.0	153.2 ± 3.6
Control	300	50	21.8 ± 1.1	139.9 ± 5.1
0.0625	600	47	20.2 ± 1.3	152.0 ± 4.7
0.0625	300	46	17.6 ± 1.2 (<0.05)**	117.4 ± 4.6 (<0.001)
0.25	600	45	21.1 ± 1.3	123.0 ± 5.7
0.25	300	43	18.7 ± 1.5	99.0 ± 4.8 (<0.05)
0.50	600	50	19.7 ± 1.4	115.3 ± 5.1
0.50	300	47	17.9 ± 1.0	92.6 ± 2.5 (<0.01)

Means ± s.e. pooled from 5 experiments.
**Figures in parentheses are *P* values for significance of difference between BCL 600 and BCL 300 at a given drug concentration.

Influence of stimulation frequency

During the first series of papillary muscle experiments, after action potentials at each drug concentration had been recorded at a basic cycle length (BCL) of 600 ms, further records were obtained after 10 min of stimulation at a BCL of 300 ms. As can be seen from Table 3 this increased rate was associated with a significant enhancement of the class I effect at all three concentrations.

Effects on Purkinje fibres

The results of five experiments on Purkinje fibres are summarized in Figure 4. In terms of class I effects, these cells were intermediate in sensitivity between atrial and ventricular myocardium. There was a marked reduction in 'overshoot' from +27 mV to −0.8 mV with no change in RP. There was a striking fall in MRD (−50% at 1 μM and −87.3% at 5 μM) while conduction velocity also fell in a dose-dependent fashion from 1.29 m/s to 0.40 m/s. The effects of the drug on repolarization in these fibres contrasted markedly with those seen in ventricular myocardium. APD_{50} and APD_{90} were not significantly shortened in the Purkinje fibres even at 5 μM but APD_{20} was greatly prolonged from a control of 13.6 ms to 50.2 ms at 1.0 μM and 75 ms at 5 μM. The reason for this latter effect is as follows. Untreated Purkinje fibres typically have action potentials with a large overshoot followed by a phase of rapid early repolarization producing a 'notch' (see Kreher, 1978, for discussion). This gives rise to a very short APD_{20} (13.6 ms), but this can be enormously prolonged if the overshoot potential is reduced, as it is by CCI 22277.

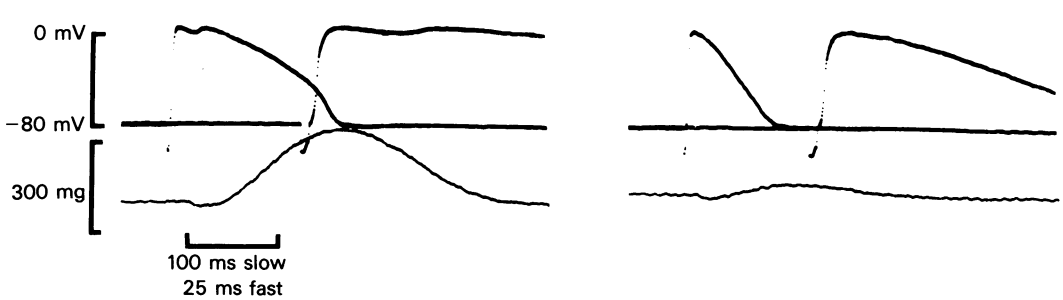


Figure 3 Alternating absence of plateau associated with markedly decreased contraction seen in two consecutive action potentials recorded from one cell. In each panel: upper traces, intracellular potentials at slow and fast sweep speeds, superimposed; lower traces, contraction. CCI 22277, 5 μM.

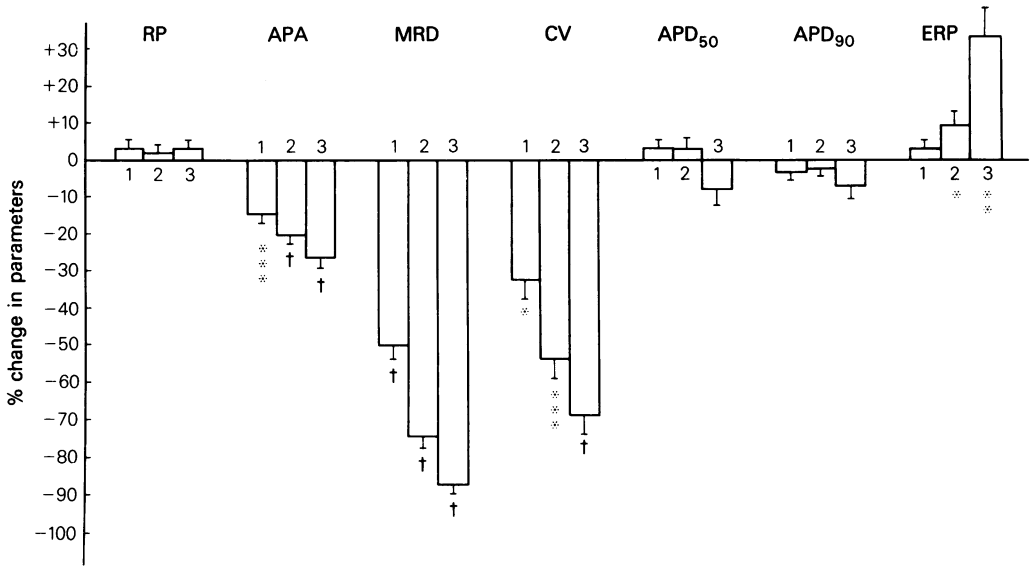


Figure 4 Effects of CCI 22277 on rabbit Purkinje fibre action potentials, expressed as % change from control values of parameters measured: (1) 1.0 μM; (2) 2.5 μM; (3) 5.0 μM. Abbreviations as for Figure 2.

The ERP of the Purkinje fibres was extended from 196 ms to 260 ms by the increasing concentrations of drug.

Adrenoceptor blocking effects: class II action

Dose-response curves for the chronotropic and inotropic responses to isoprenaline of rabbit isolated atria were obtained in control solution and in the presence of CCI 22277 0.25 and 1 μM (Figure 5). There was no evidence of any β_1-receptor blockade but there was a significant nonspecific depressant effect, indicating that CCI 22277 itself had negative inotropic and chronotropic actions in this preparation. Specifically, the contractions in the presence of no added isoprenaline were depressed by 3.1% at 0.25 μM (NS), and 17.5% at 1 μM ($P<0.02$). The spontaneous frequency was decreased from 142 ± 11 (beats per min) to 124 ± 8 by 0.25 μM CCI 22277 (NS) and to 119 ± 8 by 1 μM ($P<0.05$).

In vivo, in the pithed rat (6 experiments), intravenous doses of 0.5 mg/kg and 2.5 mg/kg had no effect on the hypertensive response to phenylephrine or on the hypotensive and positive chronotropic responses to isoprenaline. One of the six rats developed transient (2–3 min) second-degree heart block associated with hypotension immediately on being given the higher dose. Otherwise the only haemodynamic effects of the injections of CCI 22277 were minor. Mean blood pressure was reduced from 58.8 ± 5.3 mmHg to 56.0 ± 5.4 by 0.5 mg/kg ($P<0.05$) and from 52.8 ± 4.8 to 49.8 ± 4.9 by 2.5 mg/kg ($P<0.005$). The spontaneous heart ra fell from 170 ± 12 before the drug to 162 ± 11 aft 0.5 mg/kg and 156 ± 13 after 2.5 mg/kg (NS).

Calcium antagonism: class IV

Verapamil and other drugs loosely termed 'calciu antagonist' are negatively inotropic, and depress tl positive inotropic responses to increases in extr cellular calcium concentration. Org 6001, anoth steroidal antiarrhythmic drug, had no significant e fect in this test, which gave a positive result with a lc concentration (0.15 μg/ml) of verapamil (Salako *al.*, 1976). The pooled result of nine experimer with CCI 22277, presented in Figure 6, suggest th the drug does have some calcium antagonist activit as thus defined.

Discussion

The experiments described indicate that CCI 222' is predominantly a class I antiarrhythmic agent, wi a local anaesthetic activity on nerve sixty tim greater than that of procaine. The drug increas electrical threshold, decreased conduction veloci and increased refractoriness in isolated cardiac t sues. It produced a large dose-dependent reducti in maximum rate of depolarization (MRD), though, surprisingly this was associated with only small (NS) though dose-related fall in maximu follow frequency. In both atrial and ventricular pre

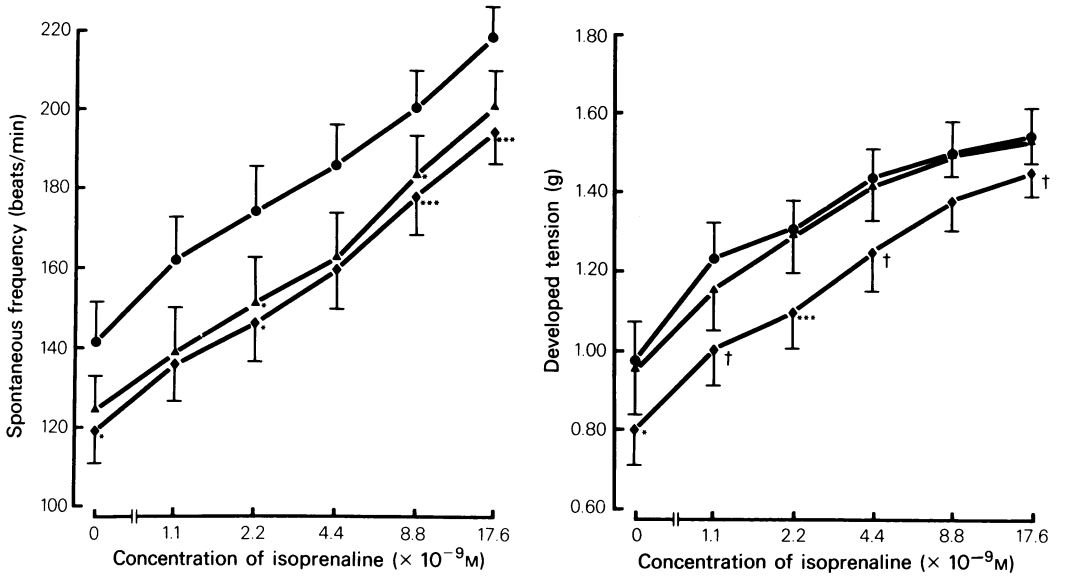


Figure 5 Effects of CCI 22277 on chronotropic and inotropic responses of rabbit atria to isoprenaline: (●) Control; (▲) CCI 22277 0.25 μM; (◆) CCI 22277 1.0 μM. $*P<0.05$; $**P<0.01$; $***P<0.001$; †$P<0.0001$ (significance of differences from control).

arations there was a slight reduction in resting potential (RP) which in itself is well known to produce a fall in MRD (Weidmann, 1955). However, these small decreases in RP were insufficient to account for the large falls in MRD. Moreover, in Purkinje fibres, in which the greatest depression of MRD was exhibited, there was no fall in RP.

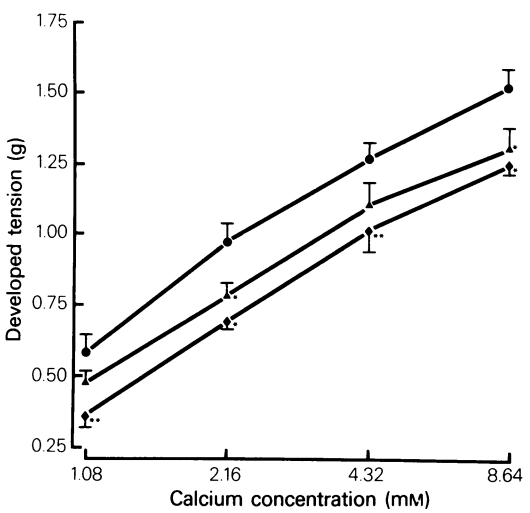


Figure 6 Effects of CCI 22277 on the positive inotropic action of increases in calcium concentration in rabbit atria: (●) Control; (▲) CCI 22277 0.25 μM; (◆) CCI 22277 1.0 μM. $*P<0.05$; $**P<0.01$ (significance of difference from control.

The fact that the class I activity of CCI 22277, at least in ventricular fibres, was augmented by increasing the driving rate is consistent with the observation of 'frequency-dependence' in other class I drugs (Johnson & McKinnon, 1957; Heistracher, 1971; Tritthart, Fleckenstein & Fleckenstein, 1971; Hondeghem & Katzung, 1980). It is likely, therefore, that CCI 22277 delays recovery from inactivation of the fast inward current (i_{Na}), as do other class I drugs. As the pacing frequency increases, successive action potentials are subject to a cumulative decline in the number of available sodium channels.

CCI 22277 produced a slowing of the spontaneous frequency of isolated atria and also a slowing of *in vivo* heart rate in pithed rats. Virtually all class I antiarrhythmic drugs depress the sino-atrial node *in vitro* though this effect may be masked *in vivo* by other factors. Quinidine and disopyramide, for example (but not CCI 22277), are antimuscarinic agents (Mirro, Manalan, Bailey & Watanabe, 1980). The mechanism of this slowing of sinus rate is not known. There is evidence in sheep Purkinje fibres (Attwell, Cohen, Eisner, Ohba & Ojeda, 1979) for a steady-state component of i_{Na} present over a potential range of -65 mV to -15 mV. As the diastolic potential of sino-atrial cells normally lies within this range and as there is evidence that these cells do possess fast inward channels (Kreitner, 1975) it has been proposed (Vaughan Williams, 1980; Cowan &

Vaughan Williams, 1981) that reduction of such an inward current by class I drugs could account for their negative chronotropic effect. Another possibility yet to be investigated is that these drugs inhibit the newly-described current, i_f, suggested to be responsible for pace-making in the sinus node and perhaps elsewhere (Brown & DiFrancesco, 1980; DiFrancesco & Ojeda, 1980; DiFrancesco, 1981).

CCI 22277 also depressed contractions, as do several class I drugs. The effect was observed in all the isolated atrial preparations, but only a minor, though significant, hypotensive effect was seen *in vivo* in the pithed rats. However, it is not possible, to assess adequately the clinical relevance of this negative inotropic effect without more detailed haemodynamic studies beyond the scope of the present investigation.

In contrast to its class I activity, CCI 22277 exhibited no significant class II (antisympathetic) or class III (APD prolongation) effects in any of the preparations studied. There was, however, some evidence of class IV activity. In the isolated atrial preparation, CCI 22277 exhibited a dose-dependent antagonism to the positive inotropic effects of increasing extracellular calcium concentrations, as does verapamil, but not Org 6001 or flecainide (Salako *et al.*, 1976; Cowan & Vaughan Williams, 1981). Whereas it is clear that a drug giving a negative result in this test could have little effect on (i_{SI}), a positive result could be attributed to actions (e.g. intracellular) other than, or additional to, restriction of slow inward current.

Another reason for supposing that CCI 22277 may restrict slow inward current was that in ventricular fibres, high concentrations of CCI 22277 shortened APD and very high concentrations completely abolished the plateau and markedly reduced APD_{90}. Some shortening of APD_{50} by other calcium antagonists has been observed (Singh & Vaughan Williams, 1972; Kass & Tsien, 1975) but a sudden abolition of the plateau in a rate-dependent manner such as was demonstrated in Figure 3 has not been described before, to our knowledge. The absence of shortening of APD by CCI 22277 in Purkinje fibres is not surprising, because their APD is mainly determined by activation of outward potassium currents (i_x) (McGuigan, 1974; Beeler & Reuter, 1977).

There is evidence that several other antiarrhythmic drugs have both class I and class IV actions. Verapamil and perhexiline, both regarded primarily as calcium antagonists, exhibit class I activity at higher concentrations (Singh & Vaughan Williams, 1972; Bayer, Kalusche, Kaufmann & Mannhold, 1975; Ehara & Daufmann, 1978; Vaughan Williams, 1980). Conversely, the primarily class I drug cibenzoline has some class IV activity (Millar & Vaughan Williams, 1982) and quinidine itself restricts i_{SI} as well as i_{Na} (Ducouret, 1976; Nawrath & Eckel, 1979; Nawrath, 1981). The combination of two classes of action could make a drug much more potent as an antiarrhythmic agent than might have been expected on the basis of either action alone (Vaughan Williams, 1980). Thus we have concluded that CCI 22277 is a potentially effective antiarrhythmic drug which merits further testing in experimental models of arrhythmias.

T.J.C. is a Nuffield Dominions Demonstrator (Australia).

References

ATTWELL, D., COHEN, I., EISNER, D., OHBA, M. & OJEDA, C. (1979). The steady state TTX-sensitive ('window') sodium current in cardiac Purkinje fibres. *Pflügers Arch.*, **379**, 137–142.

BAYER, R., KALUSCHE, D., KAUFMANN, R. & MANNHOLD, R. (1975). Inotropic and electrophysiological actions of verapamil and D-600 in mammalian myocardium. III. Effects of the optical isomers on transmembrane action potentials. *Naunyn Schmiedebergs Arch. Pharmac.*, **290**, 81–97.

BEELER, G.W. & REUTER, H. (1977). Reconstruction of the action potential of ventricular myocardial fibres. *J. Physiol.*, **268**, 177–210.

BROWN, H. & DiFRANCESCO, D. (1980). Voltage-clamp investigations of membrane currents underlying pacemaker activity in rabbit sino-atrial node. *J. Physiol.*, **308**, 331–351.

COWAN, J.C. & VAUGHAN WILLIAMS, E.M. (1981). Characterization of a new oral antiarrhythmic drug, flecainide (R818). *Eur. J. Pharmac.*, **73**, 333–342.

DiFRANCESCO, D. (1981). A study of the ionic nature of the pacemaker current in calf Purkinje fibres. *J. Physiol.* **314**, 337–393.

DiFRANCESCO, D. & OJEDA, C. (1980). Properties of the current i_f in the sino-atrial node of the rabbit compared with those of the current i_{K2} in Purkinje fibres. *J. Physiol.*, **308**, 353–367.

DODDS, M.G., DOLAMORE, P.G., SAWYER, P.R., STRAUGHAN, D.W. & TWISSELL, D.J. (1982). The activity of a novel aminosteroid CCI22277 against aconitine and coronary artery ligation-induced dysrhythmias in the rat. *Br. J. Pharmac.*, (in press).

DUCOURET, P. (1976). The effect of quinidine on membrane electrical activity in frog auricular fibres studied by current and voltage clamp. *Br. J. Pharmac.*, **57**, 163–184.

EHARA, T. & DAUFMANN, R. (1978). The voltage-and time-dependent effects of (−)-verapamil on the slow inward current in isolated cat ventricular myocardium. *J. Pharmac. exp. Ther.*, **207**, 49–55.

HEISTRACHER, P. (1971). Mechanism of action of antifibrillatory drugs. *Naunyn Schmiedebergs Arch. Pharmac.*,

269, 199–212.

HONDEGHEM, L. & KATZUNG, B.G. (1980). Test of a model of antiarrhythmic drug action. *Circulation.*, **61,** 1217–1224.

JOHNSON, E.A. & McKINNON, M.G. (1957). The differential effect of quinidine and pyrilamine on the myocardial action potential at various rates of stimulation. *J. Pharmac. exp. Ther.*, **120,** 460–468.

KASS, R.S. & TSIEN, R.W. (1975). Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. *J. gen. Physiol.*, **66,** 169–192.

KREHER, P. (1978). Role of membrane permeabilities (Calcium, chloride and potassium) in the genesis of the notch observed on simian and prosimian cardiac action potentials. *Cardiovasc. Res.*, **12,** 507–515.

KREITNER, D. (1975). Evidence for existence of a rapid sodium channel in the membrane of rabbit sinoatrial cells. *J. mol. cell Cardiol.*, **7,** 655–662.

MARSHALL, R.J. & PARRATT, J.R. (1975). Antiarrhythmic, haemodynamic and metabolic effects of 3α-amino-5α-androstan-2β-ol-17-one hydrochloride in greyhounds following acute coronary ligation. *Br. J. Pharmac.*, **55,** 359–368.

MILLAR, J.S. & VAUGHAN WILLIAMS, E.M. (1982). Effects on rabbit nodal, atrial, ventricular and Purkinje cell potentials of a new antiarrhythmic drug, cibenzoline, which protects against action potential shortening in hypoxia. *Br. J. Pharmac.*, (in press)

MIRRO, M.J., MANALAN, A.S., BAILEY, J.C. & WATANABE, A.M. (1980). Anticholinergic effects of disopyramide and quinidine on guinea pig myocardium. *Circulation. Res.*, **47,** 855–865.

McGUIGAN, J.A.S. (1974). Some limitations of the double sucrose gap, and its use in a study of the slow outward current in mammalian ventricular muscle. *J. Physiol.*, **240,** 775–806.

NAWRATH, H. (1981). Action potential, membrane currents and force of contraction in mammalian heart muscle fibers treated with quinidine. *J. Pharmac. exp. Ther.*, **216,** 176–181.

NAWRATH, H. & ECKEL, L. (1979). Electrophysiological study of human ventricular heart muscle treated with quinidine: interaction with isoprenaline. *J. cardiovasc. Pharmac.*, **1,** 415–425.

SALAKO, L.A., VAUGHAN WILLIAMS, E.M. & WITTIG, J.H. (1976). Investigations to characterize a new antiarrhythmic drug, Org 6001, including a simple test for calcium antagonism. *Br. J. Pharmac.*, **57,** 251–262.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1972). A fourth class of anti-dysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. *Cardiovasc. Res.*, **6,** 109–119.

SZEKERES, L. & VAUGHAN WILLIAMS, E.M. (1962). Antifibrillatory action. *J. Physiol.*, **160,** 470–482.

TRITTHART, H., FLECKENSTEIN, B. & FLECKENSTEIN, A. (1971). Some fundamental actions of antiarrhythmic drugs on the excitability and contractility of single myocardial fibres. *Naunyn Schmiedebergs Arch. Pharmac.*, **269,** 212–219.

VARGÄFTIG, B.B., SUGRUE, M.F., BUCKETT, W.R. & VAN RIEZEN, H. (1975). Org 6001 (3α-amino-5α-androstan-2β-ol-17-one-hydrochloride), a steroidal antiarrhythmic agent. *J. Pharm. Pharmac.*, **27,** 697–699.

VAUGHAN WILLIAMS, E.M. (1958). Some observations concerning the mode of action of acetylcholine in isolated rabbit atria. *J. Physiol.*, **140,** 327–346.

VAUGHAN WILLIAMS, E.M. (1975). Classification of antidysrhythmic drugs. *Pharmac. Ther.*, B., **1,** 115–138.

VAUGHAN WILLIAMS, E.M. (1977). Automated measurement of potentials. *J. Physiol.*, **272,** 1–2P.

VAUGHAN WILLIAMS, E.M. (1980). *Antiarrhythmic Action and the Puzzle of Perhexiline.* London: Academic Press.

VERDOUW, P.D., SCHAMHARDT, H.C., REMME, W.J. & DE JONG, J.W. (1978). Antiarrhythmic, metabolic and haemodynamic effects of Org 6001 (3α-amino-5α-androstan-2β-ol-17-one-hydrochloride) after coronary flow reduction in pigs. *J. Pharmac. exp. Ther.*, **204,** 634–644.

WEIDMANN, S. (1955). The effect of the cardiac membrane potential on the rapid availability of the sodium carrying system. *J. Physiol.*, **127,** 213–224.

(Received February 4, 1982.)