Br. J. Pharmac. (1987), **92**, 97–103

Resting, and rate-dependent depression of $\dot{V}_{max}$ of guinea-pig ventricular action potentials by amiodarone and desethylamiodarone

Regan T. Pallandi & [1]Terence J. Campbell

School of Physiology & Pharmacology, University of New South Wales, Sydney 2033, Australia

1 The cellular electrophysiological effects of amiodarone and its metabolite desethylamiodarone (DEA) were studied in guinea-pig ventricular myocardium by use of standard microelectrode techniques.

2 Both compounds produced significant increases in action potential duration (Class III antiarrhythmic effect) and decreases in maximum rate of depolarization (Class I effect), at clinically relevant concentrations.

3 The Class I effects were rate-dependent, with small (0–16%) falls in maximum depolarization rate in the absence of stimulation ('resting block') and progressively larger effects at decreasing interstimulus intervals (range 1200–300 ms).

4 The kinetics of onset and offset of the Class I effect in response to a step change in driving rate were quite fast for both drugs (comparable to those reported for Class Ib agents).

5 It is concluded that this unique combination of Class III action plus Class I effects with fast onset and offset kinetics may help explain the great efficacy of amiodarone in antiarrhythmic therapy.

Introduction

Amiodarone is a very effective antiarrhythmic drug, of value in the treatment of both supraventricular and ventricular arrhythmias (Nademanee *et al.*, 1982; Haffajee, 1985). Its predominant cellular mechanism of action is thought to be prolongation of the cardiac action potential and hence the refractory period (Class III action; Singh & Vaughan Williams, 1970; Vaughan Williams, 1984). This drug possesses very unusual pharmacokinetic properties including an extremely long biological half-life. It is metabolized in part to desethylamiodarone which frequently accumulates to higher concentrations than the parent compound during chronic dosage (Latini *et al.*, 1984). Desethylamiodarone (DEA) possesses antiarrhythmic properties of its own (Nattel, 1983; Abdollah *et al.*, 1986; Yabek *et al.*, 1986).

Recent studies have shown that in addition to Class III effects, both amiodarone and DEA possess the ability to depress the fast inward sodium current and hence slow the maximum rate of depolarization ($\dot{V}_{max}$) of the action potential (Class I action). This effect is enhanced at faster driving rates (Mason *et al.*, 1983; 1984; Varro *et al.*, 1985; Yabek *et al.*, 1986).

The aims of the present study, therefore, were to quantify the Class III properties of amiodarone and DEA and then proceed to a detailed study of their Class I actions at clinically relevant concentrations. Of particular interest was the speed with which the drugs could respond to a sudden change in driving rate by producing an increase or decrease in the Class I effect (kinetics of onset and offset of rate-dependent depression of $\dot{V}_{max}$) since this has been shown to be of relevance to the clinical electrophysiological properties of other Class I agents (Campbell, 1983a; Hill, 1985; Estes *et al.*, 1985).

Methods

Guinea-pigs of either sex weighing 500–800 g were decapitated and their hearts quickly removed. Strips (approximately 5 mm × 3 mm × 1 mm) were cut from

[1]Author for correspondence.

the right ventricular free wall and pinned to the base of a bath (volume 0.5 ml). The tissue was superfused at 3 ml min^{-1} with modified Locke solution gassed with 95% O_2 and 5% CO_2 and maintained at 37°C ± 0.2°C by a Peltier element. The composition of the Locke solution was as follows (mM): NaCl 125, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 25, $MgCl_2$ 1.0, NaH_2PO_4 0.44, glucose 11 and the pH was 7.4. Preparations were allowed to equilibrate for 2 to 3 h before taking recordings. During this time and throughout the experiment, they were driven from bipolar platinum electrodes providing rectangular pulses of sufficient amplitude to limit variations in latency (between stimulus and action potential upstroke) to less than 1 ms (Walton & Fozzard, 1979). Action potentials were recorded from cells on the surface of the preparations by use of conventional glass microelectrodes filled with 3M KCl, coupled to a high imput impedance d.c. amplifier with negative capacitance compensation (Neurolog). To study rate-dependent effects, the preparations were driven at interstimulus intervals (ISI) varying from 300–2400 ms. Trains of stimuli were of sufficient duration to achieve a stable level of effect and rest periods (unstimulated) of at least 60 s were interposed between trains to allow full recovery from rate-dependent effects. The time course of this recovery process was studied by adding a single extra-stimulus at varying intervals after a series of identical trains of stimuli at constant ISI (Campbell, 1982).

Analysis of recordings

Action potentials were monitored on an oscilloscope. Selected potentials were analysed by digital techniques using a Rockwell AIM-65 8-bit microcomputer modified by the addition of sampling hardware comprising a gain-matching amplifier, and an Analog Devices ADC 571 10-bit analog-to-digital converter connected to two parts of the AIM-65 Versatile Interface Adaptor. Parameters measured were: resting membrane potential, action potential amplitude, action potential duration to 50% and 90% repolarization and the maximum rate of depolarization ($\dot{V}_{max}$). The latter was calculated by simple differencing of 10-bit data samples taken at 20 kHz. To allow visual monitoring of changes in $\dot{V}_{max}$ from beat-to-beat, the upstrokes of the action potentials were electronically differentiated and the signal fed to a sample-and-hold peak detector (Hondeghem & Cotner, 1978). The output of this instrument was displayed as a square wave with amplitude linearly proportional to $\dot{V}_{max}$. Quantitative analyses of changes in $\dot{V}_{max}$ were performed with the values measured by the computer.

Amiodarone and desethylamiodarone were kindly supplied by Reckitt and Colman (Australia). Because these compounds are almost insoluble in physiological saline they were dissolved according to a method derived from that of Polster & Broekhuysen (1976), using ethanol and bovine serum albumin (Sigma). Thus the 50 μM solutions of either drug also contained 0.35 mg per 100 ml of albumin and 0.25 mg per 100 ml of ethanol. The 5 and 10 μM solutions were made by simple dilution with Locke solution. All these solutions were clear. Preliminary experiments showed no effect on any action potential parameter during constant stimulation at ISI = 1000 ms, of 2 h superfusion with drug-free Locke solution containing albumin 0.35 mg per 100 ml and ethanol 0.25 mg per 100 ml. This ethanol-albumin-Locke solution was used as the 'control' superfusate in the experiments to be described.

In some cases the myocardial strips were removed at the end of the experiment, enzymatically digested and assayed for amiodarone and DEA concentration using high-performance liquid chromatography (Storey *et al.*, 1983).

Data are presented as means ± standard deviation and Student's unpaired *t* test used to estimate differences between means.

Results

Class III effects

The major aim of the present study was to investigate the Class I effects of amiodarone and DEA. The Class III properties of the two agents were, however, quantified in six experiments (Figure 1). It can be seen that at concentrations of 5 μM and 10 μM, both drugs produced modest increases in action potential duration at a constant ISI of 1000 ms. Amiodarone appeared somewhat more potent (APD_{90} increased from 157 ± 10 ms, to 179 ± 11 ms for amiodarone; 153 ± 15 ms to 169 ± 16 ms for DEA) but a detailed direct comparison of the two compounds was not undertaken. There were no significant changes in any of the other action potential parameters (including $\dot{V}_{max}$) when studied at a constant ISI of 1000 ms. The class III effects were evident within 30 min of drug exposure but were not always complete after 2 h, the arbitrarily selected exposure time for each dose.

Class I effects

Class I effects were also evident within 30–60 min exposure to any of the concentrations of amiodarone or DEA used (5, 10 and 50 μM). As for the class III effects, however, quantitation was complicated by the fact that they tended to increase in magnitude for at least 3 h and did not significantly 'wash off' after 1–2 h exposure to drug-free superfusate. Unless otherwise stated, the results given below were recorded after exposure for 90–180 min to drug.

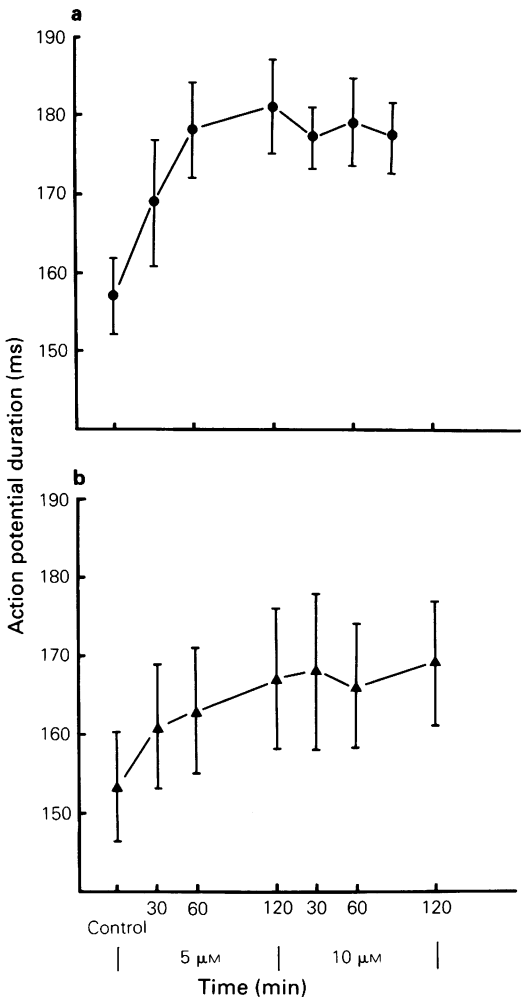

Figure 1 Prolongation of ventricular action potential duration in the presence of amiodarone, 5 and 10 μM (a) and desethylamiodarone, 5 and 10 μM (b).

Resting block

The measurement of the degree of depression of $\dot{V}_{max}$ by a drug in the absence of any stimulation of the tissue (resting block) requires that impalement of a single cell be maintained throughout the period of drug exposure (arbitrarily selected to be 2 h). This was achieved in 14 experiments (7 with amiodarone and 7 with DEA) at concentrations of 5–50 μM. The depression of $\dot{V}_{max}$ (measured as the percentage reduction compared to control value) ranged from 0–16% for amiodarone and 0–10% for DEA. In three preparations, 2 h exposure to amiodarone (1 case) or DEA (2 cases) at a concentration of 50 μM produced no change in $\dot{V}_{max}$ in the first beat of an induced train of action potentials.

Rate-dependent depression of $\dot{V}_{max}$

In addition to these small and inconsistent amounts of resting block, repetitive stimulation produced a further, much larger decline in $\dot{V}_{max}$ to a new plateau value usually within 5–10 beats (Figure 2). The extent of this 'rate dependent block' (RDB) varied with the stimulation rate, being greatest at the shortest value of ISI (300 ms; Figures 2, 3). At the highest concentration (50 μM) and fastest rate (ISI = 300 ms), $\dot{V}_{max}$ fell by 48.4 ± 17.6% for amiodarone and 45.4 ± 13.8% for DEA (n = 14 and 11 respectively).

The between-dose variation (dose-response relationship) was more marked for DEA than for amiodarone (Figure 3).

Kinetics of onset of rate-dependent block

The decline in $\dot{V}_{max}$ at the beginning of a train of action potentials (or following any sudden increase in driving rate) was quite rapid. A new steady-state level of $\dot{V}_{max}$ was usually achieved within 5–10 beats (Figure 2) and in most cases, more than 50% of the final fall in $\dot{V}_{max}$ occurred between the first and second beats (51% ± 15% for 50 μM DEA, n = 6, 64% ± 9.3% for 50 μM amiodarone, n = 7).

Kinetics of recovery from rate-dependent block

Recovery of $\dot{V}_{max}$ towards its 'resting' level at the end of a train of stimuli followed an exponential time course and was independent of drug concentration, exposure time, or ISI. For amiodarone the time constant of recovery (τ_{re}) was 1.48 ± 0.28 s (n = 11) and for DEA, it was 1.20 ± 0.25 s (n = 7).

Myocardial drug levels

In 12 experiments (6 amiodarone and 6 DEA) the myocardial strips were assayed for drug concentration at the end of the study (approx 4 h exposure to 10 μM amiodarone or DEA).

The levels of DEA (mean 9.6 μg 100 mg^{-1}; range 5.8–13.7) were consistently higher than amiodarone (mean 3.0 μg 100 mg^{-1}; range 2.2–4.3; $P < 0.01$).

Discussion

The initial series of experiments, performed at a fixed driving rate (ISI = 1000 ms), confirmed that DEA shares the ability of amiodarone to prolong ventricular action potentials without significantly altering resting potential or other parameters (Class III effect).

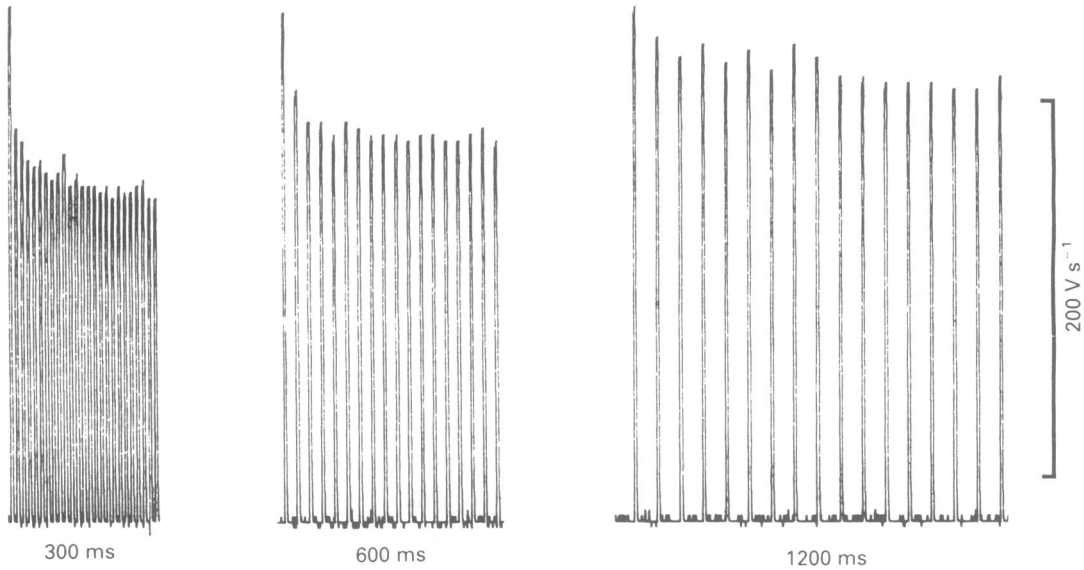

Figure 2 The effect on $\dot{V}_{max}$ of a train of action potentials in previously quiescent tissue in the presence of desethylamiodarone, 10 μM. The spikes represent the $\dot{V}_{max}$ of successive action potentials. The 3 trains shown were produced at decreasing rates (interstimulus interval = 300, 600 and 1200 ms). It can be seen that the degree of depression of $\dot{V}_{max}$ is greater at faster rates and that 50% or more of the final effect is produced within the first interstimulus interval.

In particular neither drug produced a significant reduction in $\dot{V}_{max}$ at this (relatively slow) rate of stimulation. These findings are consistent with those of others (Singh & Vaughan Williams, 1970; Yabek *et al.*, 1986).

Resting block

A number of groups have recently reported depression of $\dot{V}_{max}$ (Class I effect) at faster driving rates, for amiodarone (Mason *et al.*, 1983; 1984; Varro *et al.*, 1985; Yabek *et al.*, 1986) and DEA (Yabek *et al.*, 1986) and, indeed a small degree of depression of $\dot{V}_{max}$ in rabbit atria after 6 weeks' treatment with amiodarone was noted by Singh & Vaughan Williams (1970). These workers did not, however, quantitate the ability of the drugs to reduce the maximum rate of depolarization of myocardial cells in the absence of prior stimulation ('resting block'). This is technically difficult to do as prolonged exposure times and hence prolonged impalements are required. As might be expected from the absence of significant Class I effects seen at ISI = 1000 ms, the degree of resting block seen in the present study was small (0–16%). It was also variable and showed no obvious dose-dependence in the relatively few preparations in which it could be measured.

Amiodarone and DEA are highly lipid-soluble compounds but they are also far larger molecules than other antiarrhythmic drugs in clinical use (mol wt = 646 and 618 respectively). While the ability to produce resting depression of $\dot{V}_{max}$ correlates well with lipophilicity for relatively low molecular weight drugs (Sada & Ban, 1981), this relationship does not hold for antiarrhythmic drugs with molecular weights above about 350 (Campbell, 1983b). For these compounds it has been suggested that their physical size somehow limits access to the resting sodium channel (Courtney, 1980a).

Rate-dependent block

It is well-established that the ability of Class I antiarrhythmic agents to block the fast inward sodium channel and hence depress $\dot{V}_{max}$ of cardiac action potentials is enhanced at faster driving rates (Hondeghem & Katzung, 1980; Campbell, 1982; 1983c; Courtney, 1980a). Several models of drug interaction with the sodium channel have been proposed to explain this behaviour (Hondeghem & Katzung, 1977; 1980; 1984; Courtney, 1980b,c; Grant *et al.*, 1984).

Mason *et al.* (1983; 1984) showed similar effects for high concentrations (44 and 88 μM) of amiodarone and provided evidence that the drug selectively

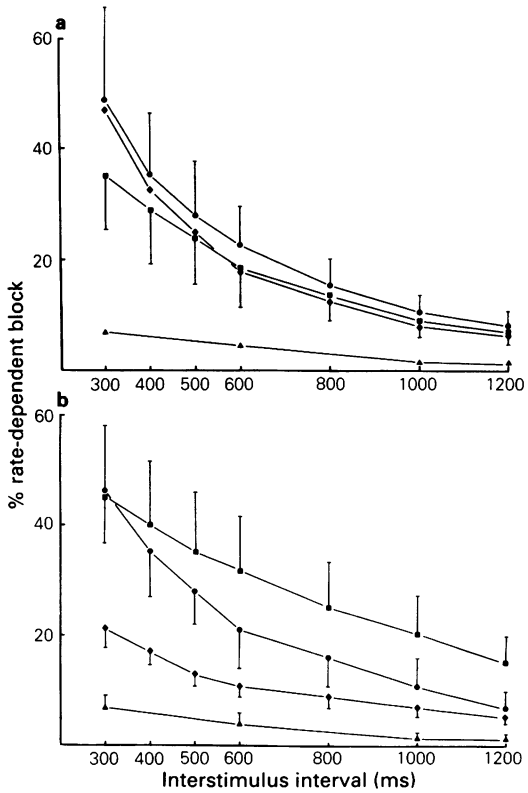

Figure 3 Relationship between rate expressed as interstimulus interval and % rate-dependent reduction of $\dot{V}_{max}$ produced by three concentrations of amiodarone (a) and desethylamiodarone (b). In (a): amiodarone 50 μM (●), 10 μM (◆), 5 μM (■), control (▲); in (b): desethylamiodarone 50 μM (■), 10 μM (●), 5 μM (◆), control (▲).

blocked inactivated sodium channels. More recent studies have confirmed significant rate-dependent depression of $\dot{V}_{max}$ by concentrations of amiodarone of 50 μM (Yabek *et al.*, 1986) and 7.3 μM (Varro *et al.*, 1985). Only this last study demonstrated Class I effects for amiodarone in concentrations in or near the range seen in patients taking the drug (approximately 0.1–7.6 μM for amiodarone and DEA; Harris *et al.*, 1983; Raeder *et al.*, 1985).

The present study confirms marked rate-dependent reductions in $\dot{V}_{max}$ by amiodarone in clinically relevant concentrations and demonstrates very similar behaviour for DEA, the main metabolite of amiodarone. Both drugs produce increasing depression of $\dot{V}_{max}$ over a clinically relevant range of increasing drive rates (Figure 3).

The onset of rate-dependent block following a step increase in driving frequency was quite rapid for both drugs with the fall to a new steady-state level of $\dot{V}_{max}$ being achieved within 5–10 beats. At concentrations producing approximately 50% rate-dependent depression of $\dot{V}_{max}$ at ISI = 300 ms, amiodarone and DEA achieved 64 ± 9% and 51 ± 15% respectively of the final reduction in $\dot{V}_{max}$ between the first and second beats of a train. These speeds of onset of rate-dependent block are comparable with those produced by lignocaine, mexiletine and tocainide and much faster than seen with other Class I agents in routine clinical use (Campbell, 1983b). Such fast onset kinetics are consistent with the finding that amiodarone binds strongly and selectively to inactivated sodium channels (Mason *et al.*, 1984).

The speed of recovery of $\dot{V}_{max}$ from rate-dependent depression was also quite fast (τ_{re} = 1.48 s for amiodarone and 1.20 s for DEA). These values fall between those reported for the Class Ib drugs lignocaine, mexiletine and tocainide (τ_{re} = 200–500 ms; Courtney, 1980a; Campbell, 1983b) and for the Ia drugs quinidine, disopyramide and procainamide (τ_{re} = 2.3–12.2 s; Courtney, 1980a; Grant *et al.*, 1982; Campbell, 1983a). This value for the time constant of recovery of $\dot{V}_{max}$ from block by amiodarone (1.48 s) agrees well with that found by Mason and co-workers (1984) who also used guinea-pig ventricular cells. Varro *et al.* (1985) found the recovery process to be considerably faster (τ_{re} = 282 ms). They too used guinea-pig ventricular cells but the mean resting potential of their preparations was −95 mV (cf. −85 mV in Mason *et al.* (1984) and −84 ± 1.8 mV in the present study), and the recovery process for other Class I drugs is known to be voltage-dependent. Furthermore, the technique used by these workers to measure τ_{re} (interpolated extra beats during a basic drive cycle of 1000 ms) differed from that employed in the present study and by Mason *et al.* (1984). It is interesting to note that the time course of recovery of $\dot{V}_{max}$ in the presence of DEA (which has not been reported before), was found to be very similar to that of amiodarone.

On the basis of previous studies which showed a strong correlation between increasing molecular weight and τ_{re}, for drugs with mol. wt 190 to about 450 (Courtney, 1980a,b,c; Campbell, 1983b), amiodarone and DEA would be expected to have extremely slow recovery kinetics. In fact recovery from rate-dependent depression induced by these drugs is faster than for almost all other antiarrhythmic agents studied. This suggests a need for a reassessment of current ideas about the influence of physico-chemical properties on the interaction between antiarrhythmic drugs and the sodium channel.

Whatever the physical basis for the observed behaviour of amiodarone and DEA, they exhibit a group of properties so far unique among antiarrhyth-

mic drugs (Varro *et al.*, 1985). They are able to prolong action potential duration (Class III effect) and they have Class I effects with fast onset and offset kinetics so they respond rapidly to step increases in heart rate with an increased degree of depression of $\dot{V}_{max}$. Thus in therapeutic concentrations, they have minimal influence on $\dot{V}_{max}$, and hence conduction at normal heart rates (Finerman *et al.*, 1982; Zipes *et al.*, 1984) but are able to respond to a premature beat or tachycardia with a rapid decrease in $\dot{V}_{max}$ (Campbell, 1983a).

There are other Class I drugs with Class III effects, notably the Ia subgroup (quinidine, disopyramide and procainamide) but these have slower onset and offset kinetics. On the other hand the Class I drugs with comparable speed of response to heart rate changes, the Ib agents (lignocaine, tocainide and mexiletine) tend to shorten the action potential duration, an effect which is probably not antiarrhythmic and may even contribute to proarrhythmic effects (Vaughan Williams, 1977).

It is possible that this unique combination of properties may help explain the fact that amiodarone is the most effective antiarrhythmic agent available against a wide range of arrhythmias (Haffajee, 1985).

Finally it is interesting to note the apparent higher myocardial uptake seen with DEA than amiodarone during acute exposure. This finding is consistent with reports of higher levels of DEA than amiodarone in all tissues except fat in patients on chronic amiodarone therapy (Holt *et al.*, 1983a,b; Latini *et al.*, 1984).

The assistance of Mr R. Troughear in modifying the AIM-65 microcomputer and writing the programmes used for analysis of action potentials and Mr J. Ray in performing the myocardial drug assays is gratefully acknowledged.

References

ABDOLLAH, H., BRENNAN, F.J. & BRIEN, J.F. (1986). The effect of intravenous desethylamiodarone in dogs with myocardial infarction and inducible ventricular arrhythmias. *J. Am. Coll. Cardiol.*, **7**, 82A.

CAMPBELL, T.J. (1982). Voltage- and time-dependent depression of maximum rate of depolarisation of guinea-pig ventricular action potentials by two steroidal antiarrhythmic drugs, CCI 22277 and Org 6001, *J. Pharmac.*, **77**, 541–548.

CAMPBELL, T.J. (1983a). Kinetics of onset of rate-dependent effects of Class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. *Cardiovasc. Res.*, **17**, 344–352.

CAMPBELL, T.J. (1983b). Importance of physico-chemical properties in determining the kinetics of the effects of Class I antiarrhythmic drugs on maximum rate of depolarization in guinea-pig ventricle. *Br. J. Pharmac.*, **80**, 33–40.

CAMPBELL, T.J. (1983c). Resting and rate-dependent depression of maximum rate of depolarisation ($\dot{V}_{max}$) in guinea-pig ventricular action potentials by mexiletine, disopyramide and encainide. *J. cardiovasc. Pharmac.*, **5**, 291–296.

COURTNEY, K.R. (1980a). Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. *J. Molec. Cell. Cardiol.*, **12**, 1273–1286.

COURTNEY, K.R. (1980b). Structure-activity relations for frequency-dependent sodium channel block in nerve by local anesthetics. *J. Pharmac. exp. Ther.*, **213**, 114–119.

COURTNEY, K.R. (1980c). Antiarrhythmic drug design: frequency dependent block in myocardium. In *Molecular Mechanisms of Anesthesia* (Progress in Anesthesiology, Vol. 2) ed. Fink, B.R. pp. 111–118. New York: Raven Press.

ESTES, N.A.M., GARAN, H., McGOVERN, B. & RUSKIN, J.N. (1985). Class I antiarrhythmic agents: Classification, electrophysiologic considerations, and clinical effects. In *Mechanisms and Treatment of Cardiac Arrhythmias; Relevance of Basic Studies to Clinical Management*. ed. Reiser, H.J. & Horowitz, L.N. pp. 183–199. Baltimore: Urban & Schwarzenberg.

FINERMAN, W.B., HAMER, A. & PETER, T. (1982). Electrophysiologic effects of chronic amiodarone therapy in patients with ventricular arrhythmias. *Am. Heart. J.*, **104**, 987–993.

GRANT, A.O., STARMER, C.F. & STRAUSS, H.C. (1984). Antiarrhythmic drug action: blockade of the inward sodium current. *Circulation Res.*, **55**, 428–439.

GRANT, A.O., TRANTHAM, J.L., BROWN, K.K. & STRAUSS, H.C. (1982). pH-dependent effects of quinidine on the kinetics of dV/dt_{max} in guinea-pig ventricular myocardium. *Circulation Res.*, **50**, 210–217.

HAFFAJEE, C.I. (1985). Clinical effects of class III antiarrhythmic agents. In *Treatment of Cardiac Arrhythmias: Relevance of Basic Studies to Clinical Management*. ed. Reiser, H.J. & Horowitz, L.N. pp. 283–293. Baltimore: Urban & Schwarzenberg.

HARRIS, L., McKENNA, W.J., ROWLAND, E., HOLT, D.W., STOREY, G.C.A. & KRIKLER, D.M. (1983). Side effects of long-term amiodarone therapy. *Circulation*, **67**, 45–51.

HILL, J.L. (1985). Class I antiarrhythmic agents: Diversity of Action. In *Mechanisms and Treatment of Cardiac Arrhythmias: Relevance of Basic Studies to Clinical Management*. ed. Reiser, H.J. & Horowitz, L.N. pp. 173–182. Baltimore: Urban & Schwarzenberg.

HOLT, D.W., STOREY, C.A., McKENNA, T., ADAMS, P.C. & CAMPBELL, R.W.F. (1983a). Unwanted effects of amiodarone in relation to tissue concentration. (Abstract). *J. Am. Coll. Cardiol.*, **1**, 630.

HOLT, D.W., TUCKER, G.T., JACKSON, P.R. & STOREY, G.C.A. (1983b). Amiodarone pharmacokinetics. *Am. Heart J.*, **106**, 840–847.

HONDEGHEM, L.M. & COTNER, C.L. (1978). Measurement of $\dot{V}_{max}$ of the cardiac action potential with a sample/hold peak detector. *Am. J. Physiol.*, **234**, H312–314.

HONDEGHEM, L.M. & KATZUNG, B.G. (1977). Time and voltage-dependent interactions of antiarrhythmic drugs

with cardiac sodium channels. *Biochim. biophys. Acta*, **472,** 373–398.

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

HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: the modulated receptor mechanisms of actions of sodium and calcium channel-blocking drugs. *A. Rev. Pharmac. Tox.*, **24,** 387–423.

LATINI, R., TOGNONI, G. & KATES, R.E. (1984). Clinical pharmacokinetics of amiodarone. *Clin. Pharmacokin.*, **9,** 136–156.

MASON, J.W., HONDEGHEM, L.M. & KATZUNG, B.G. (1983). Amiodarone blocks inactivated cardiac sodium channels. *Pflugers Arch.*, **396,** 79–81.

MASON, J.W., HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Block of inactivated sodium channels and of depolarization-induced automaticity in guinea-pig papillary muscle by amiodarone. *Circulation Res.*, **55,** 277–285.

NADEMANEE, K., HENDRICKSON, J., KANNAN, R. & SINGH, B.N. (1982). Antiarrhythmic efficacy and electrophysiologic actions of amiodarone in patients with life-threatening ventricular arrhythmias: potent suppression of spontaneously occurring tachyarrhythmias versus inconsistent abolition of induced ventricular tachycardia. *Am. Heart. J.*, **103,** 950–959.

NATTEL, S. (1983). Pharmacodynamic studies of amiodarone and its active n-desethyl metabolite. *J. cardiovasc. Pharmac.*, **32,** 2473–2477.

POLSTER, P. & BROEKHUYSEN, J. (1976). The adrenergic antagonism of amiodarone. *Biochem. Pharmac.*, **25,** 131–134.

RAEDER, E.A., PODRID, P.J. & LOWN, B. (1985). Side effects and complications of amiodarone therapy. *Am. Heart J.*, **109,** 975–983.

SADA, H. & BAN, T. (1981). Time-independent effects on cardiac action potential upstroke velocity (resting block) and lipid solubility of beta adrenergic blocker. *Experientia*, **37,** 171–172.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1970). The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. *Br. J. Pharmac.*, **39,** 657–667.

STOREY, G.C.A., ADAMS, P.C., CAMPBELL, R.W.F. & HOLT, D.W. (1983). High-performance liquid chromatographic measurement of amiodarone and desethylamiodarone in small tissue samples after enzymatic digestion. *J. clin. Path.*, **36,** 785–789.

VARRO, A., NAKAYA, Y., ELHARRAR, V. & SURAWITCZ, B. (1985). Use-dependent effects of amiodarone on $\dot{V}_{max}$ in cardiac Purkinje and ventricular muscle fibres. *Eur. J. Pharmac.*, **112,** 419–422.

VAUGHAN WILLIAMS, E.M. (1977). Mexiletine in isolated tissue models. *Postgrad. Med. J.*, **53** (Suppl. 1), 30–34.

VAUGHAN WILLIAMS, E.M. (1984). A classification of antiarrhythmic actions reassessed after a decade of new drug. *J. clin Pharmac.*, **24,** 129–147.

WALTON, M. & FOZZARD, H.A. (1979). The relation of $\dot{V}_{max}$ to I_{Na}, $\overline{G}_{Na}$ and h-infinity in a model of the cardiac Purkinje fiber. *Biophys. J.*, **25,** 407–420.

YABEK, S.M., KATO, R. & SINGH, B. (1986). Effects of amiodarone and its metabolite, desethylamiodarone, on the electrophysiologic properties of isolated cardiac muscle. *J. cardiovasc. Pharmac.*, **8,** 197–207.

ZIPES, D.P., PRYSTOWSKY, E.N. & HEGGER, J.J. (1984). Amiodarone: electrophysiologic actions, pharmacokinetics and clinical effects. *J. Am. Coll. Cardiol.*, **3,** 1059–1068.

(Received January 16, 1987.
Accepted May 12, 1987.)