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Alterations to the electrical activity of atrial muscle isolated from the rat heart, produced by exposure *in vitro* to amiodarone

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1 Glass microelectrodes were used to record transmembrane electrical activity from cells located just beneath the endocardial surface of the left atrial free wall of rat hearts during superfusion and electrical stimulation *in vitro* at 37°C.

2 Availability of the fast sodium channel for current flow was inferred from the maximum rate of rise of membrane potential during phase O of the action potential ($\dot{V}_{max}$).

3 Muscle exposed to polysorbate 80 (10 to 80 $\mu g\,ml^{-1}$) showed a concentration-dependent lengthening of action potential duration (APD) but no detectable change in $\dot{V}_{max}$.

4 Amiodarone (1 to 20 $\mu g\,ml^{-1}$) was dissolved in physiological salt solution with the aid of polysorbate 80 (50 $\mu g\,ml^{-1}$) and caused a concentration-dependent prolongation of APD and a decrease in $\dot{V}_{max}$, both of which were slow to develop and extremely slow to wash-out. The speed of onset of action of amiodarone varied with drug concentration and ranged from a few minutes with high concentrations to many hours with low concentrations.

Introduction

Amiodarone is an effective anti-arrhythmic drug, particularly against supraventricular tachyarrhythmias, but its therapeutic usefulness is limited by a high incidence of toxic side-effects, some of which are serious (Fogoros *et al.*, 1983). The drug has been designated a member of class 3 on the basis of its ability to prolong the duration of the cardiac action potential (Singh & Vaughan Williams, 1970). However, uncertainties remain regarding details of its mode of action. In particular, it is not known whether action potential prolongation is the only relevant electrophysiological action which the drug possesses. Indeed, blockade of the fast sodium channel, a class 1 action, was first noted by Singh & Vaughan Williams (1970), but was considered by them to contribute little to the overall antiarrhythmic action of the drug. Recent studies, using isolated cardiac tissues exposed *in vitro* to the drug have shown a more prominent block of both the fast sodium and slow calcium channels (Néliat *et al.*, 1982a, b; Mason *et al.*, 1983). Unfortunately, it is difficult to see the relevance of some of these reported class 1 and 4 actions, as the drug concentrations used *in vitro* have been considerably higher than are ever achieved in the blood during therapeutic use (Harris *et al.*, 1981; Debbas *et al.*, 1983; Saksena *et al.*, 1983). The present experiments were undertaken to explore the extent to which lower, therapeutic concentrations of amiodarone block the fast sodium channel in rat atrial muscle exposed *in vitro* to the drug. In addition, an attempt has been made to account for the enormously varied speeds of onset of action of this drug which have been reported by previous workers. When administered orally, for example, despite being well absorbed and reaching peak plasma concentrations within 4 h (Andreasen *et al.*, 1981), there is a lag period of 4 to 8 days before an anti-arrhythmic effect is obtained (Rosenbaum, *et al.*, 1976). After intravenous administration, on the other hand, the effects on cardiac rhythm are maximally established within 15 min (Singh & Vaughan Williams, 1970; Touboul *et al.*, 1975, 1976a, 1979, 1982; Demoulin & Legrand, 1979; Lubbe *et al.*, 1979; Marcus *et al.*, 1981).

Methods

Male rats of the Sprague Dawley strain weighing 320–350 g were killed by a blow to the head. Hearts were excised rapidly and placed, unless otherwise stated, in a solution of the following composition

(mM): NaCl 138, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 10, glucose 10 and gassed with a mixture of 95% O_2 plus 5% CO_2, giving a pH of 7.2. The left atrium was attached, endocardial face upwards, to the base of a superfusion trough maintained at 37°C. Glass microelectrodes filled with a 3M solution of KCl and having an electrical resistance of $1.0-1.5 \times 10^7$ ohms were used to record membrane potentials (V_m) from muscle cells situated just beneath the endocardial surface. These voltages were transmitted via a cathode-follower circuit to a dual channel oscilloscope equipped with a camera, the second channel of which displayed a time-differentiated derivative of the V_m signal ($\dot{V}_{max}$) during phase O of the action potential. Values of $\dot{V}_{max}$ provide a convenient measure of the availability of the fast sodium channel for current flow (Gettes & Reuter, 1974). The muscle was stimulated via a pair of platinum wires with square wave pulses, each of 10 V and 1 ms duration, at a rate of 1 Hz unless otherwise specified in the text. Facilities were available to stimulate the muscle not only with single pulses but also, once every 20 s, with a pair of stimuli, the members of which could be separated from each other by any chosen interval between 5 and 1000 ms. In this way it was possible to study the kinetics of recovery of excitability immediately following the end of the refractory period (RP).

Amiodarone was kindly supplied by Labaz: Sanofi UK Ltd. of Stockport, as the commercially available 'Cordarone' injection, containing 150 mg of amiodarone hydrochloride in 3 ml of an aqueous vehicle containing 10% polysorbate 80 and 2% benzyl alcohol as solubilisers. Dilution of this injection fluid with physiological salt solution to a final drug concentration of between 1 and 20 $\mu g\,ml^{-1}$ unfortunately caused precipitation. However, if additional polysorbate 80 was included at a final concentration of 50 $\mu g\,ml^{-1}$ the amiodarone remained in solution indefinitely. In the experiments to be described here, therefore, additional polysorbate 80 was included to a final cocentration of 50 $\mu g\,ml^{-1}$ in all amiodarone solutions.

Results

Atrial muscle exposed to the standard superfusate for an hour had a V_m of 81 ± 6 mV, inside negative. When stimulated electrically, the muscle yielded action potentials having a duration (APD) of 72 ± 11 ms, measured to the point of 90% repolarization. The muscle first became re-excitable after an action potential when it had repolarized to -64 mV, which represents a RP of 40 ± 8 ms.

Polysorbate 80 is included in commercial injection solutions of amiodarone as a solubiliser. It has been shown in the present experiments, however, to exert electrophysiological actions of its own, causing an increase in APD (ΔAPD) that was concentration-dependent (Figure 1), and rapid in onset (Figure 2). The relationship between APD and RP produced by polysorbate 80 is shown by the closed symbols in Figure 3, where ΔAPD is plotted against the corresponding increments in RP produced by the various

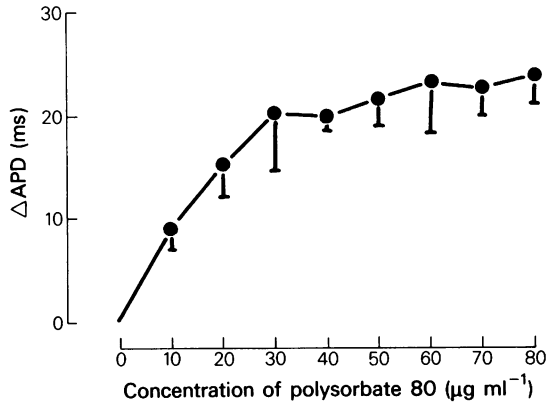

Figure 1 Prolongation of action potential duration (ΔAPD) produced by exposure for 8 h to varying concentrations of polysorbate 80. Each point is the mean of between 12 and 28 measurements. Increments in APD were measured by comparison with observations made in the basic superfusate immediately prior to surfactant exposure. Vertical lines represent s.e.

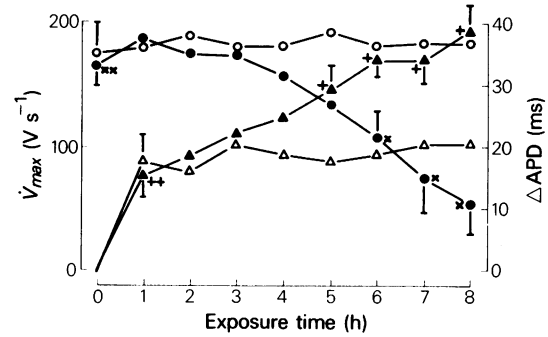

Figure 2 Effect of exposure for varying times to polysorbate 80 alone at 50 $\mu g\,ml^{-1}$ (open symbols) or to a combination of amiodarone at 5 $\mu g\,ml^{-1}$ plus polysorbate 80 at 50 $\mu g\,ml^{-1}$ (closed symbols). Changes in action potential duration (ΔAPD) and values of phase 0 $\dot{V}_{max}$ are shown as triangles and circles respectively. Values of ΔAPD were measured by comparison with observations made in the basic superfusate immediately prior to drug or surfactant exposure. Each point is the mean of between 10 and 32 measurements. A significant difference exists between the value marked xx and those marked x, and between the value marked ++ and those marked +. Vertical lines represent s.e.

concentrations of polysorbate 80. In each case, the values of APD and RP used for comparison were measured immediately prior to surfactant exposure. The best-fitting straight line through these closed symbols is almost exactly superimposable upon the line of equality shown in the Figure. This suggests that the prolongation of RP caused by polysorbate 80 is entirely accounted for by the corresponding prolongation of APD. This fact, together with the failure of polysorbate 80 to alter $\dot{V}_{max}$ (Figure 2), suggests that this agent is unable to block the fast sodium channel in atrial muscle.

Benzyl alcohol is also included as a solubiliser in commercial injection solutions of amiodarone. At a final concentration of 20 $\mu g\,ml^{-1}$, it failed to alter any of the electrophysiological parameters in the present study. Since the final concentration of benzyl alcohol in the superfusate used in the present experiments was always less than 20 $\mu g\,ml^{-1}$, this agent was considered to be inert.

Amiodarone was always tested in the present experiments in the presence of polysorbate 80. It is necessary, therefore, to examine the extent to which amiodarone produced actions additional to those caused by the polysorbate 80 with which it was dissolved. Figure 2 shows that amiodarone caused a slowly developing prolongation of APD which was quite distinct from that attributable to the contained polysorbate 80. Thus, the prolongation of APD during the first 3 h of drug exposure was fully accounted for by the effects of the contained polysorbate 80. Beyond that time, however, there was a gradually developing additional prolongation of APD which is attributable to the amiodarone itself. From the same time, amiodarone also caused an equally slowly developing decrease in $\dot{V}_{max}$ (Figure 2).

Amiodarone caused a considerable prolongation of RP, and in contrast to the action of polysorbate 80, this was much greater than could be accounted for solely by prolongation of APD. Thus, the best-fitting straight line through the amiodarone points in Figure 3 is significantly displaced towards the RP axis compared with the line of equality. By analogy with classical class 1 drugs, it seems appropriate to ascribe

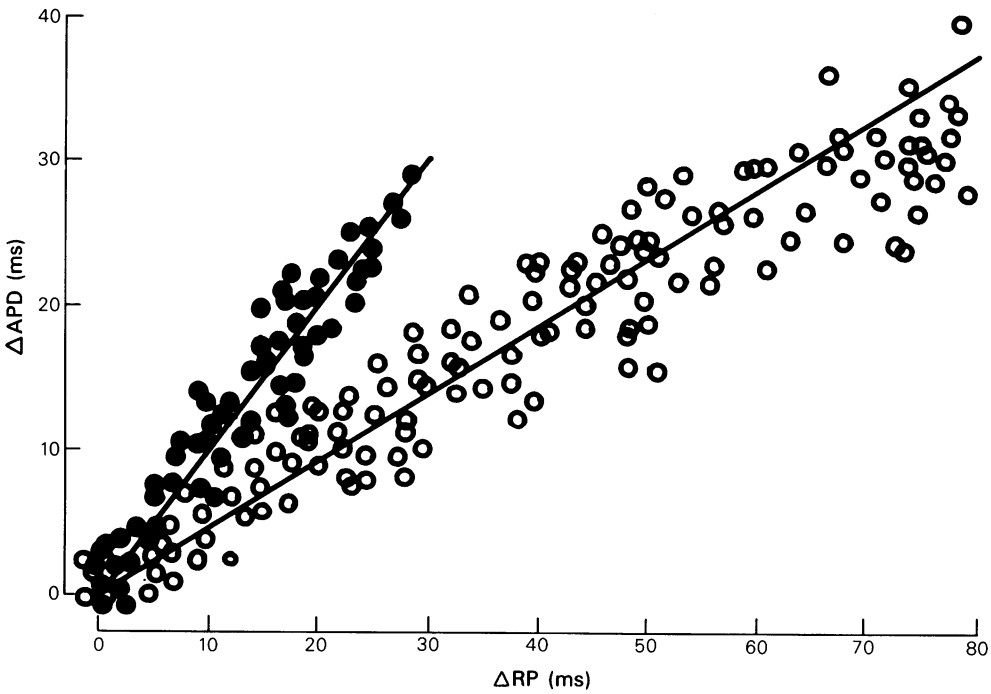

Figure 3 Effects of exposure for 8 h to varying concentrations of polysorbate 80 alone (closed symbols) or to various concentrations of amiodarone in polysorbate 80 at 50 $\mu g\,ml^{-1}$ (open symbols). Changes in action potential duration (Δ APD) and in refractory period (Δ RP) were measured by comparison with observations made in the basic superfusate immediately prior to drug or surfactant exposure. Each point is the mean of between 3 and 16 observations from a single cell. The straight line through the polysorbate 80 points is the theoretical line of equality. This line differs significantly in gradient from the best-fitting straight line through the amiodarone points ($P<0.001$).

this extra prolongation of RP to block of the fast sodium channel. This is also reflected in the amiodarone-induced decrease in $\dot{V}_{max}$ shown in Figure 2.

A striking feature of the action of amiodarone was the slowness with which sodium channel blockade and prolongation of APD developed during exposure to the lower drug concentrations tested, but the rapid development of both features with the higher drug concentrations (Figures 4 and 5). Washing the tissue with drug-free superfusate for up to 4 h produced less than 20% restoration of both sodium channel availability and APD in the 6 experiments where this was studied after the tissue had been exposed to amiodarone at 10 $\mu g\,ml^{-1}$ for 3 h. Tissues exposed to amiodarone at 4 $\mu g\,ml^{-1}$ or less for 8 h showed no significant change in the diastolic value of V_m, although decreases were noted with higher concentrations shortly before electrical inexcitability occurred.

The ability of amiodarone to block the fast sodium channel was greatly potentiated by raising the concentration of KCl in the superfusate (Figure 6). The reverse was seen when the concentration of KCl was lowered. Prolongation of APD by amiodarone was much less influenced by KCl concentration. It is noteworthy also that in the experiments shown in Figures 2 and 6 depression of $\dot{V}_{max}$ occurred simultaneously with the prolongation of APD if the superfusate contained the normal concentration of KCl, namely 5 mM. In contrast, at an elevated concentration of 10 mM, a significant depression of $\dot{V}_{max}$ occurred sooner than the amiodarone-induced prolongation of APD. When the experiment was conducted with KCl at 2.5 mM, however, amiodarone failed to depress $\dot{V}_{max}$ during the 8 h experiment (Figure 6) but caused a significant prolongation of APD after only 4 h of drug exposure. These results suggest that the class 1 action of amiodarone is more prominent at high concentrations of KCl and the class 3 action at low concentrations of KCl.

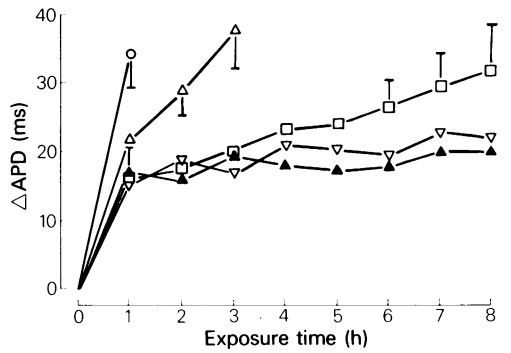

Figure 5 Changes in action potential duration (ΔAPD) produced by exposure for varying lengths of time to polysorbate 80 alone at 50 $\mu g\,ml^{-1}$ (▲), or to a combination of polysorbate 80 at 50 $\mu g\,ml^{-1}$ plus amiodarone at concentrations of 20 (○), 10 (△), 4 (□) or 2 (▽) $\mu g\,ml^{-1}$. Vertical lines represent s.e. and are shown only where the mean of an amiodarone-treated tissue differs significantly ($P<0.05$) from that of the control tissue exposed for 1 h to polysorbate alone.

Discussion

The present experiments have shown that *in vitro* exposure of rat atrium to therapeutically relevant concentrations of amiodarone produced several dis-

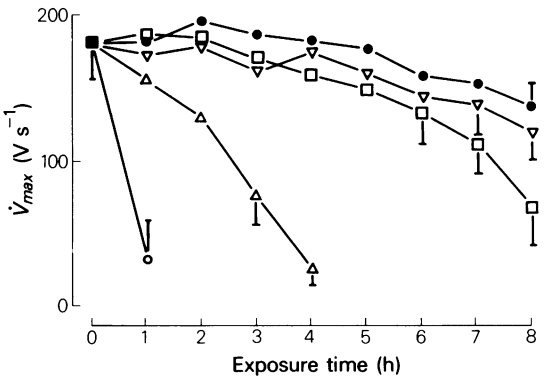

Figure 4 Effect on phase 0 $\dot{V}_{max}$ of exposure for varying times to amiodarone at concentrations of 1 (●), 2 (▽), 4 (□), 10 (△) and 20 (○) $\mu g\,ml^{-1}$. Vertical lines represent s.e. and are shown only where a mean differs significantly ($P<0.05$) from that of control measurements (■) recorded in the basic superfusate. Each point is the mean of between 8 and 35 observations.

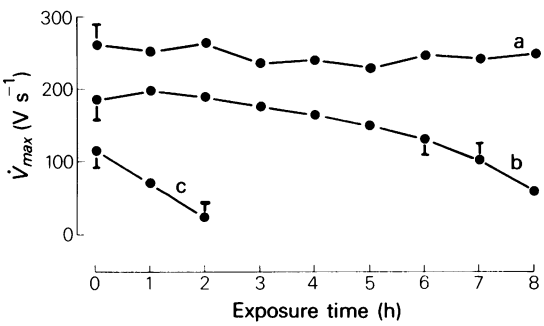

Figure 6 Effect on phase 0 $\dot{V}_{max}$ of exposure to amiodarone (4 $\mu g\,ml^{-1}$) for varying times in the presence of KCl at 2.5 (a), 5.0 (b) and 10 (c) mM. Each point is the mean of between 14 and 22 observations. Vertical lines represent s.e. and are shown only where mean values differ significantly ($P<0.05$) from the mean of control measurements recorded at time zero at the same concentration of KCl but in the absence of amiodarone.

tinct electrophysiological effects. In addition to prolongation of APD there was block of the fast sodium channel reflected in a reduction in the value of phase 0 $\dot{V}_{max}$. There was also prolongation of RP to a degree that was considerably greater than was attributable to the delay in phase 3 action potential repolarization. These effects of amiodarone confirm and extend earlier findings of Néliat *et al.* (1982a, b) and Mason *et al.* (1983).

Sodium channel block caused by amiodarone was shown in the present experiments to depend upon the prevailing concentration of KCl. This action resembles that of the classical anti-arrhythmic drugs of class 1 (Bein, 1948). Extracellular concentrations of potassium govern the diastolic value of V_m in cardiac muscle, and thereby govern not only the opening and closing of the fast sodium channel during phase 0 of the action potential but also regulate the affinity of the gates within the channel for anti-arrhythmic drug molecules (Chen *et al.*, 1975).

The class 1 action of amiodarone described in the present paper may or may not be relevant to the clinically useful anti-arrhythmic effects produced by the drug. One must remember that amiodarone was introduced into medicine first because it reduced myocardial ischaemia *in vivo* (Charlier *et al.*, 1962). The extent to which relief of ischaemia contributes to the anti-arrhythmic effects of the drug *in vivo*, is unknown. Relief of ischaemia would be less likely to explain the atrial effects of the drug than effects upon the ventricle, and it is with the former that the present paper is concerned. Another uncertainty concerns the relative contributions of class 1 and 3 actions to the overall anti-arrhythmic effects of amiodarone. If a class 1 action operates in human atrial muscle *in vivo* then amiodarone-treated patients might be expected to display slowing of intra-atrial conduction. Indeed, some investigators have found such a slowing (Coutte *et al.*, 1976; Touboul *et al.*, 1976b; Waleffe, *et al.*, 1978), albeit sometimes only a slight slowing and one which was not always statistically significant (Finerman *et al.*, 1982). The relevant literature has been reviewed recently by Camm & Bexton (1983). Failure of therapeutic concentrations of class 1 agents to slow action potential conduction velocity during normal sinus rhythm, or to do so only modestly, however, may be accounted for by the fact that depression of $\dot{V}_{max}$ is often dependent upon the time interval between action potentials, as was pointed out for lignocaine by Sarec, *et al.* (1981). Indeed, one of the therapeutically most valuable features of a class 1 agent such as lignocaine is the ability to retard selectively the restoration of sodium channel availability following an action potential. This prolongs the refractory period, selectively blocks conduction of early-cycle extrasystoles, and slows the maximally attainable rates of paroxysmal tachycardias, without significantly reducing either $\dot{V}_{max}$ or conduction velocity of action potentials produced by normal sinus beats. Were the sodium channel-blocking action of amiodarone to be of this type, therefore, one would expect atrial conduction velocity to be more strongly reduced at high rates of atrial stimulation, and this feature was actually observed by Finerman *et al.* (1982). A systematic search for a contribution of a class 1 action to the overall anti-arrhythmic effectiveness of this drug now seems to be needed.

The rapidity of the onset of action of amiodarone was shown in the present experiments to be highly dependent upon the concentration of drug in the superfusate. This probably accounts for the great variations in previously reported rates of onset of action of the drug *in vivo*. Amiodarone concentrations in the blood following oral doses of 200 to 400 mg are in the range 0.5–5.0 $\mu g\,ml^{-1}$, and usually less than 1 $\mu g\,ml^{-1}$ (Andreasen *et al.*, 1981; Debbas *et al.*, 1983; Holt & Storey, 1983). At concentrations of 1 and 2 $\mu g\,ml^{-1}$, several hours were required in the present experiments before electrophysiological effects were detected (Figure 4) and the effects were still increasing after 8 h of exposure to the drug. One may readily understand, therefore, why previous workers have found a delay of more than 24 h between the oral administration of conventional doses and the appearance of cardiac effects. On the other hand, at concentrations of 10 and 20 $\mu g\,ml^{-1}$ in the present experiments there was a decline in $\dot{V}_{max}$ values within a few minutes of exposure to the drug. Peak plasma concentrations of amiodarone following the intravenous administration of 400 mg of the drug to man were about 20 $\mu g\,ml^{-1}$ (Andreasen *et al.*, 1981: Holt & Storey, 1983). After the intravenous administration of doses of this magnitude, therefore, one can readily see why there is a delay of only a few minutes before cardiac effects begin. Plasma concentrations of amiodarone fall rapidly after administration of intravenous bolus doses to values of less than 1 $\mu g\,ml^{-1}$ after 5 h (Andreasen *et al.*, 1981). Presumably this prevents excessive effects of the drug on the myocardium such as were found in the present experiments with the continued presence of 20 $\mu g\,ml^{-1}$ of the drug (Figure 4).

Few previous workers have studied the electrophysiological effects obtained by exposing cardiac tissue to amiodarone *in vitro*, probably because of the low solubility of the drug in physiological salt solution (Vaughan Williams, 1979). The solubility problem has been overcome, however, in the present experiments by adding polysorbate 80 at a final concentration of 50 $\mu g\,ml^{-1}$. Néliat *et al.* (1982a) applied amiodarone to muscle isolated from the hearts of frogs and ferrets. To minimize drug precipitation they diluted the commercially available injection solution with physiological salt solution 'immediately

prior to addition to the tissue bath'. Precipitation would probably still have occurred while their experiments were in progress. Indeed, this may account for the 100 fold higher concentrations of amiodarone needed to produce electrophysiological effects in their experiments compared with the concentrations shown to be effective in the present experiments. Mason *et al.* (1983) avoided drug precipitation by adding albumin to all their drug solutions. Unfortunately, however, they reported the effects of only a single high concentration of the drug, namely 60 $\mu g\,ml^{-1}$, and only after 30 min exposure to the drug, with sodium channel blockade being the most prominent feature.

Polysorbate 80 caused a concentration-dependent increase in APD in the present experiments, without causing a detectable change in the value of phase 0 $\dot{V}_{max}$. This class 3 action does not seem to have been reported previously, although there are reports of a negative inotropic action *in vivo* (Gough, *et al.*, 1982). This raises the possibility that part of the observed clinical anti-arrhythmic action following intravenous injection of an ampoule of 'Cordarone' may be due to its 300 mg content of polysorbate 80. At concentrations as low as 10 $\mu g\,ml^{-1}$ in the present experiments this agent prolonged APD. If the volume of distribution of polysorbate 80 was 30 litres or less, as it is likely to be in man, a class 3 anti-arrhythmic effect is to be expected. This possibility warrants further study. Clearly, however, 'Cordarone' injection contains more than one substance with a class 3 anti-arrhythmic action, and the ostensibly active ingredient possesses a class 1 action in addition to its better-known class 3 action. The class 1 action of amiodarone is likely to be more prominent at elevated extracellular concentrations of potassium.

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