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Effect of verapamil and diltiazem on calcium-dependent electrical activity in cardiac Purkinje fibres

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1 The effects of verapamil and diltiazem on normal action potentials, abnormal automaticity at depolarized membrane potential and oscillatory afterpotentials were compared in sheep cardiac Purkinje fibres.

2 Concentrations of verapamil and diltiazem exerting the same action on abnormal automaticity due to slow action potentials, caused different effects on action potential characteristics and on oscillatory afterpotentials.

3 Diltiazem significantly shortened action potential duration whereas verapamil slightly lengthened it (NS).

4 Diltiazem appeared to be more effective than verapamil in preventing the development of oscillatory afterpotentials induced by barium or by strophanthidin.

5 In 50% of barium-treated preparations, verapamil caused the appearance of spontaneous activity due to enhanced normal diastolic depolarization, while diltiazem had no such effect.

6 The observed differences were explained in terms of the different effects of the two drugs on currents other than the slow inward current, since diltiazem was more potent than verapamil in depressing $\dot{V}_{max}$.

Introduction

There is increasing evidence that the electrophysiological effects of cardiac ischaemia are mediated by Ca^{2+}. Calcium-antagonists, which are known to reduce calcium entry into the cardiac cell, have been reported to protect the ischaemic myocardium. Such a protection can occur independently of changes in coronary blood flow (Higgins *et al.*, 1980; Jolly *et al.*, 1981), and is accompanied by suppression of early ischaemic arrhythmias in experimental animals. The antifibrillatory effect of verapamil was first demonstrated by Kaumann & Aramendia in 1968, and subsequently confirmed by several other studies (Fondacaro *et al.*, 1978; Brooks *et al.*, 1980; Thandroyen, 1982). A similar effect has been suggested for other calcium antagonists including diltiazem (Clusin *et al.*, 1982). However, the antifibrillatory efficacy of diltiazem has recently been denied (Sheehan & Epstein, 1982; Patterson *et al.*, 1983). The direct antiarrhythmic effect of this class of drugs is attributed to their ability to block calcium entry into the cell. In fact, a rise in intracellular calcium causes many effects that are potentially arrhythmogenic, such as the uncoupling of gap junctions between cells (Dahl & Isenberg, 1980), or the activation of a Na/K channel (Colquhoun *et al.*, 1981). Through these channels the oscillatory inward current recorded under different experimental conditions in cardiac Purkinje fibres (Lederer & Tsien, 1976; Vassalle & Mugelli, 1981) and underlying the oscillatory afterpotentials (delayed afterdepolarizations), is thought to flow. Basically there are two forms of calcium-dependent electrical activity that might be potentially important in the genesis of acute ischaemic ventricular arrhythmias: slow action potentials (Cranefield & Dodge, 1980) and oscillatory afterpotentials (delayed afterdepolarizations) (El-Sherif *et al.*, 1983). Slow action potentials have been recorded during ventricular fibrillation from the heart *in situ* (Akiyama, 1981) and oscillatory afterpotentials have been recorded from infarcted tissues (Rosenthal *et al.*, 1983; El-Sherif *et al.*, 1983). We thought it important to study the effects of verapamil and diltiazem on these cellular mechanisms. Our primary objective was to compare their actions on abnormal automaticity due to slow action potentials and on oscillatory afterpotentials. At the same time, it appeared necessary to evaluate in some detail their

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effects on the electrical activity of normal Purkinje fibres.

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Methods

Sheep hearts were brought from the slaughterhouse to the laboratory in cool oxygenated Tyrode solution. Purkinje strands were excised from the ventricles and kept in oxygenated Tyrode solution at room temperature until used. One strand was mounted in a tissue bath and superfused with Tyrode solution at a rate of $8\,ml\,min^{-1}$. The Tyrode solution had been equilibrated with a mixture of 97% O_2 and 3% CO_2 and warmed to 37°C; the pH of the solution was 7.3–7.4. The composition of the Tyrode solution was as follows (mM): NaCl 137, KCl 4, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, $MgCl_2$ 0.5, $CaCl_2$ 2.7, glucose 5. The concentration of KCl and $CaCl_2$ was changed in different experiments, as indicated when appropriate. The preparations were stimulated with rectangular pulses (0.5 to 1 ms in duration and 1.5 times the threshold) through bipolar silver electrodes that were electrically insulated except for the tip. Transmembrane action potentials were recorded by means of two glass microelectrodes filled under vacuum with 3 M KCl (resistance about 10 MΩ). The tip of one electrode was inserted intracellularly and the other was placed in the solution near the preparation. The membrane potential was measured differentially by means of two high-input impedance guard electrometer amplifiers (Bigongiari, Florence). The potential was displayed on a Tektronix model 5113 dual beam storage oscilloscope and recorded on a FM tape recorder (Racal 14 DS) at 3.75 and 7.5 inches s^{-1}. The records were played back into a chart recorder (Gould Brush 2400). For studies in normal Purkinje fibres, the preparations were stimulated at constant rate (1 Hz) and an automated analysis of action potential was performed as previously described (Fusi *et al.*, 1984). The evaluation of the following parameters was carried out: action potential amplitude (AP), overshoot (OS), maximum diastolic potential (MDP), maximum upstroke velocity ($\dot{V}_{max}$), action potential duration at -60 mV (APD_{-60}) and at 90% repolarization (APD_{90}).

Studies on fibres exposed to barium chloride or strophanthidin

It has been shown that in Purkinje fibres, barium chloride causes the appearance of sustained spontaneous activity, which occurs when the fibre fails to repolarize completely after a normal action potential upstroke (Mugelli *et al.*, 1983). Such abnormal automaticity is characterized by a maximum diastolic potential in the range of -50 to -40 mV, typical phase 4 depolarization and action potentials with a slow upstroke and small overshoot. We used $BaCl_2$ in concentrations between $1-5 \times 10^{-4}$ M ($[K^+]_o = 2.7$ mM) to induce abnormal automaticity. Once stable abnormal automaticity was obtained, the effect of different concentrations of verapamil and diltiazem was studied. Concentrations of verapamil and diltiazem which had the same effect on abnormal automaticity were selected for further study on normal action potentials and oscillatory afterpotentials. Oscillatory afterpotentials were induced by exposing

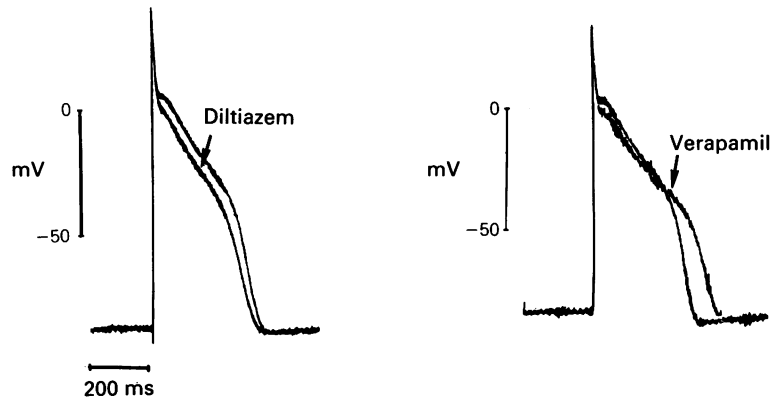

Figure 1 Effect of diltiazem and verapamil on action potential configuration. The records are taken from two different preparations superfused with normal Tyrode solution, before and 30 min after the indicated drug. In this and the following Figures the concentrations of diltiazem and verapamil were 4.4 μM and 2 μM, respectively. Driving rate: 1 Hz.

Table 1 Effects of verapamil and diltiazem on action potential characteristics

	AP (mV)	*OS* (mV)	*MDP* (mV)	APD_{-60} (ms)	APD_{90} (ms)	$\dot{V}_{max}$ ($V\,s^{-1}$)
Control ($n = 10$)	125.6 ± 1.6	39.4 ± 1.7	85.2 ± 1.0	356 ± 25	405 ± 27	575 ± 47
Verapamil (2 μM)	121.9 ± 2.3**	37.8 ± 1.9*	83.1 ± 1.5*	378 ± 23	422 ± 23	559 ± 52
Control ($n = 12$)	125.5 ± 1.9	38.4 ± 1.3	87.1 ± 0.9	370 ± 18	412 ± 19	604 ± 32
Diltiazem (4.4 μM)	121.5 ± 1.8***	36.6 ± 1.0	84.9 ± 1.4*	334 ± 16*	375 ± 16*	546 ± 40**

AP = amplitude, OS = overshoot, MDP = maximum diastolic potential, APD_{-60} and APD_{90} action potential duration measured at − 60 mV and at 90% of repolarization respectively, $\dot{V}_{max}$ = maximum rate of upstroke. Driving rate 1 Hz. Data are presented as means ± s.e.mean $*P<0.05$; $**P<0.01$, $***P<0.001$.

the preparations to low barium concentrations ($1-3 \times 10^{-5}$ M, $[Ca^{2+}]_o = 7.2$ mM) as previously described (Mugelli *et al.*, 1983) or to strophanthidin ($3-5 \times 10^{-7}$ M). The drive stimulus was interrupted periodically (usually every 1 min for 30 s) to assess the presence of oscillatory afterpotentials and eventually of spontaneous activity. The drugs used were as follows: strophanthidin (Sigma), verapamil hydrochloride (Knoll), diltiazem hydrochloride (Parke Davis), $BaCl_2$.

Analysis of data

All the data are presented as means ± s.e.mean. The analysis of data for significance was performed by means of Student's *t* test for paired or grouped data. The amplitude of oscillatory afterpotentials was measured from the peak to the maximum membrane potential immediately following the oscillatory afterpotentials.

Results

Studies on normal Purkinje fibres

Each Purkinje fibre preparation was driven at 1 Hz and allowed to equilibrate for at least 60 min to obtain stable control records. Verapamil (2 μM) or diltiazem (4.4 μM) were then superfused; records were monitored for 30–40 min. Typical records showing the effects of the drugs on the action potential configuration are given in Figure 1. Both drugs depressed the plateau but affected the action potential duration in opposite directions. A summary of the results obtained in 22 experiments is shown in Table 1.

Effects on abnormal automaticity

Abnormal automaticity was induced in 10 preparations by superfusing them with Tyrode solution containing $BaCl_2$ in concentrations of 1 to 5×10^{-4} M. When spontaneous activity began, the driving stimulus was turned off. As shown in Figure 2, barium caused marked changes in the electrical activity: the fibre failed to repolarize completely, leading to sustained rhythmic activity at depolarized membrane level (the maximum diastolic potential was − 43.5 ± 1.7 mV, $n = 10$). Under control conditions, the barium-treated preparations generated spontaneous action potentials of small amplitude (48.1 ± 4.2 mV, $n = 10$) at a steady rate (44.0 ± 4.1 beats min^{-1}, $n = 10$) for periods of several hours. After a sufficient period had elapsed, the superfusate was changed to one containing verapamil or diltiazem. Verapamil (2 μM) and diltiazem (4.4 μM) arrested the abnormal automaticity in less than 2 min. Usually the total amplitude of the action potentials decreased before abolition of automaticity (Figure 3).

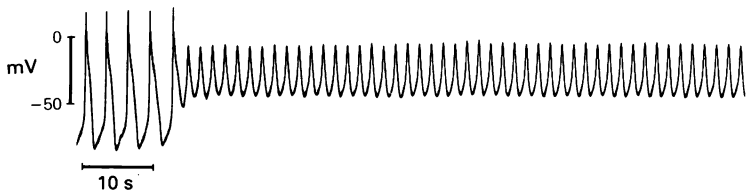

Figure 2 Typical experiment showing the appearance of abnormal automaticity at depolarized membrane potential in the presence of barium (5×10^{-4} M). $[K^+]_o = 2.7$ mM. The figure shows four normal spontaneous action potentials; after the 5th, the fibre fails to repolarize and a sustained rhythm ensues.

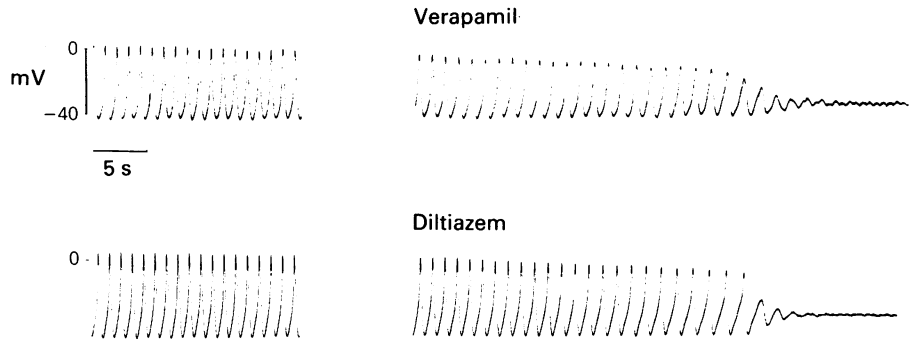

Figure 3 Effect of verapamil and diltiazem on abnormal automaticity at depolarized membrane potential, induced as in Figure 2.

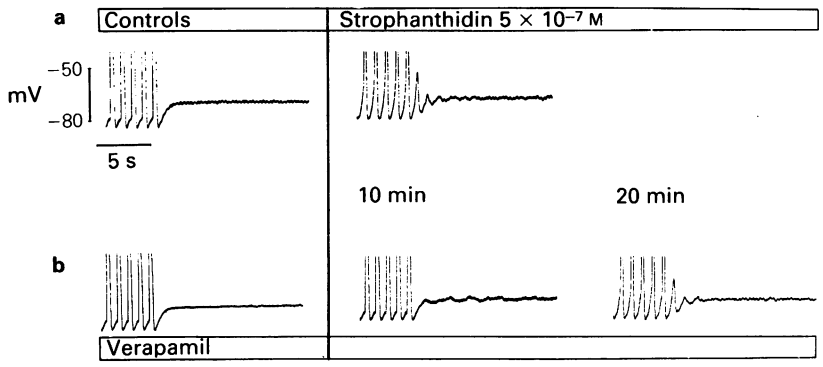

Figure 4 Induction of oscillatory afterpotentials by strophanthidin in the absence (a) and in the presence (b) of verapamil. Each panel shows the lower part of the last driven action potentials and the electrical activity during the interruption of the stimulation. Records obtained from the same fibre.

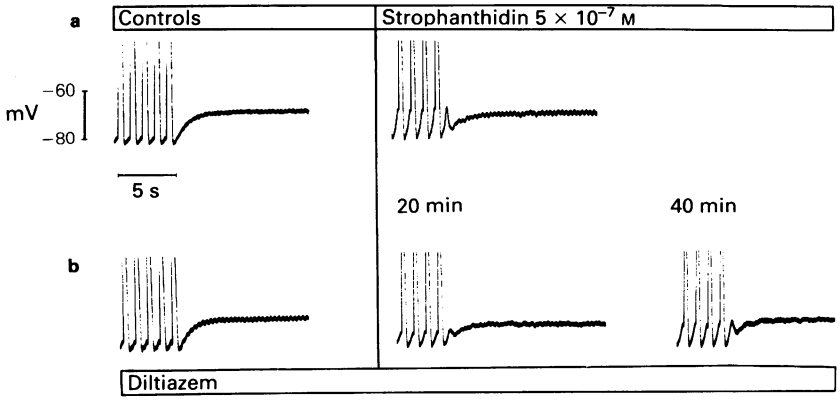

Figure 5 Induction of oscillatory afterpotentials by strophanthidin in the absence (a) and in the presence (b) of diltiazem. Each panel shows the lower part of the last driven action potentials and the electrical activity during the interruption of the stimulation. Records obtained from the same fibre.

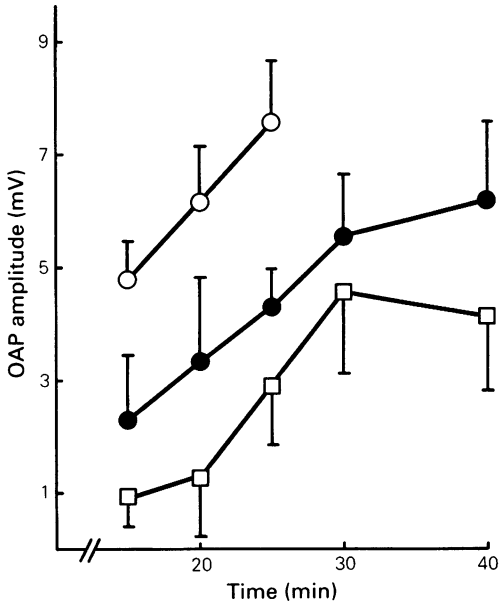

Figure 6 Effect of verapamil and diltiazem on the development of strophanthidin-induced oscillatory afterpotentials. Each point represents the mean of 4 to 9 experiments; vertical lines show s.e.mean. (○) Strophanthidin; (●) strophanthidin plus verapamil; (□) strophanthidin plus diltiazem.

Effects on oscillatory afterpotentials

The preparations were superfused, after the control period, with Tyrode solution containing strophanthidin; the development of the oscillatory afterpotentials was monitored for at least 25 min. The preparation was then superfused with drug-free solution for 1.5 h, until complete recovery was obtained. Superfusion for 30 min with verapamil or diltiazem was started; at this time the preparation was superfused again with strophanthidin (in the presence of verapamil or diltiazem). Figures 4 and 5 show typical records of the effects of verapamil and diltiazem on strophanthidin-induced oscillatory afterpotentials. In each panel, the lower part of the last driven action potentials and the electrical activity during the interruption of the stimulation are recorded.

Superfusion with strophanthidin caused a marked steepening of diastolic depolarization; that this effect is due to the development of an oscillatory afterpotential superimposed on the diastolic depolarization is clearly demonstrated by the records after the interruption of the stimulation. Thus the reduction in the slope of diastolic depolarization observed with both verapamil and diltiazem (in the presence of strophanthidin) can be attributed to the reduction they caused on the oscillatory potential amplitude. This is also confirmed by the lack of effect of the two drugs on the slope of diastolic depolarization in the absence of strophanthidin (compare the upper and lower left-hand panels of Figures 4 and 5).

The effects of verapamil (2 μM) and diltiazem (4.4 μM) on strophanthidin-induced oscillatory afterpotentials are summarised in Figure 6. It is apparent that both drugs markedly delayed the development of the strophanthidin-induced oscillatory afterpotentials. Diltiazem appears to be more active than verapamil.

Similar results were obtained on barium-induced oscillatory afterpotentials. Barium ($1-3 \times 10^{-5}$ M) in

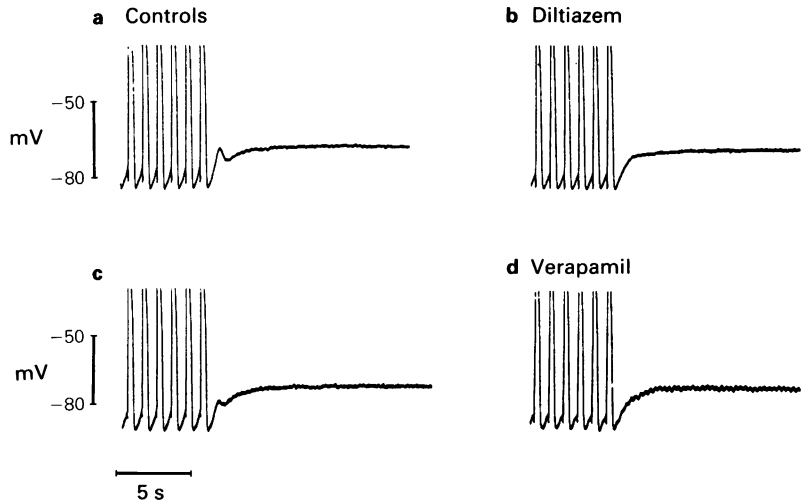

Figure 7 Effect of diltiazem and verapamil on barium-induced oscillatory after-potentials. Each panel shows the lower part of the last driven action potentials and the electrical activity during the interruption of the stimulation.

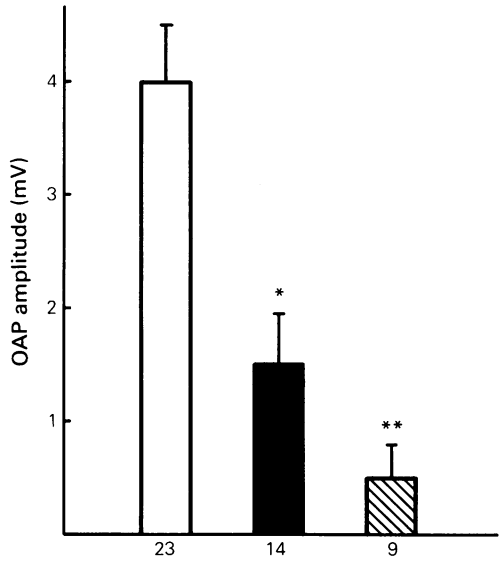

Figure 8 Effects of verapamil and diltiazem on the amplitude of barium-induced oscillatory afterpotentials: open column, Ba^{2+} alone; solid column, Ba^{2+} plus verapamil; hatched column, Ba^{2+} plus diltiazem. Figures on abscissa scale = number of experiments. $*P<0.005$, $**P<0.0001$.

the presence of high $[Ca^{2+}]_o$, induces oscillatory afterpotentials which present the characteristic features of the oscillatory afterpotentials induced by different experimental procedures (see Mugelli *et al.*, 1983). The barium-induced oscillatory afterpotentials developed rapidly (the maximal effect was obtained in 10 min) and promptly disappeared on washing. Diltiazem consistently prevented their appearance (Figure 7b and Figure 8); verapamil had a similar effect (Figure 7d and Figure 8). However, in 7 out of 14 preparations verapamil, in the presence of barium, caused the appearance of spontaneous activity when the stimulation was interrupted (Figure 9). Such activity is not due to an oscillatory afterpotential which reaches the threshold, but instead to the enhanced normal diastolic depolarization. In fact, as shown in Figure 9a, the oscillatory afterpotential peaks after approximately 1 s, that is the cycle length of the previous drive. In the presence of verapamil (Figure 9b), no oscillatory afterpotential is recorded after 1 s. Instead, the potential oscillation recorded later on may be considered as an oscillatory prepotential which is known to be closely associated with the normal generation of impulses (Vassalle, 1965).

Discussion

The present results show that verapamil (2 μM) and diltiazem (4.4 μM) exert the following actions: (1) they affect the action potential duration in normal Purkinje fibres in opposite directions; (2) they block the abnormal automaticity at depolarized membrane potentials with the same efficacy; (3) they delay the development of oscillatory afterpotentials induced by strophanthidin (diltiazem appearing more effective than verapamil); (4) they consistently prevent the appearance of oscillatory afterpotentials induced by barium; (5) in the presence of barium, verapamil may cause the appearance of spontaneous activity due to enhanced normal diastolic depolarization, while diltiazem has no such effect.

Abnormal automaticity at depolarized membrane potential is characterized by action potentials of small amplitude and overshoot, and by fairly marked phase 4 depolarization. Barium induced such abnormal automaticity mainly by reducing the conductance of the membrane to potassium (Mugelli *et al.*, 1983), i.e. by a mechanism which plays no role in naturally-occurring disrhythmias. The action potentials generated under these conditions appear to be slow responses. Slow responses depend on an inward depolarizing current, carried primarily by Ca^{2+}, which flows through the slow channel in partially depolarized cells (see Carmeliet, 1980). The ability to suppress slow responses is one of the fundamental actions of calcium antagonists (Fleckenstein, 1981).

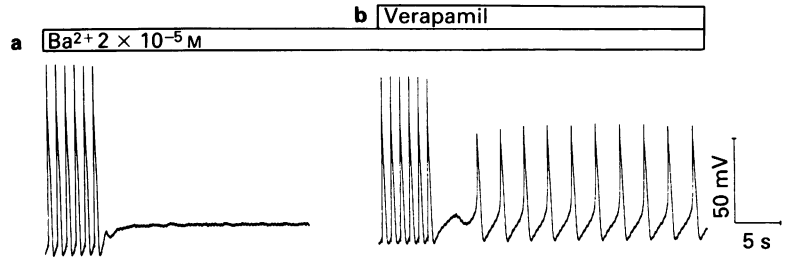

Figure 9 Verapamil abolishes the barium-induced oscillatory afterpotential but causes the appearance of spontaneous activity. $[Ca^{2+}]_o = 7.2$ mM. See text for details.

We found that verapamil (2 μM) and diltiazem (4.4 μM) produced the same effect on abnormal automaticity, thus reflecting their efficacy in abolishing slow action potentials (Fleckenstein, 1981).

Oscillatory afterpotentials are transient depolarizations, which occur after phase 3 repolarization has restored maximum diastolic potential, and thus necessarily follow an action potential (Hoffman & Rosen, 1981). If large enough to reach the threshold, these oscillatory afterpotentials might initiate a premature response or a self-sustained rhythm (Cranefield, 1977). Oscillatory afterpotentials (transient depolarizations, delayed afterdepolarizations) have been recorded under different experimental conditions, such as in the presence of toxic amounts of cardiac glycosides (Ferrier, 1977) or in the presence of low barium concentrations (Mugelli *et al.*, 1983). They are due to a transient inward oscillatory current which has been recorded in the presence (Lederer & Tsien, 1976) and in absence (Vassalle & Mugelli, 1981) of cardiac steroids. The transient inward oscillatory current is independent of the pacemaker current (Vassalle & Mugelli, 1981; Mugelli, 1982), which is responsible for the normal diastolic depolarization. It is not directly dependent on the slow inward current, but is enhanced by all procedures which supposedly increase calcium (Vassalle & Mugelli, 1981). Oscillatory afterpotentials are in fact considered as an expression of 'calcium overload'. It has been suggested recently that there is a direct correlation between calcium overload, oscillatory afterpotentials and early ischaemic cardiac arrhythmias (Clusin *et al.*, 1983).

Calcium antagonists, by antagonising calcium entry into the cell, delay the occurrence of the calcium overload and thus the development of the oscillatory afterpotentials. The observation that diltiazem appears to be more effective than verapamil (Figure 6 and Figure 8), may be explained by its effect on $\dot{V}_{max}$. In fact (Table 1) at the concentration used, diltiazem caused a significant reduction in rate of rise of the upstroke of the action potential, thus reducing Na entry into the cells. It has been reported that drugs able to reduce fast Na conductance, such as lidocaine, or tetrodotoxin, also decrease the transient inward current (Eisner & Lederer, 1979) and oscillatory afterpotential amplitude in Purkinje fibres (Rosen & Danilo, 1980). However, it is not known if this effect is the consequence of a decreased Na entry or may result from more direct action in mechanisms controlling intracellular calcium. At present it is not known whether the transient inward current flows through the Ca-activated non-specific channels described by Colquhoun *et al.* (1981) or whether it is due to the Na/Ca exchanger (Noble, 1984).

An important difference between verapamil and diltiazem concerns their interaction with barium. Verapamil, while preventing the barium-induced oscillatory afterpotentials, causes the appearance of spontaneous activity in 50% of the preparations superfused with barium and high $[Ca^{2+}]_o$. The barium-induced enhancement of normal diastolic depolarization on Purkinje fibres may be attributed to reduced background K^+ conductance (Mugelli *et al.*, 1983). Since verapamil also reduces K^+ conductance (Kass & Tsien, 1975; Posner *et al.*, 1975), this additional reduction in K^+ conductance is likely to result in a decrease of maximum diastolic potential, the steepening of diastolic depolarization and the induction of spontaneous activity (see Figure 9). Diltiazem, which has been reported to increase K^+ conductance (Morad *et al.*, 1982), never caused such an effect. The opposite effects caused on action potential duration by verapamil and diltiazem in normal Purkinje fibres are consistent with their opposite effects on K^+ conductance.

In conclusion our results demonstrate that verapamil and diltiazem possess different direct electrophysiological effects. However, these differences do not appear to justify the likelihood that the two drugs have different direct antifibrillatory efficacies.

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