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Comparative antiarrhythmic and electrophysiological effects of drugs known to inhibit calmodulin (TFP, W7 and bepridil)

E. Barron, R.J. Marshall, M. Martorana & E. Winslow[1]

Organon Laboratories Limited, Newhouse, Lanarkshire ML1 5SH

1 The potential antiarrhythmic and electrophysiological actions of drugs known to inhibit calmodulin, i.e. trifluoperazine (TFP) and N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W7) have been compared with bepridil, whose antiarrhythmic actions have previously been ascribed to blockade of the fast inward sodium current in cardiac tissue.

2 Like bepridil, both TFP and W7 reduced the severity of arrhythmias evoked by 30 min of coronary artery occlusion in tha anaesthetized rat.

3 TFP (2.5–10 mg kg^{-1}, i.v.), W7 (2.5–10 mg kg^{-1}, i.v.) and bepridil (1–5 mg kg^{-1}, i.v.) also antagonized the development of ventricular fibrillation induced by 5 min of occlusion followed by reperfusion. All three drugs also reduced mortality. TFP and bepridil also reduced the incidence of reperfusion-induced ventricular tachycardia whilst all 3 drugs reduced its duration.

4 Although TFP was shown to possess α-adrenoceptor blocking properties, the classical α-blocker, phentolamine, failed to reduce significantly the incidence or severity of reperfusion arrhythmias.

5 In contrast to bepridil (2–20 μM), which markedly reduced the maximum rate of depolarization (V_{max}) of guinea-pig isolated papillary muscle, W7 (5–50 μM) showed only weak effects on V_{max} and was at least 10 times less potent than bepridil whilst TFP only reduced V_{max} in high concentrations (40–100 μM) which lowered resting membrane potential.

6 Unlike bepridil, neither TFP (4–40 μM) nor W7 prolonged the absolute refractory period.

7 The results suggest that drugs which inhibit calmodulin confer protection against both ischaemia – and reperfusion-induced arrhythmias in the rat. Although the electrophysiological actions of bepridil would adequately account for its antiarrhythmic activity, the same cannot be said of W7 and especially TFP.

8 In conclusion, calmodulin antagonism may constitute a mechanism of antiarrhythmic activity.

Introduction

Bepridil is an antianginal agent which also protects against arrhythmias of varying aetiology (for recent reviews see Marshall *et al.*, 1984; Alpert *et al.*, 1985). The mechanisms through which bepridil exerts these effects appear to be complex since this agent inhibits both the fast (class I action) and the slow inward (class IV action) depolarizing currents in cardiac tissue. In addition, bepridil, accumulates in both vascular smooth muscle and cardiac cells (Pang & Sperelakis, 1983; Cramb & Dow, 1983) and indirect evidence suggests that bepridil may inhibit cardiac contractility partly via an intracellular mechanism (Vogel *et al.*, 1979; Brown *et al.*, 1985). More recent evidence has implicated calmodulin as an intracellular target site for bepridil. Several workers have shown that bepridil inhibits Ca^{2+}/calmodulin-activated enzymes from bovine brain, chicken gizzard and erythrocytes and displaces the calmodulin antagonists trifluoperazine (TFP) and N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W7) from calmodulin (Itoh *et al.*, 1984; Agré *et al.*, 1984; Lugnier *et al.*, 1984).

The ventricular antiarrhythmic efficacy of bepridil has largely been attributed to its class I action since similar antiarrhythmic efficacy has not been consistently demonstrated for more specific inhibitors of the slow inward current (Nayler & Horowitz, 1983; Marshall, 1985). In addition, some calcium antagonists possess non-specific actions such as fast ion-channel

[1] Author for correspondence.

or α-adrenoceptor blockade which has led to controversy regarding the precise mechanism of action of Ca^{2+} antagonists in those studies where antiarrhythmic efficacy has been demonstrated (Rosenberger & Triggle, 1978; Karliner *et al.*, 1982; Motulsky *et al.*, 1983; Marshall, 1985).

In the present study the calmodulin antagonists TFP and W7 were investigated for potential antiarrhythmic activity in an attempt to ascertain whether or not calmodulin antagonism may play a role in the antiarrhythmic efficacy of bepridil. Reperfusion-induced arrhythmias in the rat were chosen since sodium channel blocking agents have consistently shown activity against this type of arrhythmia in the rat heart, both *in vivo* and *in vitro*, whereas in most studies to date, calcium channel blockers, especially the more specific dihydropyridines, have not proved effective (Bergey *et al.*, 1982; Winslow *et al.*, 1983; Manning *et al.*, 1983; Kane *et al.*, 1984).

Phentolamine was also included in our study because a large body of evidence suggests that drugs possessing α-adrenoceptor blocking properties protect against reperfusion-induced arrhythmias in cats, dogs and guinea-pigs (Sheridan, 1982; Corr & Witowski, 1983; Penny *et al.*, 1985) and there is evidence to suggest that TFP may possess α-adrenoceptor blocking properties (Cocks *et al.*, 1981).

Little is known of the possible electrophysiological actions of TFP or W7. Indeed we are only aware of one such study (Colatsky & Jurkiewicz, 1984) reported in abstract form. Some basic electophysiological studies were therefore carried out on guinea-pig papillary muscle.

Part of this work has already been presented to the British Pharmacological Society (Barron *et al.*, 1985).

Methods

Coronary artery occlusion and reperfusion

Male Wistar rats (250–300 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1} i.p.) and artificially ventilated with room air (stroke volume, 6 ml; 48 strokes min^{-1}). Arterial blood pressure (BP) was recorded from the right carotid artery and the electrocardiogram (ECG) (Lead II) recorded from subcutaneous steel needle electrodes. BP and the ECG were displayed on a Mingograph 82 ink-jet recorder. A left thoracotomy was performed, the heart exteriorized and a 6/0 silk suture placed under the main left coronary artery. The heart was repositioned in the thoracic cavity and the ligature loosely tied around a fine piece of polythene tubing. A stabilisation period of 15 min was allowed.

Drugs or vehicle (distilled water, 0.05 ml 100 g^{-1}) were given via the left femoral vein, 15 min before tightening the ligature. Five min later the ligature was released by sliding a scalpel blade over the polythene tubing. The incidence of premature ventricular systoles (PVS), ventricular tachycardia (VT), ventricular fibrillation (VF) and electrical deaths were noted together with the time to the onset of arrhythmias, the total duration of the arrhythmic period and the maximum rate of VT (calculated over at least a 1 s period).

In a separate series of experiments the ligature (tied without the polythene tubing) was left in place for 30 min and the number of ischaemia-induced PVS counted. The incidence of VF, VT and electrical deaths was also noted.

Electrophysiological studies

Right papillary muscles were removed from male guinea-pigs (Dunkin-Hartley strain) and pinned to the base of a recording chamber. The preparations were superfused with modified Krebs solution containing (mmol l^{-1}) NaCl 119, KCl 4.7, MgCl 0.56, NaH_2PO_4 1.0, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11, gassed with carbogen and maintained at a temperature of 36 ± 0.5°C. The tissues were stimulated at a frequency of 2 Hz with rectangular pulses of 1 ms duration delivered at 3 times threshold voltage. After a 1 h equilibration period, action potentials were recorded using 3 M KCl filled glass microelectrodes. The action potentials were displayed on an oscilloscope and the signals fed into a Hewlitt Packard 85 microcomputer for measurement and data analysis. The parameters measured were resting membrane potential (RMP), action potential amplitude (APH), the maximum rate of depolarization (V_{max}) and the times taken to reach 50% and 90% repolarization levels. Absolute refractory periods (ARP) were determined by applying a second pulse (1 ms duration) at 3 times threshold voltage through the stimulating electrode after every sixth driving stimulus.

Six to ten action potentials were recorded from each tissue before and 45–60 min after addition of the drugs to the superfusate. The cumulative method of drug addition was used and each preparation was exposed to only one drug.

Drugs and statistics

Drugs used were bepridil dihydrochloride monohydrate (Organon), phentolamine mesylate (CIBA), trifluoperazine dihydrochloride, W7 [N-(6-amino hexyl)-5-chloro-1-naphthalene sulphonamide) and angiotensin (Sigma).

Significant differences between control and drug-treated groups regarding the incidences of VT, VF and death were obtained by the chi-square test.

Drug-induced significant changes in ARP were

determined by the paired t test.

All other comparisons were made using Student's t test.

Results

Effects of bepridil, TFP, W7 and phentolamine on reperfusion-induced arrhythmias

Reperfusion of the main left coronary artery after 5 min of occlusion (during which time ischaemia-induced arrhythmias were absent) resulted immediately in the development of premature ventricular systoles and ventricular tachycardia in all untreated animals. A typical reperfusion-induced arrhythmia is shown in Figure 1. Ventricular fibrillation developed in 73% of control animals and 32% suffered electrical deaths. Neither bepridil (1–5 mg kg^{-1}), W7 (2.5–10 mg kg^{-1}) nor TFP (2.5–10 mg kg^{-1}) significantly prevented the development of PVS although the incidence of this arrhythmia in the 2.5 and 5.0 mg kg^{-1} bepridil groups was only 63%. All 3 drugs did however protect against reperfusion-induced VF and death although the mortality in the control groups was too low to allow statistical significance to be shown for this index of arrhythmia severity (Figure 2). In addition, bepridil and TFP also reduced the incidence of VT (Figure 2) whereas W7 did not. However, like bepridil and TFP, W7 did shorten the duration of VT. Figure 3 shows that 73% of control animals developed at least one episode of VT lasting for longer than 10 s whilst only 4% showed a maximum VT duration of less than 2 s. Fewer animals given bepridil, W7 or TFP developed long runs of VT and in contrast to the controls, a number of these drug-treated animals either did not develop VT at all or showed VT runs lasting no longer than 2 s (Figure 3).

The antiarrhythmic effects of bepridil and TFP were essentially dose-dependent whereas no clear cut dose-dependency was observed for W7. Of interest, however, was the observation that W7 did reduce the maximum rate of tachycardia in a dose-dependent fashion. The mean maximum VT rate in control animals was 911 ± 33 beats s^{-1}. This was reduced to 907 ± 59, 832 ± 43 and 767 ± 37 ($P < 0.05$) in animals given 2.5, 5 and 10 mg kg^{-1} W7 respectively. The highest dose of TFP used also reduced the maximum VT rate (to 445 ± 95 beats min^{-1}, $n = 2$) whereas a significant increase was seen in animals given 5 mg kg^{-1} bepridil (1085 ± 48 beats min^{-1}, $P < 0.05$).

TFP caused a significant delay in the onset of the arrhythmias. The mean delay in onset in control animals was 17.3 ± 3.1 s and in animals pretreated with 5 and 10 mg kg^{-1} TFP was 37.3 ± 8.8 s ($P < 0.05$) and 132 ± 17 s ($P < 0.001$) respectively. Onset times in the high dose bepridil and W7 groups were 31.1 ± 9.7 and 52.3 ± 26.8 s but neither result was statistically significant.

Only bepridil (5 mg kg^{-1}) significantly ($P < 0.05$) shortened the duration of the arrhythmic period (from 161 ± 21 s in the controls to 65 ± 22 s).

Phentolamine (0.5 mg kg^{-1}) failed to modify any index of reperfusion-induced arrhythmias (Figures 2 and 3). The higher dose (2 mg kg^{-1}) did appear to reduce the incidence of VF (to 29%), to prevent electrical deaths and to somewhat shorten the duration of VT but none of these results attained statistical significance. Neither dose of phentolamine influenced the time to the onset of arrhythmias (15.9 ± 6.7 and 15.7 ± 4.3 s in animals given 0.5 and 2.0 mg kg^{-1} respectively), the total duration of the arrhythmic episodes or the maximum VT rate (969 ± 12 and 916 ± 47 beats s^{-1}).

The α-adrenoceptor blocking potency of 0.5 and 2.0 mg kg^{-1} phentolamine was confirmed by obtaining log dose-response curves for phenylephrine-induced increases in diastolic blood pressure before and after administration of phentolamine. The smaller dose of phentolamine caused an approximate 4 fold

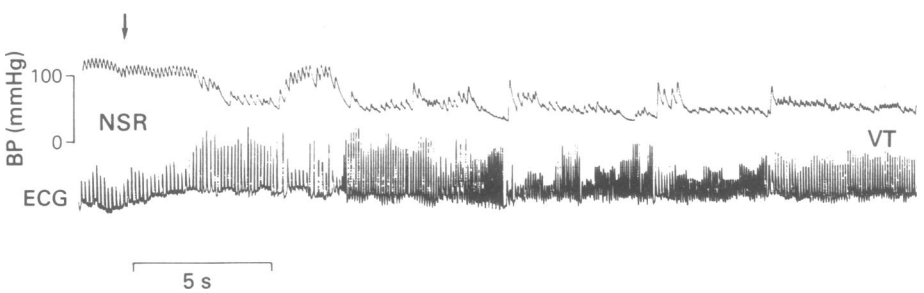

Figure 1 Reperfusion-induced ventricular tachycardia (VT) in a typical control rat. The top trace is arterial blood pressure and the lower trace is the lead II electrocardiogram, NSR is normal sinus rhythm and the arrow indicates the point at which the ligature was released.

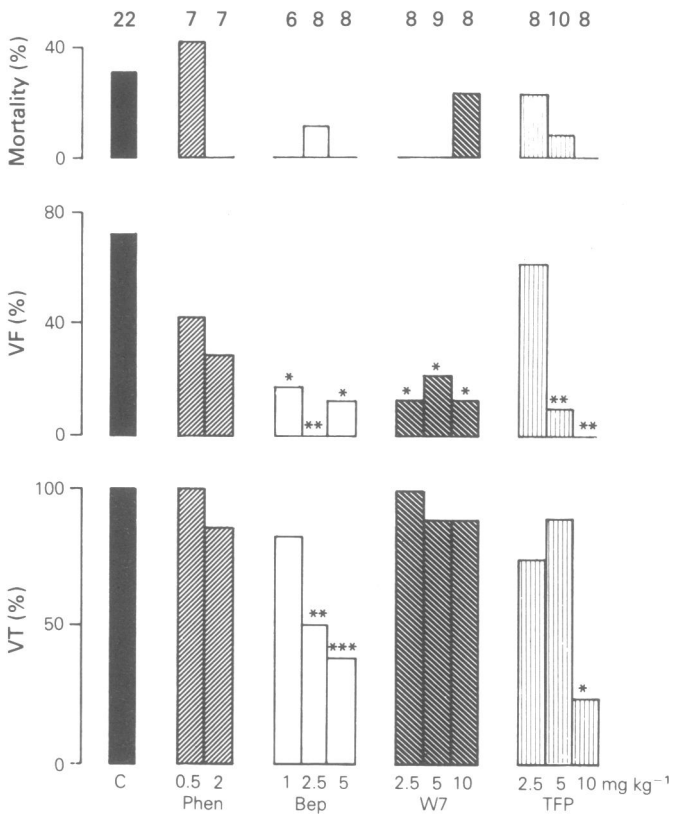

Figure 2 Percentage mortality and the percentage incidence of reperfusion-induced ventricular fibrillation (VF) and ventricular tachycardia (VT) in control animals (C) and in animals pretreated with phentolamine (Phen), bepridil (Bep), W7 and TFP. The numbers of animals used in each group are shown above the top row of columns. $*P < 0.05$; $**P < 0.01$ and $***P < 0.001$ denote significant differences from the appropriate control groups.

parallel shift to the right whilst the higher dose produced an approximate 40 fold shift in the log dose-response curve to phenylephrine. TFP (5 and 10 mg kg^{-1}) caused approximate 5 and 7 fold shifts respectively. The α-blocking properties of TFP were further confirmed by comparing its effects on diastolic blood pressure responses to phenylephrine 5 μg kg^{-1} and angiotensin II 0.25 μg kg^{-1} in the same animals. Like phentolamine, TFP inhibited responses to phenylephrine but not those to angiotensin. In contrast we found no evidence of α-adrenoceptor blockade by W7.

Haemodynamic effects of test drugs

Bepridil, W7 and TFP all produced a transient decrease in systemic arterial blood pressure (Table 1) but by 15 min after administration (i.e. immediately before ligation) blood pressure had regained pretreatment values and in the case of W7 exceeded them. After coronary artery occlusion, blood pressure fell in all groups. In the bepridil (1 and 2.5 mg kg^{-1}) and W7 groups, blood pressure was again approaching pretreatment values after 5 min of occlusion (i.e. immediately before reperfusion). However, recovery in the animals treated with TFP was less marked and blood pressure remained significantly below pretreatment values at the time of reperfusion. The blood pressure response to phentolamine was similar to that to TFP although hypotension immediately following administration was less marked.

Bepridil, W7 and TFP all induced a bradycardia (Table 2). This response to W7 was transient but heart rate was still significantly reduced 15 min after bepridil (2.5 and 5 mg kg^{-1}) and TFP (10 mg kg^{-1}) administration.

Bradycardia in response to phentolamine developed more slowly, only reaching significance 15 min after administration of the lower dose (0.5 mg kg^{-1}). Following ligation, heart rate fell slightly (although not significantly) compared to pre-ligation levels in the

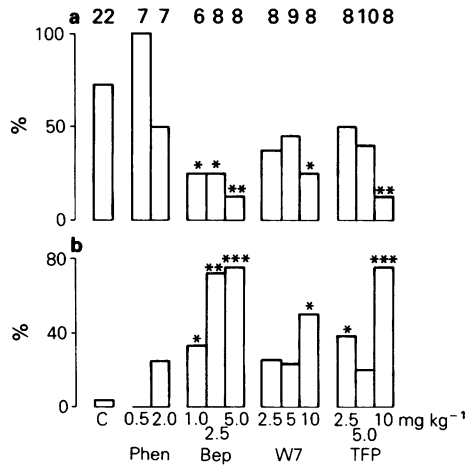

Figure 3 Percentage incidence of animals showing at least one episode of reperfusion-induced ventricular tachycardia (VT) lasting longer than 10 s (a) and percentage of animals which either did not develop VT or developed runs of VT lasting no longer than 2 s (b). C, Phen and Bep denote control, phentolamine and bepridil pretreated animals. The numbers of animals used in each group are shown above the top row of columns. $*P<0.05$; $**P<0.01$ and $***P<0.001$, denote significant differences from the appropriate control groups.

control, W7 and TFP groups but tended to increase in the bepridil and phentolamine groups.

Arrhythmias during sustained coronary artery occlusion

TFP and W7 were tested at doses of 2.5–10 mg kg^{-1}. The results are summarized in Figure 4. Both drugs reduced the expected number of PVS, the incidence of VT and VF and prevented electrical deaths. The antiarrhythmic effect of TFP was particularly dramatic. Control animals often developed runs of VT lasting more than 10 s. The longest run of VT recorded in the 10 mg kg^{-1} TFP group was only 3 s and the majority of the rats developed only single PVS or bigeminal or trigeminal rhythms (Figure 5).

Electrophysiological effects

The electrophysiological effects of bepridil, TFP and W7 are summarized in Figure 6. Bepridil (2–20 μM) reduced the maximum rate of depolarization in a concentration-dependent manner, whereas resting membrane potential was unchanged. APH was also reduced by the higher concentrations (from 106.4 ± 1.0 to 88.0 ± 1.6 mV; $P<0.001$ in the presence of 10 μM bepridil). In addition, bepridil prolonged both action potential duration and the absolute refractory period. Two out of four preparations were rendered unresponsive to electrical stimulation in the presence of 20 μM bepridil.

High concentrations of TFP (40–100 μM) were required to reduce significantly V_{max} and APH (to 90.3 ± 2.1 and 53.9 ± 4.4 mV in the presence of 42 and 104 μM respectively). However, these concentrations also substantially reduced RMP. APD was largely unaffected by TFP except for a moderate shortening

Table1 Effects of bepridil, W7, TFP and phentolamine on arterial blood pressure (systolic/diastolic)

Group	*Dose* (mg kg^{-1})	*Preatment* (mmHg)	*Maximum* (mmHg)	*Preligation* (mmHg)	*Prereperfusion* (mmHg)	n
Controls		90 ± 5/66 ± 4		99 ± 4/75 ± 5	85 ± 4/62 ± 5	22
Bepridil	(1)	81.6 ± /52 ± 3	68 ± 6/32 ± 5^b	98 ± 6/65 ± 7	83 ± 8/51 ± 7	6
	(2.5)	86 ± 5/64 ± 5	53 ± 4^c/28 ± 3^c	91 ± 7/64 ± 5	78 ± 7/47 ± 8	8
	(5)	96 ± 4/71 ± 5	58 ± 7/31 ± 3^c	102 ± 7/71 ± 6	83^a ± 4/54 ± 4^a	8
W7	(2.5)	78 ± 5/53 ± 6	59 ± 3/29 ± 2^b	95 ± 5/71 ± 6^a	71 ± 6/48 ± 7	8
	(5)	89 ± 6/66 ± 6	65 ± 4^b/38 ± 2^b	101 ± 7/77 ± 7	82 ± 9/60 ± 9	8
	(10)	94 ± 1/63 ± 4	66 ± 5^b/33 ± 3^c	113 ± 7^a/87 ± 7^a	87 ± 6/64 ± 6	8
TFP	(2.5)	94 ± 10/71 ± 11	78 ± 9/55 ± 10	88 ± 12/66 ± 12	68 ± 8/47 ± 7	8
	(5)	79 ± 4/53 ± 4	54 ± 4^c/25 ± 3^c	79 ± 4/57 ± 5	62 ± 5^a/37 ± 5^a	9
	(10)	82 ± 7/64 ± 7	53 ± 5^b/35 ± 3^b	73 ± 3/52 ± 3	57 ± 5^a/41 ± 5^a	8
Phentolamine	(0.5)	82 ± 4/59 ± 4	74 ± 6/51 ± 6	77 ± 3/52 ± 4	62 ± 4^b/41 ± 5^a	7
	(2.0)	82 ± 6/58 ± 5	70 ± 5/45 ± 4	81 ± 4/55 ± 3	67 ± 6/46 ± 5	7

Each result is the mean ± s.e.mean of n observations. $^a P<0.05$; $^b P<0.01$ and $^c P<0.001$ denote significant differences from the pretreatment values.

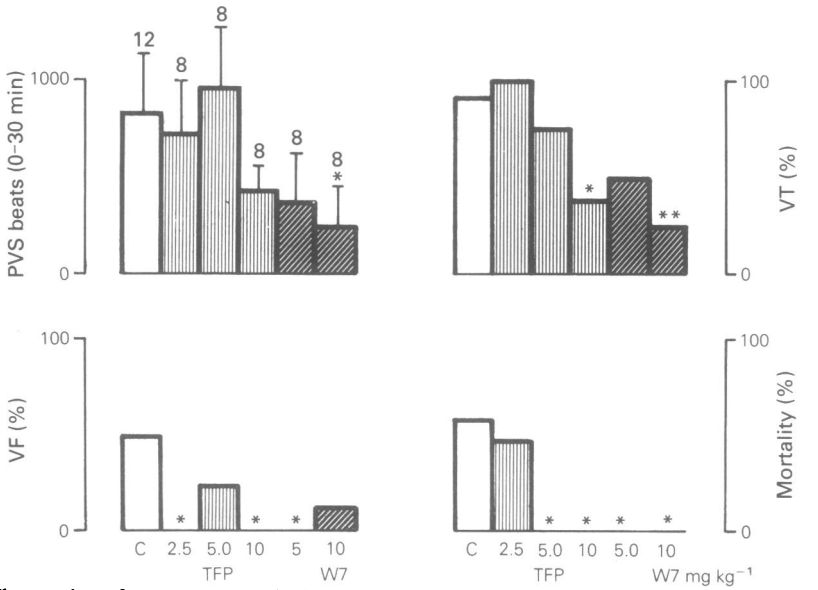

Figure 4 The number of premature ventricular systoles (PVS)(s.e.mean shown by vertical lines) and the percentage incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality observed during 30 min of coronary artery occlusion in control (C), TFP-(vertical hatching) and W7-(cross hatching) treated animals. The numbers of animals used in each group are shown above the PVS columns. $*P < 0.05$ and $**P < 0.01$ denote significant differences from the appropriate control group.

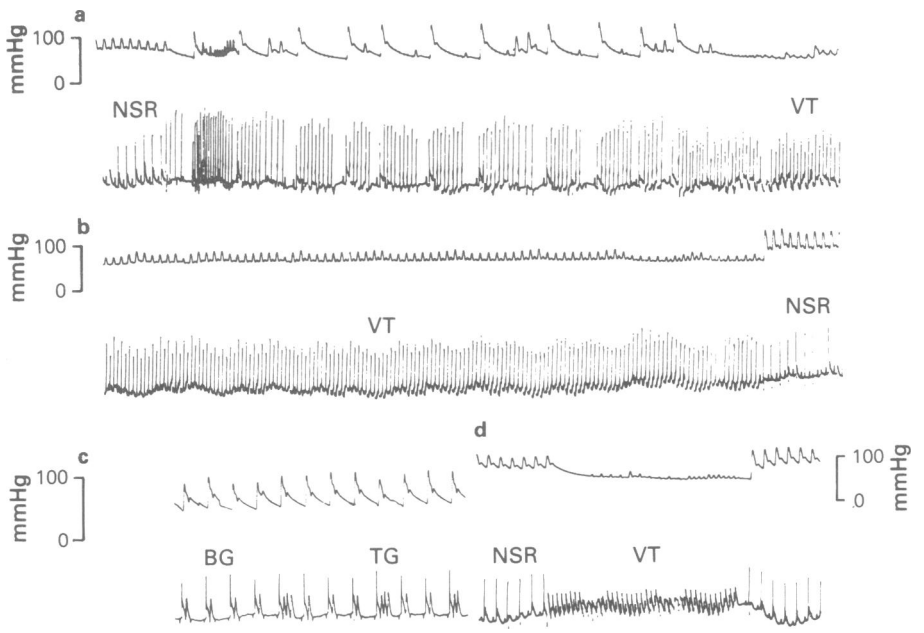

Figure 5 Typical occlusion-induced arrhythmias in control rat (a and b), which are continuous, and show long runs of ventricular tachycardia (VT) and in 2 rats pretreated with 10 mg kg^{-1} TFP (c). The top trace in each panel shows arterial blood pressure and the lower panel shows the lead II ECG. (c) Shows the predominant rhythms bigeminy (BG) and trigeminy (TG) seen in TFP-treated animals. Shows the longest run of ventricular tachycardia (VT) recorded in any animal given 10 mg kg^{-1} TFP. NSR is normal sinus rhythmn.

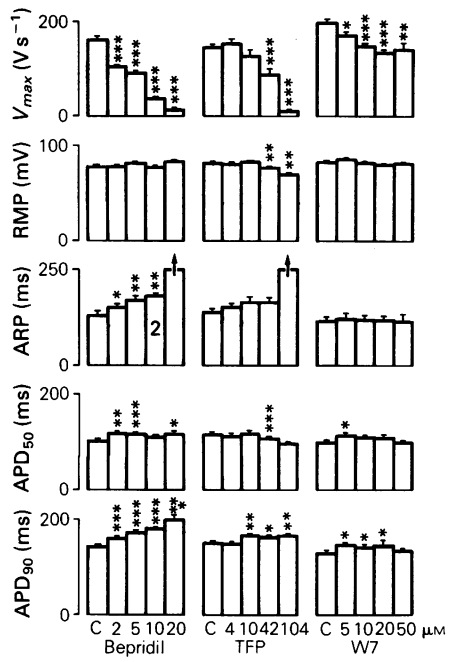

Figure 6 Action potential characteristics recorded from guinea-pig papillary muscle driven at 2 Hz in the absence (C) and in the presence of increasing concentrations of bepridil, TFP and W7. V_{max}, RMP, ARP, APD_{50} and APD_{90} denote the maximum rate of phase 0 depolarization, resting membrane potential, the absolute refractory period, and the times taken to reach 50 and 90% repolarization respectively. Each result is the mean of from 30 to 44 impalements taken from 4 to 5 tissues with s.e.mean shown by vertical lines. The arrows drawn on the ARP bars denote refractory periods of duration longer than that which could be measured using the paired stimulus technique (250 ms). $^{*}P<0.05$; $^{**}P<0.01$ and $^{***}P<0.001$ denote significant differences from the appropriate control groups.

of APD_{50} at high concentrations and a small prolongation of the tail of the action potential at concentrations of 10 μM and above. ARP was only significantly lengthened at the highest concentration of TFP used and was probably due to the accompanying marked fall in RMP.

W7 induced only a very modest depression of V_{max} (− 27% at 50 μM). However no marked or significant ($P<0.01$) changes in RMP, APD or ARP were observed. A significant increase in APH (from 107.0 ± 1.4 to 115.1 ± 1.8 mV, $P<0.01$) was seen at the lowest concentration (5 μM) of W7 used but was not observed in the presence of the higher concentrations.

Figure 7 illustrates the comparative effects of bepridil, TFP and W7 on action potential characteristics recorded from typical experiments.

Discussion

The results of the present study clearly show that the calmodulin antagonists TFP and W7 (2.5–10 mg kg^{-1}) and bepridil (1–5 mg kg^{-1}) are all effective in protecting against reperfusion-induced arrhythmias following 5 min of coronary artery occlusion in the anaesthetized rat. All 3 drugs reduced the incidence of reperfusion-induced VF and the number of electrical deaths. TFP and bepridil also reduced the incidence of VT whilst all 3 drugs shortened its duration. In addition, like bepridil (Kane & Winslow, 1980), both W7 and TFP were also highly effective in antagonizing the development of arrhythmias observed during 30 min of sustained coronary artery occlusion.

In addition to inhibiting calmodulin-dependent cellular processes, some calmodulin antagonists have been reported to exert additional actions. Cocks *et al.* (1981) reported α-adrenoceptor blockade by TFP, an action confirmed in the present study. TFP in antiarrhythmic doses induced a 5 to 7 fold shift in the log dose-response curve to phenylephrine without reducing vasopressor responses to angiotensin. However, phentolamine at a dose sufficient to shift the log dose-response curve to phenylephrine by a factor of 4, failed to modify any index of the severity of reperfusion-induced arrhythmias. Indeed even in a dose that produced a 40 fold shift in the phenylephrine curve, phentolamine exerted only a modest antiarrhythmic action. Although this high dose of phentolamine did appear to reduce the incidence of reperfusion-induced VF, the number of electrical deaths and the duration of VT, statistical significance was not attained. Kane & Williams (personal communication) have also shown prazosin to be inactive in this model. It seems unlikely therefore that α-adrenoceptor blockade could account for the pronounced antiarrhythmic effects of TFP observed in the present study. In contrast to TFP, W7 was without α-adrenoceptor blocking actions.

Colatsky & Jurkiewicz, (1984) have recently reported that both TFP and W7, in concentrations (3–30 μM) close to those known to inhibit calmodulin, inhibit the fast inward sodium current (class I action) in dog Purkinje fibres and that W7 is the more potent of the two. In the present study on guinea-pig papillary muscle, we found only a modest class I effect in response to W7. This agent in concentrations of 20–50 μM reduced V_{max} (in the absence of a fall in RMP) by a maximum of only 32%. Significant (but marked) decreases in V_{max} were only observed in response to high concentrations (40–100 μM) of TFP but these were accompanied by substantial decreases

Table 2 Effects of bepridil, W7, TFP and phentolamine on heart rate (beats min^{-1})

Group	*Dose* ($mg\,kg^{-1}$)	*Pretreatment*	*Maximum*	*Preligation*	*Prereperfusion*	n
Controls		369 ± 13		412 ± 13	396 ± 12	22
Bepridil	(1)	391 ± 13	355 ± 14	370 ± 11	359 ± 15	6
	(2.5)	432 ± 17	360 ± 19[a]	358 ± 7[b]	381 ± 8[a]	8
	(5)	425 ± 9	269 ± 11[c]	306 ± 10[c]	329 ± 17[c]	8
W7	(2.5)	381 ± 15	371 ± 13	380 ± 18	371 ± 15	8
	(5)	409 ± 18	343 ± 10[b]	380 ± 11	360 ± 18	8
	(10)	386 ± 12	328 ± 16[a]	369 ± 15	359 ± 17	8
TFP	(2.5)	416 ± 20	377 ± 21	372 ± 18	367 ± 15	8
	(5)	404 ± 11	341 ± 19[a]	372 ± 18	366 ± 17	9
	(10)	411 ± 24	252 ± 9[c]	318 ± 11[b]	323 ± 24[a]	8
Phentolamine	(0.5)	406 ± 13	381 ± 12	361 ± 12[a]	378 ± 11	7
	(2.0)	403 ± 13	363 ± 17	367 ± 20	394 ± 18	7

Each result is the mean ± s.e.mean of n observations. [a] $P<0.05$; [b] $P<0.01$ and [c] $P<0.001$ denote significant differences from the pretreatment values.

in RMP suggesting that such concentrations may be toxic. Both drugs induced a modest prolongation of the tail of the action potential (10–13%) whilst APD_{50} and the absolute refractory period were essentially unchanged. These results were in marked contrast to those obtained with bepridil. At concentrations as low as 2 μM, bepridil reduced V_{max} by 36%. At 20 μM V_{max} was reduced by 89% in the absence of a fall in RMP in two preparations and action potentials in two further preparations were abolished. These results suggest that bepridil is, at the very least, ten times more potent than W7 in inhibiting the fast inward sodium current. In addition to its class I effects, bepridil also lengthened the action potential, especially the tail, and increased the absolute refractory period. All of these effects would be expected to contribute to antiarrhythmic activity. In view of these results and the similar antiarrhythmic potency of W7, TFP and bepridil observed *in vivo*, it seems very unlikely that inhibition of the fast inward sodium current in cardiac muscle cells plays a role in the antiarrhythmic efficacy of TFP or W7 although, a class I action *in vivo* for W7 cannot be totally ruled out.

Taken together, the results suggest that neither α-adrenoceptor blockade nor inhibition of the fast inward sodium current are likely to play a role in the observed and marked antiarrhythmic efficacy of TFP and W7 seen in the present study. It is therefore tempting to speculate that their antiarrhythmic effects are mainly due to calmodulin inhibition. Also since concentrations of bepridil which exert class I actions (2–20 μM) overlap with those reported to inhibit calmodulin (EC_{50} values range between 8 and 18 μM), it is difficult to exclude calmodulin antagonism as an additional possible mechanism of action of bepridil. It is thought that calmodulin-stimulated processes may reduce cell to cell coupling via conformational changes in gap junctions (Perrachia & Bernardini, 1984) and Anno *et al.* (1986) have recently suggested that hypoxia-induced conduction disturbances in rabbit isolated hearts may be due to electrical uncoupling of AV nodal cells. These workers showed that calmodulin antagonists (including W7 and chlorpromazine) prevent hypoxia-induced prolongation of atrio-His (AH) conduction and prevent AH block. It is therefore possible that the antiarrhythmic actions of calmodulin antagonists seen in the present study may be explained by improved cardiac cell to cell coupling with a consequent reduction in inhomogeneity of refractoriness which occurs during both ischaemia and reperfusion.

Another possible site of action of calmodulin inhibition may be the sympathetic nerve terminals. It is generally accepted that myocardial ischaemia is accompanied by an increase in sympathetic outflow (reviewed by Ceremuzynski, 1981) which may initiate or exacerbate arrhythmias and Schomig *et al.* (1984) have recently demonstrated an 18 fold increase in noradrenaline release following 15 min of global ischaemia and reperfusion in the isolated non-beating rat heart. There is also good evidence to suggest that calmodulin regulates both the biosynthesis and the release of monoamine neurotransmitters (De Lorenzo *et al.*, 1979; Fujisawa *et al.*, 1984). It is therefore possible that the antiarrhythmic effects of TFP and W7 might be related to a reduced release of noradrenaline from sympathetic nerve terminals during or even before coronary artery occlusion. In this context it is perhaps of interest that both these drugs reduced the expected rate of ventricular tachycardia and delayed the onset of reperfusion induced arrhythmias (although the delay in response to W7 was not significant).

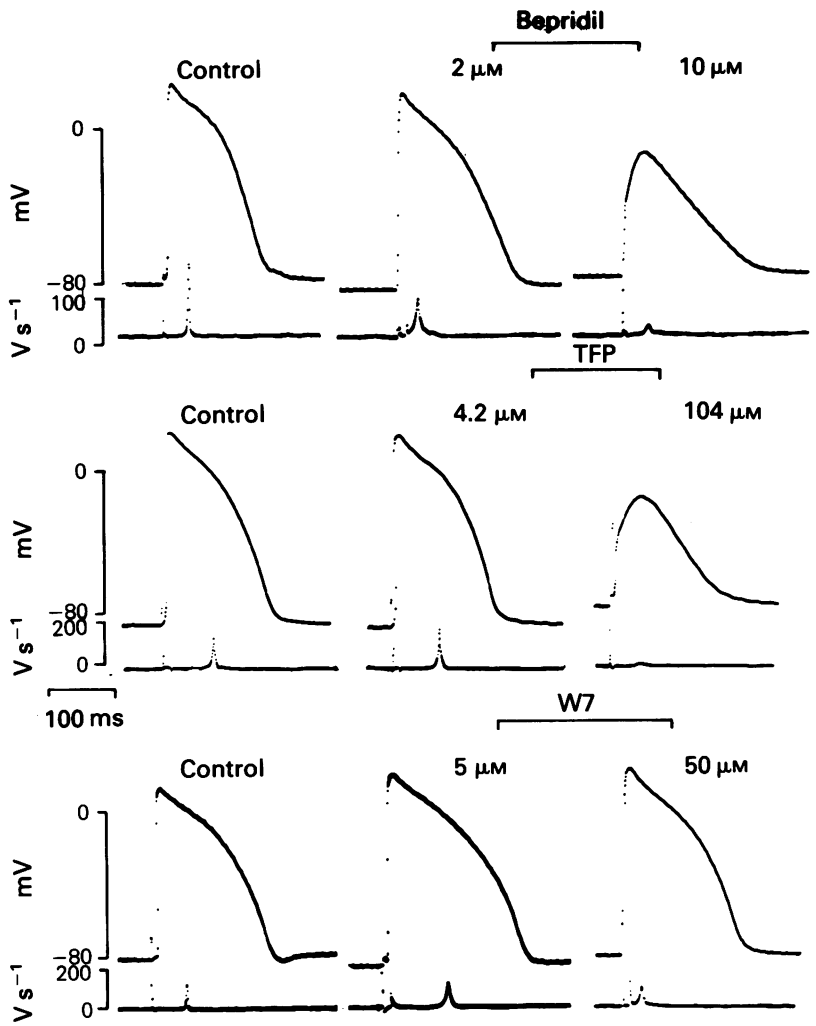

Figure 7 Action potentials recorded from typical experiments in the absence (control) and in the presence of bepridil, TFP or W7. The lower trace in each panel shows the maximum rate of phase 0 depolarization obtained by electronic differentiation.

Antisympathetic actions might also be suggested by the observed bradycardia seen in response to TFP and W7. Bepridil also exerted negative chronotropic effects but the evidence available suggests that this is due, at least in part, to a direct action on sinus nodal cells (Goto & Sperelakis, 1984). Bepridil did not reduce the rate of reperfusion-induced VT, in fact, a significant increase was seen. However, the electrophysiological actions of bepridil would be expected to alter the conditions of any existing re-entrant pathway, the outcome of which, in terms of VT rate, would be unpredictable and beyond the scope of this paper.

In conclusion, the calmodulin antagonists TFP and W7 exerted pronounced antiarrhythmic actions against both ischaemia-induced and reperfusion-induced arrhythmias. α-Adrenoceptor blockade by TFP and fast sodium current inhibition by W7 seem unlikely to play a role in the mechanism of their antiarrhythmic actions. The results suggest that inhibition of calmodulin may confer protection against the development of these arrhythmias. The antiarrhythmic effects of bepridil can be adequately explained by its actions on fast Na^+ ion channels but calmodulin antagonism may constitute a second antiarrhythmic mechanism.

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