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Pharmacological mapping of regional effects in the rabbit heart of some new antiarrhythmic drugs

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1 *In vitro* preparations of rabbit heart were made from which measurements of effective refractory period (ERP), atrio-Hisian (A-H) and His-Purkinje (H-P) conduction times could be obtained, analogous to electrophysiological measurements customarily carried out *in vivo*.

2 Intracellular potentials also were recorded from the sino-atrial (SA) node, atrium, bundle of His, preterminal Purkinje fibres and papillary muscles.

3 The effects of a range of concentrations of three new antiarrhythmic drugs, melperone, cibenzoline and alinidine were compared, the lower concentrations studied corresponding to clinical levels.

4 At low concentrations the effects of melperone, inducing bradycardia and lengthening ERP, could be attributed to prolongation of action potential duration (APD) in the sinus node and atrial and ventricular tissues. The slope of slow diastolic depolarization was not altered, nor was there any change in A-H or H-P conduction time, or in maximum rate of depolarization (MRD).

5 At higher concentrations melperone had a substantial class 1 action, but there was no negative inotropic effect, or other evidence of restriction of slow inward current.

6 Cibenzoline was primarily a class 1 agent but also lengthened APD to some extent in the SA node and in atrial and ventricular muscle, but not in Purkinje fibres. APD thus became more uniform along the ventricular conducting pathway.

7 Cibenzoline also depressed contractions and increased A-H conduction time, implying restriction of slow inward current. The bradycardia could thus be attributed to a slowing of both depolarization and repolarization in the SA node, without any change in slope of the slow diastolic depolarization. Conduction time was increased in all tissues.

8 Alinidine greatly reduced the slope of the slow diastolic depolarization and slightly lengthened APD in the SA node. MRD was also reduced in the SA node, and A-H conduction time was increased, implying some restriction of slow inward current. However, there was no negative inotropic effect.

9 Alinidine had no significant effect on MRD in atrium, ventricle or Purkinje cells, nor was H-P conduction time altered, implying absence of effect on fast inward current. APD was moderately lengthened in atrium and ventricle but not in Purkinje cells.

10 It was concluded that the effects of the drugs in the sinus node and on ERP and on A-H and H-P conduction times could be accounted for by their selective cellular actions in different regions of the heart.

Introduction

Clinical electrophysiological studies with intracavitory stimulating and recording electrodes can provide information about sinus node function, by measurement of recovery time from overdrive pacing, and about conduction times across the A-V node (atrio-Hisian, A-H), and in the ventricular Purkinje system (H-V), and measurements can be made by programmed stimulation of effective and functional refractory periods in atrium and ventricle (Curry, 1975). Consequently the actions of drugs are often described in anatomical terms. For example, sinus node function may be depressed indirectly (β-blockers) or directly (verapamil, alinidine), and A-H conduction may be accelerated indirectly (anticholinergic agents) or directly (sympathomimetics) or depressed indirectly (β-blockers, digitalis) or directly (ver-

apamil, digitalis). H-V conduction is slowed and QRS is widened by class 1 drugs, but with the fastest dissociating drugs (mexiletine, lignocaine) the effect may not be apparent unless the heart is paced at a high frequency.

In contrast, in animal experiments, effects of drugs on cellular electrophysiology have been widely studied. Effective refractory period, for example, could be prolonged either by a delay in the recovery of cells from inactivation, without change of action potential duration (APD), or by a prolongation of APD itself. In fact, all the antiarrhythmic drugs in current clinical use have been shown to possess one or more of four types of action, restricting fast inward current, having antisympathetic properties, delaying repolarization, or restricting slow inward current (Vaughan Williams, 1980).

In man it is apparent that there are marked differences between individual drugs within the same group. For example some class 1 drugs have a fast (lignocaine, mexiletine, tocainide), intermediate (quinidine, disopyramide, procainamide) or slow (encainide, lorcainide, flecainide) rate of attachment to and release from the Na-channel (Campbell, 1982), or have subsidiary properties; e.g. an anticholinergic action (quinidine, disopyramide) which can counteract the direct depressant effect of these drugs on A-V conduction (Birkhead & Vaughan Williams, 1977). Overall responses to antiarrhythmic drugs *in vivo* are also influenced by extracardiac factors, such as rapid metabolism (lignocaine), CNS effects (diphenylhydantoin, digitalis, melperone), or vasodilatation (verapamil, amiodarone). In consequence the classification of drugs on the basis of cellular effects does not provide sufficient information to be other than a rather broad guide to indicate the type of arrhythmia for which a particular drug might be most efficacious clinically.

We have, therefore, expanded studies of *in vitro* preparations in an attempt to provide the kind of information which is obtained in clinical electrophysiological studies. It has become apparent that individual compounds, although still being categorized on the basis of the four main classes of action, exhibit quantitative differences in potency in various cardiac tissues. Such an anatomical selectivity could be responsible for differences in clinical efficacy of drugs of the same main class. We have chosen to study three new antiarrhythmic drugs, alinidine, melperone and cibenzoline which our previous work had shown to exhibit some regional selectivity (Millar & Vaughan Williams, 1981; 1982a and b). Alinidine acts primarily on the sinus node; in atrial muscle it appeared to have no effect on depolarization or repolarization. The main effect of melperone was to prolong action potential duration, whereas cibenzoline was a powerful class 1 agent, with the interesting property of preventing APD from being shortened by hypoxia. The lower concentrations used *in vitro* here correspond to the upper levels employed *in vivo* (Platou, Refsum, Amlie & landmark, 1982), but higher concentrations also have been studied because it is useful to know the possible consequences of overdosage, which may produce effects qualitatively different from those observed at clinical concentrations.

Methods

Intracellular potentials and contractions

Sino-atrial and atrial records Rabbits of either sex, weighing 1–1.5 kg, were stunned and their hearts rapidly removed. The atria were separated from the ventricles, and were suspended horizontally to facilitate recording with microelectrodes from the endocardial surface. Contractions were measured simultaneously, and in these experiments the temperature of the solution was 32°C. For recording sino-atrial node potentials, the node was removed together with 3–4 mm of surrounding tissue, and mounted on a perspex ring, permitting access of the fast-flowing oxygenated physiological saline at 37°C to both surfaces. The oxygenation was external to the bath, to avoid disturbance of microelectrodes by oxygen bubbles.

Ventricular records The ventricles were immersed in ice-cold physiological saline continuously oxygenated during the dissection. After the left atrium and the part of the right atrium containing the sino-atrial node (SA node) had been separated, the left ventricular free wall was removed. The right ventricular wall was cut free anteriorly and peeled back, revealing the papillary muscles. A thread was tied to one of the chordae tendineae. Dissection was continued, to leave a preparation consisting of (1) a portion of right atrium and interatrial septum within 3–4 mm of the A-V node, (2) the A-V node and His bundle, (3) a strip of interventricular septum containing the right bundle branch and two papillary muscles, (4) the moderator band and other free-running strands of Purkinje cells. The septum was anchored to a grid to stabilize the origin of one papillary muscle, the thread attached to the tendon being tied to the strain gauge. The other muscle was left slack for microelectrode recordings. Stimuli (S1) of twice threshold strength and at a frequency just fast enough to 'capture' spontaneously beating preparations (usually 1.5 to 1.8 Hz) were applied either to the atrium (SA) or to the bundle of His (SH). Intracellular records were obtained from His bundle cells, terminal Purkinje cells and papillary muscle cells. Atrio-Hisian (A-H)

interval was the time between S1 and the upstroke of the His potential. The His-Purkinje (H-P) interval was measured from the start of the His bundle potential to the start of the Purkinje cell potential. The programmed effective refractory period (ERP) was the shortest interval between a His bundle stimulus (S1) and a second, premature, stimulus (S2), introduced after every 8th basal S1, which evoked a potential conducted to the distal Purkinje cells.

The physiological solution contained (mM): NaCl 125, KCl 5.6, $NaHCO_3$ 25, Na_2HPO_4 0.4, $MgCl_2$ 1.0, $CaCl_2$ 2.16 and glucose 11. The solution was equilibrated with 95% O_2 and 5% CO_2.

During the experiments involving microelectrodes, potentials and contractions were displayed on a digital-storage oscilloscope (Gould 4002) and recorded on tape (Racal Store 4). The stored records were later replayed and analysed statistically by a computer (HP 9830A) programme which incorporated a Student's *t* test (Vaughan Williams, 1977a).

Drugs

Alinidine (ST567: 2-(N-allyl-N-(2,6-dicloro-phenyl)-amino))-2-imidazoline) was supplied by Boehringer Ingelheim; melperone (Buronil, γ-(4-methylpiperidino)-*p*-fluorobutyrophenone) was supplied by AB Ferrosan, Malmo, Sweden; cibenzoline (Ritmalan, (diphenyl-2',2'-cyclopropyl) 2 imidazoline succinate) was supplied by UPSA Laboratories, Rueil-Malmaison, France.

Results

Sino-atrial node

All three drugs slowed the spontaneous frequency of the sinus node, but by different mechanisms, as has already been briefly described (Millar & Vaughan Williams, 1982c). Bradycardia can be caused by a reduction in the slope of the slow diastolic depolarization, by a delay of repolarization, or, at least to a minor extent, by a slowing of the upstroke of the sinus node action potential. The extent to which these effects account for the bradycardia induced by the three drugs is illustrated in Figure 1 (alinidine, A, cibenzoline, C, and melperone, M). It is apparent that almost the whole of the bradycardia induced by

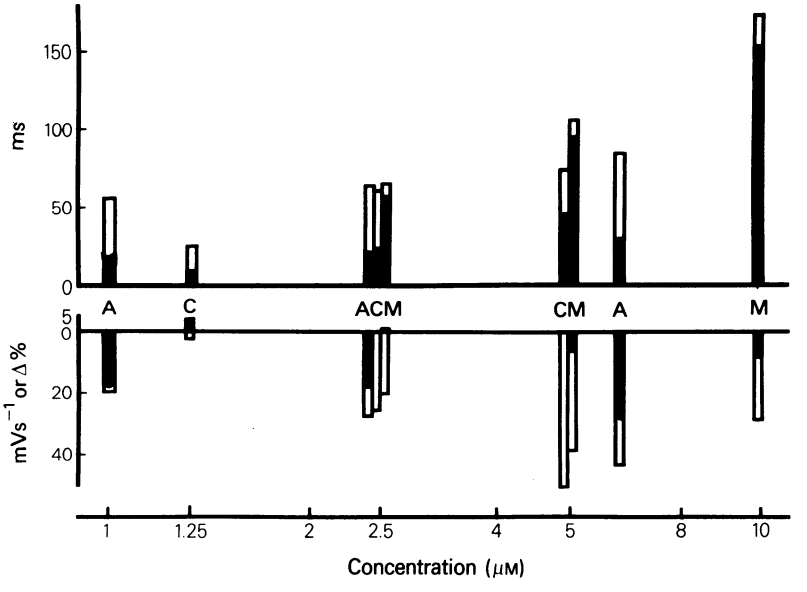

Figure 1 Effects of alinidine (A), cibenzoline (C) and melperone (M) responsible for bradycardia. Upper panel: increases in peak-to-peak interval between sinus node potentials (open columns) and in action potential duration from peak to maximum diastolic potential (solid columns). Lengthening of APD accounted for most of the bradycardia caused by melperone, but for less than half that caused by the other drugs. Lower panel. The slope of the slow diastolic potential (solid columns) in mVs^{-1}; % changes in maximum rate of depolarization in atrial muscle have been superimposed as open columns (so that they do not represent proportional contributions to P-P intervals). Cibenzoline and melperone had little effect on the slow diastolic depolarization, but reduction in the slope of this parameter was responsible for about two thirds of the bradycardic effect of alinidine. Mean baseline control values (s.e.mean) were as follows: P-P interval ($n = 221$ for SA node fibres) 324.5 (5.0) ms; APD, 108.0 (0.93) ms; diastolic depolarization 55.5 (1.46) mVs^{-1}. Atrial MRD ($n = 75$) 110.2 (4.6) Vs^{-1}.

melperone was caused by delayed repolarization, but less than half of the lengthening of diastole induced by the other drugs was attributable to this effect. In the lower part of the figure, the filled columns indicate the change in slope of the slow diastolic depolarization, in mVs^{-1}. Delay in the upstroke could also add a few milliseconds to the diastolic interval; decreases in the maximum rate of depolarization (MRD) have been plotted (open columns) as % decreases from control values. Cibenzoline did not alter the slow diastolic depolarization at any concentration, but decreased MRD in a dose-related manner. In contrast, alinidine reduced both MRD and the slope of the slow diastolic depolarization. At a concentration of 2.5 μM, all three drugs induced similar degrees of bradycardia. Neither cibenzoline nor melperone had any effect on slow diastolic depolarization at this concentration. The main effect of melperone was to delay repolarization, with only a small contribution from depressed MRD, while cibenzoline reduced MRD predominantly, with a minor contribution from delayed APD. Alinidine, on the other hand, greatly decreased the slope of slow diastolic depolarization, but only moderately prolonged APD. Baseline control values in the SA node were as follows; max. diastolic potential −60.4 (±s.e.mean 0.85)mV; take-off potential −43.6 (0.77)mV; MRD, 9.13 (0.32)Vs^{-1} ($n = 221$). Other values given in Figure 1.

Effects on atrial myocardium

The effective refractory period can be prolonged because the action potential duration is prolonged (class 3 action) without any change occurring in fast inward current. Alternatively it may be prolonged by persistent inactivation of sodium channels, without any change, or even in spite of a shortening, of APD. The effects of the three drugs on atrial myocardium are presented in Table 1. Alinidine caused no significant change in MRD at sinus frequency, nor was there any effect on ERP, as calculated from the maximum driven frequency, or on APD at concentrations of 2.5 μM and below. A small, just significant, increase in APD at 6.25 μM, could account for the small increase in ERP.

In contrast, cibenzoline produced a large, dose-related increase in ERP, attributable almost entirely to its class 1 action, with a small contribution from a lengthened APD at the higher concentrations. The large and dose-related increase in ERP produced by melperone, on the other hand, was entirely attributable to the class 3 effect, since no significant reduction of MRD was observed except at the high concentration of 10.7 μM. Thus if attention is focussed on the effects of the three drugs at 2.5 μM concentration, melperone had exclusively a class 3 action, cibenzoline exclusively a class 1 action, and alinidine had neither.

Table 1 Effects of alinidine, cibenzoline and melperone on effective refractory period (ERP), action potential duration (APD) and maximum rate of depolarization (MRD) in rabbit atrium

Alinidine					
Concentration (μM):	0.0		1.0	2.5	6.25
	Mean (s.e.mean)		*Differences from controls*		
ERP (ms)	124.5	(3. 8)	− 3.5	− 3.3	+ 3.2
APD_{90} (ms)	83.0	(2.55)	+ 3.3	+ 5.57	+ 8.67*
MRD (Vs^{-1})	140.4	(11.5)	+ 13.1	+ 7.2	− 28.7
Cibenzoline					
Concentration (μM):	0.0		1.32	2.63	5.26
	Mean (s.e.mean)		*Differences from controls*		
ERP (ms)	122.7	(4.6)	+ 20.1***	+ 50.8***	+ 83.5***
APB_{90} (ms)	83.2	(2.1)	+ 5.9	+ 13.6**	+ 15.0***
MRD (Vs^{-1})	111.1	(6.4)	− 38.3**	− 58.7***	− 79.0***
Melperone					
Concentration (μM):	0.0		2.67	5.34	10.68
	Mean (s.e.mean)		*Differences from controls*		
ERP (ms)	118.3	(3.6)	+ 25.2***	+ 44.7***	+ 61.3***
APD_{90} (ms)	74.5	(2.2)	+ 28.5***	+ 33.5***	+ 31.3***
MRD (Vs^{-1})	82.0	(6.05)	+ 10.1	− 7.0	− 31.6*

ERP = effective refractory period estimated from the maximum frequency at which a stimulus was followed. APD_{90} = action potential duration from peak to 90% repolarization. MRD = maximum rate of depolarization. Statistical significance of differences: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Conduction times

In our previous studies of alinidine (Millar & Vaughan Williams, 1981) no ventricular records were made and these have now been obtained for comparison with the other two drugs. Results from all three compounds on A-H and H-P conduction times are presented in Table 2. Alinidine caused a significant and dose-related increase in A-H conduction time.

The significant effects of the lower concentration of cibenzoline on both A-H and H-P times are consistent with restriction by this drug of slow and fast inward currents, respectively, as previously reported. Melperone, on the other hand, had little effect on conduction times except at higher concentrations.

Duration of ventricular action potentials

Alinidine, which, as seen above, prolonged APD consistently and in a dose-related manner in the sinus node, but had only a marginal effect in the atrium, actually shortened APD in the His bundle and especially the plateau phase of the Purkinje cells, with less effect on APD_{90}. There was some lengthening of papillary muscle APD, but it was not dose-related.

Cibenzoline consistently prolonged APD in ventricular muscle (Table 3), as in atrial muscle, but had little effect on APD in His bundle or terminal Purkinje cells. This selective action would reduce the differences between Purkinje and ventricular action potential durations, the latter being normally much shorter than the former.

Melperone greatly prolonged APD throughout the ventricular pathway, with relatively less effect in preterminal Purkinje cells, thus also reducing the difference between APD in the two tissues.

Effective refractory period and maximum rate of depolarization in ventricular pathway

Effective refractory period, as measured by programmed stimulation in the bundle of His, was not significantly altered by alinidine (Table 4). This is as would be expected from the absence of change of APD or MRD in this tissue. There was no significant change of MRD in the preterminal Purkinje fibres or papillary muscle. It may be concluded, therefore, that alinidine has no effect on sodium channels in any part of the heart.

In contrast cibenzoline reduced MRD in all parts of the ventricle, as it did in the atrium. Melperone, which did not reduce MRD in the atrium except at the highest concentration, had a highly significant class 1 action at all concentrations in the terminal Purkinje cells.

Discussion

In this paper an attempt has been made to discover the extent to which some of the electrophysiological drug effects such as are customarily observed in man (bradycardia, prolongation of ERP or of A-H and A-V conduction time) may be attributed to effects on intracellularly recorded potentials. It was observed

Table 2 Effects of alinidine, cibenzoline and melperone on conduction times: atrium to ventricle

Alinidine					
Concentration (μM):	0.0		1.0	2.5	6.25
	Mean (s.e.mean)		*Differences from controls*		
A-H (ms)	43.3	(1.14)	+3.4	+6.1**	+10.1***
H-P (ms)	13.6	(0.7)	−3.5	−4.1	+1.36
Cibenzoline					
Concentration (μM):	0.0		1.32	2.63	5.26
	Mean (s.e.mean)		*Differences from controls*		
A-H (ms)	40.6	(1.0)	+6.2**	+15.6**	+10.2**
H-P (ms)	14.7	(0.3)	+2.17*	+2.1*	+7.6***
Melperone					
Concentration (μM):	0.0		2.67	5.34	10.68
	Mean (s.e.mean)		*Differences from controls*		
A-H (ms)	49.2	(1.34)	−0.075	+9.02***	+12.62***
H-P (ms)	13.2	(0.4)	+1.35	+5.13	+10.40

A-H = Atrium to His Bundle; H-P = His bundle to terminal Purkinje cell.
Significance of differences indicated as in Table 1.

Table 3 Effects of alinidine, cibenzoline and melperone on action potential duration in ventricle

	Bundle of His		*Purkinje fibre*		*Ventricle*	
	APD_{50}	APD_{90}	APD_{50}	APD_{90}	APD_{50}	APD_{90}
Control						
Mean	138.9	173.7	124.6	210.5	87.8	125.3
(s.e.mean)	(2.2)	(1.9)	(3.9)	(3.3)	(2.2)	(2.0)
Alinidine						
1.0 μM	− 9.8**	− 5.0	− 16.1	− 3.9	+ 13.1***	+ 26.1***
2.5 μM	− 11.7***	− 3.2	− 16.6*	− 4.1	+ 6.3*	+ 17.9***
6.25 μM	− 18.4***	− 8.2*	− 18.4*	− 12.2	0.0	+ 11.5***
Control						
Mean	155.4	192.2	133.8	201.5	88.0	122.0
(s.e.mean)	(2.2)	(2.5)	(2.7)	(2.3)	(22.5)	(2.2)
Cibenzoline						
1.32 μM	0.0	0.0	− 3.9	− 7.5	+ 9.2	+ 12.7**
2.63 μM	− 1.4	+ 9.0	− 6.7	− 3.2	+ 27.0***	+ 29.2***
5.62 μM	+ 6.3	+ 11.8*	+ 1.62	+ 8.2	+ 27.4***	+ 30.1***
Control						
Mean	126.4	162.0	129.6	179.1	70.2	105.9
(s.e.mean)	(2.8)	(3.3)	(4.9)	(5.8)	(1.8)	(2.2)
Melperone						
2.67 μM	+ 29.5***	+ 49.1***	+ 18.9	+ 35.4*	+ 19.3***	+ 26.0***
5.34 μM	+ 22.3***	+ 58.3***	− 17.6	+ 1.1	+ 26.5***	+ 35.2***
10.68 μM	+ 41.9***	+ 86.9***	0.8	+ 28.1*	+ 30.4***	+ 35.2***

APD_{50} = time from peak to 50% repolarization; APD_{90} = time from peak to 90% repolarization. Differences from controls are given in text. Significance of differences indicated as in Table 1.

Table 4 Effects of alinidine, cibenzoline and melperone on maximum rate of depolarization (MRD) and programmed effective refractory period (ERP), in ventricle

	Bundle of His		*Purkinje fibre*	*Ventricle*
	ERP (ms)	MRD (Vs^{-1})	MRD (Vs^{-1})	MRD (Vs^{-1})
Control				
Mean	152.0	110.5	217.8	115.9
(s.e.mean)		(5.1)	(10.7)	(5.1)
Alinidine				
1.0 μM	+ 4.7	+ 9.0	+ 17.4	+ 3.3
2.5 μM	+ 7.9	+ 7.5	+ 10.4	+ 5.2
6.25 μM	− 6.5	− 2.4	− 8.7	− 2.0
Control				
Mean	145.2	160.4	307.6	128.1
(s.e.mean)		(6.6)	(12.7)	(5.1)
Cibenzoline				
1.32 μM	+ 24.6*	− 46.4***	− 95.8***	− 12.3
2.63 μM	+ 31.9*	− 97.9***	− 148.9***	− 41.9***
5.26 μM	+ 42.1*	− 104.4***	− 172.3***	− 46.6***
Control				
Mean	102.1	151.3	317.2	128.1
(s.e.mean)		(7.2)	(9.0)	(5.1)
Melperone				
2.67 μM	+ 20.3	− 19.1	− 81.2***	− 22.9*
5.34 μM	+ 23.3	− 26.2*	− 63.6***	− 32.1**
10.68 μM	+ 70.0	− 54.4**	− 64.0***	− 18.7

The effects of the drugs are given as differences from control, and the significance of differences is indicated as in Table 1.

that at a concentration of 2.5 μM, all the three drugs studied here produced approximately the same degree of bradycardia. The effect of alinidine was attributable mainly to a slowing of the rate of diastolic depolarization in the sinoatrial node. The other two drugs, however, did not alter the slope of the diastolic depolarization. The bradycardic action of melperone was caused by a prolongation of sinus node action potential duration, whereas that of cibenzoline was due partly to prolongation of APD and partly to slowing of the upstroke of the action potential. Bradycardia can, of course, be caused by autonomic effects, e.g. blockade of sympathetic action or by cholinoceptor agonist action. Our results have shown that even when bradycardia is due to a direct action on the isolated sinus node, it may be produced by quite different cellular electrophysiological effects.

Effective refractory period also may be lengthened in different ways, by prolongation of APD or by delay in recovery from inactivation of sodium channels. Some class 1 drugs are rapidly released from the channels after repolarization, so that at low stimulation frequencies no effect on MRD may be seen, because sodium channels have fully recovered from inactivation during the long diastolic interval. A prolongation of ERP may, nevertheless, be demonstrable by programmed stimulation, because the premature stimulus arrives early enough for drug still to be attached to the channel, retaining it in its inactivated form. If effective refractory period is calculated from the maximum frequency at which a stimulus can be followed, however, a prolongation of ERP is observed both with drugs which do (quinidine, flecainide) or do not (lignocaine, mexiletine) reduce MRD at low stimulation frequency. In the present experiments both methods were employed.

Melperone had no class 1 action in the atrium at a concentration of 2.7 μM, and the lengthening of ERP was entirely accounted for by the prolongation of APD, a result in accord with previous evidence from *in vivo* studies (Refsum, Amlie, Platou, Owren & Landmark, 1981). At higher concentrations, however, melperone did reduce MRD, which explains why the lengthening of ERP is greater than the prolongation of APD, illustrating how ERP may be prolonged by the same drug in two distinct ways. Similarly, in the bundle of His, APD_{50} and ERP were prolonged to the same extent by melperone at 2.7 μM, without significant effect on MRD, but ERP was prolonged more than APD_{50} at higher concentrations, which significantly depressed MRD; (inactivation of sodium channels starts to disappear at potentials negative to −55 mV, close to APD_{50} in ventricle, nearer to APD_{90} in atrium.) It was concluded, therefore, that at concentrations likely to occur *in vivo*, melperone would be acting on the heart as a class 3 agent but that class 1 effects might be an additional factor at higher concentrations. One interesting observation was that the prolongation of APD was relatively greater in the bundle of His than in the ventricle.

Action potential duration is normally much longer in the free-running preterminal Purkinje cells than in the bundle of His, and is longer in the bundle of His than in ventricular muscle cells (Meyerberg, Steward & Hoffman, 1970). Drugs may have different effects on these regions (Wittig, Harrison & Wallace, 1973). Mexiletine (Vaughan Williams, 1977b) and lignocaine, for example, shorten APD throughout, but shorten it more in the preterminal fibres, in which APD is normally longest, than in the His bundle or ventricular muscle. In contrast, amiodarone and its derivative, L9146, lengthen APD throughout the system but exert the effect preferentially on those regions in which APD was normally shorter (Vaughan Williams, Salako & Wittig, 1977). The finding that melperone lengthened APD throughout the ventricular conduction pathway, but that the effect was greatest in the His bundle and papillary muscle, suggests the possibility that melperone, like amiodarone, could be effective in pre-excitation arrhythmias of the Wolff-Parkinson-White type.

In vitro we found that melperone at 2.67 μM had no effect on A-H conduction time; at higher concentrations, however, there was a small increase in A-H conduction time, which was attributed to depressed conduction in atrium and His bundle rather than in the A-V node itself (Millar & Vaughan Williams, 1982a). However, at the lower concentrations, below the threshold for class 1 action, it has been suggested that melperone may actually reduce inactivation of sodium channels. This would be consistent with the increased atrial MRD we observed at 2.67 μM, but the shortening of A-V conduction time observed *in vivo* even at high doses is more difficult to explain (Platou, Refsum, Amlie & Landmark, 1979; 1981). The difference between the lengthening of A-H time *in vitro* and the shortening *in vivo* might be attributed to a sympathetic reflex response to the hypotensive action of melperone *in vivo*. The shortening of A-V time was still observed in the presence of atenolol, however, implying that β_1-adrenoceptor stimulation was not responsible for it. The possibility that reflex sympathetic effects could be mediated by α or β_2-adrenoceptors has not been investigated.

Cibenzoline had primarily a class 1 action in atrium and ventricle but there was also a significant dose-related prolongation of APD in both tissues. In contrast with melperone, the class 3 action of cibenzoline was greater in the ventricle than in the bundle of His. Cibenzoline had no effect at all on APD in the free-running preterminal Purkinje cells, however, which illustrates that if cellular electrophysiological studies had been carried out in Purkinje fibres only,

the class 3 effect would have been missed. The lengthening of A-V conduction time by cibenzoline is consistent with the 'calcium antagonist' action previously reported (Millar & Vaughan Williams, 1982b), presumably due to restriction of slow inward current. The bradycardia induced by cibenzoline in the sinus node was thus due partly to slowing of depolarization and partly to lengthening of APD, but there was no change in the slope of the slow diastolic depolarization.

Alinidine has been shown to cause bradycardia and hypotension in man (Harron, Jady, Riddell & Shanks, 1982) and to reduce arrhythmias induced in animals by adrenergic-stimulation plus halothane, but not those precipitated by coronary ligation. The implication was that alinidine affected pacemaking cells in the sinus node, and cells of similar type elsewhere, but not abnormal ventricular pacemakers (Harron, Allen, Wilson & Shanks, 1982). We found that the bradycardia caused by alinidine was due mainly to a reduction in the slope of the slow diastolic depolarization. Alinidine did not reduce fast depolarizing current in atrial or ventricular muscle or Purkinje cells, nor was there any negative inotropic action or shift in the relation between force of contraction and extracellular calcium concentration (Millar & Vaughan Williams, 1981). Nevertheless, the upstroke of the sinus node action potential, widely believed to be due to activation of slow inward current (Bonke, 1978), was retarded, and A-V conduction time lengthened, with the implication that slow inward current in both nodes was affected, but not the slow inward current associated with contraction coupling in cardiac muscle (Millar & Vaughan Williams, 1981).

In conclusion, these studies have shown that the effects of the three drugs on the sinus node, on A-H and A-V conduction times, and on ERP in atrial and ventricular tissue are explicable in terms of selective quantitative cellular electrophysiological effects in different regions of the heart on fast and slow depolarizing currents and on the currents responsible for repolarization.

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