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EFFECTS ON RABBIT NODAL, ATRIAL, VENTRICULAR AND PURKINJE CELL POTENTIALS OF A NEW ANTIARRHYTHMIC DRUG, CIBENZOLINE, WHICH PROTECTS AGAINST ACTION POTENTIAL SHORTENING IN HYPOXIA

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1 The effects of cibenzoline (UP 339–01), a new anti-arrhythmic drug, have been investigated in various cardiac tissues.

2 UP 339–01 produced a bradycardia, due partly to prolongation of the intracellularly recorded sinus node action potential duration (APD) and partly to depression of the maximum rate of depolarization (MRD). The slope of the slow diastolic depolarization was not significantly reduced.

3 UP 339–01 was not a β-adrenoceptor antagonist.

4 UP 339–01 was negatively inotropic, and shifted the relation between $[Ca^{2+}]_o$ and force of contractions to the right, and increased A–H conduction time. It was concluded that UP 339–01 restricted slow inward current.

5 In all cardiac tissues depolarized by fast inward current, UP 339–01 caused a reduction in MRD and conduction velocity. The reduction was similar in atrial muscle, His and terminal Purkinje fibres, but in papillary muscle the effect was about half as great. On desheathed frog nerve UP 339–01 had a local anaesthetic potency slightly greater than that of procaine.

6 APD was significantly prolonged in a dose-related manner in ventricular muscle but to a lesser extent in the bundle of His and atrial tissue. In terminal Purkinje fibres APD_{50} and APD_{90} were unaltered, but the transient outward current ('notch') was abolished, resulting in a lengthening of APD_{20}.

7 The effective and functional refractory periods of the A–V node and right bundle branch were both lengthened by UP 339–01 in a dose-related manner, and the difference between them was greatly increased.

8 UP 339–01 (2.63 μM) completely prevented the shortening of APD_{90} induced by hypoxia, and the shortening of APD_{50} and APD_{20} was much attenuated. There was no protection against hypoxic depression of contractions.

9 It was concluded that UP 339–01 is a highly active class 1 anti-arrhythmic agent with additional class 3 and 4 properties.

Introduction

Shortening of action potential duration (APD) is associated with an increased incidence of arrhythmias in man (Olsson, Cotoi & Varnauskas, 1971; Gavrilescu & Cotoi, 1972), and it is possible that the APD shortening induced by hyperthyroid states (Freedberg, Papp & Vaughan Williams, 1970) or by hypoxia is responsible, at least in part, for facilitating the development of re-entry arrhythmias. Conversely, the anti-anginal drug amiodarone lengthens APD in animals (Singh & Vaughan Williams, 1970) and man (Olsson, Brorson & Varnauskas, 1973) and has become established as a valuable antiarrhythmic drug (Ferrero & Benabderhamane, 1972; Rosenbaum, Chiale, Ryba & Elizari, 1974; Rosenbaum, Chiale, Halpern, Nau, Przybylski, Levi, Lazzari & Elizar, 1976). Thus if a drug were to prevent or diminish the APD shortening induced by hypoxia, it might have a protective action against arrhythmias associated with myocardial ischaemia.

Cibenzoline (UP 339–01) is a new antiarrhythmic drug at present undergoing clinical trial, and its electrophysiological effects have been investigated in different cardiac tissues. A study of its influence on APD in hypoxia was added to other tests customarily undertaken to characterize the mode of action of antiarrhythmic drugs (Vaughan Williams, 1980).

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Methods

Local anaesthesia

Sciatic nerves were removed from pithed frogs, and the perineural sheaths were stripped from the central portions. The nerve was enclosed in a three compartment chamber; supramaximal stimuli were applied at the proximal end, and action potentials were recorded from the distal end, the nerve being supported on platinum wires in moist air. Logarithmically increasing concentrations of procaine were applied to the stripped portion of the nerve, immersed in physiological solution in the central chamber, as previously described (Dohadwalla, Freedberg & Vaughan Williams, 1969). After washing with drug-free solution the action potential returned to its pre-drug value, and a cumulative dose-response curve was obtained for UP 339–01.

Intracellular potentials

Atrial and sino-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 of the atrial myocardium. 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 (Szekeres & Vaughan Williams, 1962).

Ventricular records The heart was immersed in ice-cold physiological saline continuously oxygenated during the dissection. The left atrium, the part of the right atrium containing the sino-atrial node (SAN), and the left ventricular free wall were removed. The right ventricular wall was cut free anteriorly and peeled back, revealing the anterior papillary muscles which, in the rabbit, both originate from the septum. A thread was tied to one of the chordae tendineae and attached to the strain gauge. Details of the dissection have been described previously (Millar & Vaughan Williams, 1982). The other papillary muscle was left slack and was used for microelectrode recordings. Stimuli, 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) Figure 1c) or to the bundle of His (SH). Intracellular records were obtained from bundle of His cells, terminal Purkinje cells and papillary muscle cells. Atrio-Hisian (A–H) interval was the time between S_1 and the upstroke of the His potential. The His-Purkinje potential (H–P) interval was measured from start of the His bundle potential to the start of the Purkinje cell potential, and the P–V interval from the start of the Purkinje to the start of the papillary potential. The effective refractory period (ERP) of the A–V node was the shortest interval between an atrial stimulus (S_1) and a second stimulus (S_2) which would evoke a premature His bundle potential. The functional refractory period (FRP) of the A–V node was the interval between the His bundle action potential responses (AP_1–AP_2) to S_1 and to S_2. Likewise the effective and functional refractory periods of the H–V conducting system were the S_1–S_2 intervals and AP_1–AP_2 intervals respectively, when stimuli were applied to the bundle of His and action potentials were recorded from the papillary muscle.

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. To produce hypoxia the gas was changed to 20% O_2, 5% CO_2, 75% N_2, and the atria were paced at 3 Hz throughout.

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

Drugs used

UP 339.01 (cibenzoline) (diphenyl-2',2'-cyclopropyl) 2 imidazoline succinate was supplied by UPSA Laboratories, Rueil-Malmaison, France; isoprenaline sulphate (Burroughs Wellcome); procaine HCl (B.D.H.).

Results

Local anaesthesia

A comparison of the local anaesthetic potency of procaine and UP 339–01 (hereinafter called UP) was carried out on six desheathed frog nerves, the procaine always being applied first, because UP had a long duration of action. The results showed that UP was slightly more potent than procaine.

Rabbit isolated atria

UP reduced the spontaneous frequency of rabbit isolated atria (at 32°C) in a dose-related manner, concentrations of 1.32, 2.63 and 5.26 μM inducing

mean falls in heart rate of 3.8, 8.4 and 15.6% respectively ($n = 5$). UP also reduced the maximum frequency at which the atria would follow a stimulus, in a dose-related manner, concentrations of 1.32, 2.63, 5.26 and 10.52 μM causing reductions of 16, 29, 39 and 51% respectively. The bradycardia was very persistent, the heart rate still being 10% below control after washing for an hour in drug-free solution.

The bradycardia was not due to an anti-adrenoceptor action. In control solution the mean threshold concentration of isoprenaline required to increase rate and force was 3.5 nM, and in the presence of UP 5.26 μM it was 2.8 nM (the difference was not significant). Since UP had a negative inotropic effect as well as inducing bradycardia, both the inotropic and the chronotropic isoprenaline dose-response curves were depressed downwards, but there was no shift to the right, and it was concluded that UP had no β-adrenoceptor blocking action.

Sino-atrial node (SAN)

The sinus node was explored and potentials were accepted as coming from sinus node cells if they exhibited a slow diastolic depolarization, and if the maximum rate of depolarization of the action potential was not greater than 13 V/s (this was a more rigorous selection than in our previous paper, in which MRD up to 18 V/s was accepted; Millar & Vaughan Williams, 1981b).

The mean effects of logarithmically increasing concentrations of UP on the parameters of intracellularly recorded SAN potentials are presented in Table 1. Rows 2 to 9 correspond to the measurements indi-

Table 1 Effect of UP 339.01 on sinus node potentials

UP 339-01 conc.	1 0.0	2 1.316 μM		3 2.63 μM		4 5.26 μM		5 0.0 (Recovery)	
Parameters as shown in Figure 1a	Mean (s.e.mean)	Mean (s.e.mean)	Δ	Mean (s.e.mean)	Δ	Mean (s.e.mean)	Δ	Mean (s.e.mean)	Δ from 5.26 μM
1 Heart rate (beats/min)	162.6	152.6	−10.0	139.6	−23.0	135.3	−27.3	149.1	+13.8
2 Spontaneous peak-to-peak interval between potentials (ms)	369.0 (3.19)	393.3 (5.16)	+24.3**	429.7 (1.65)	+60.7***	443.5 (3.42)	+74.3***	402.4 (2.88)	−41.1***
3 Peak amplitude (mV)	−1.19 (0.93)	−2.34 (0.64)	−1.14	−2.26 (0.80)	−1.06	−4.56 (0.82)	−3.36 ($P = 0.063$)	−3.62 (0.97)	+0.95
4 Maximum diastolic potential (mV)	−62.78 (1.16)	−62.47 (1.60)	+0.31	−59.91 (1.26)	+2.87	−56.99 1.31	+5.78*	−57.51 1.20	−0.52
5 Slope of slow diastolic depolarization (mV/s)	53.90 (2.37)	57.39 (2.49)	+3.48	52.57 (2.79)	−1.33	52.64 3.16	−1.27	64.15 5.00	+11.52 ($P = 0.17$)
6 'Take-off' potential (mV)	−42.37 (1.89)	−43.65 (1.64)	−1.28	−36.03 (1.69)	+6.34*	−38.85 1.33	+3.52	29.21 1.49	+9.64***
7 Maximum rate of depolarization (V/s)	4.35 (0.30)	4.24 (0.36)	−0.11	3.21 (0.37)	−1.13 ($P = 0.10$)	2.17 0.24	−2.18***	1.95 0.20	−0.22
8 Mean rate of repolarization (V/s)	0.55 (0.012)	0.49 (0.01)	−0.05**	0.42 (0.01)	−0.12***	0.33 0.01	−0.22***	0.44 0.01	+0.107***
9 Total duration of repolarization (ms)	115.6 (1.88)	125.7 (1.49)	+10.1**	137.8 (2.15)	+22.2***	162.2 2.48	+46.6***	125.2 2.04	−37.0***

Statistical significance. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Where $P > 0.05$ but approached significance, the actual P-value has been given. Δ in columns 2, 3 and 4 represents differences from pre drug controls. In column 5 it represents differences from column 4.

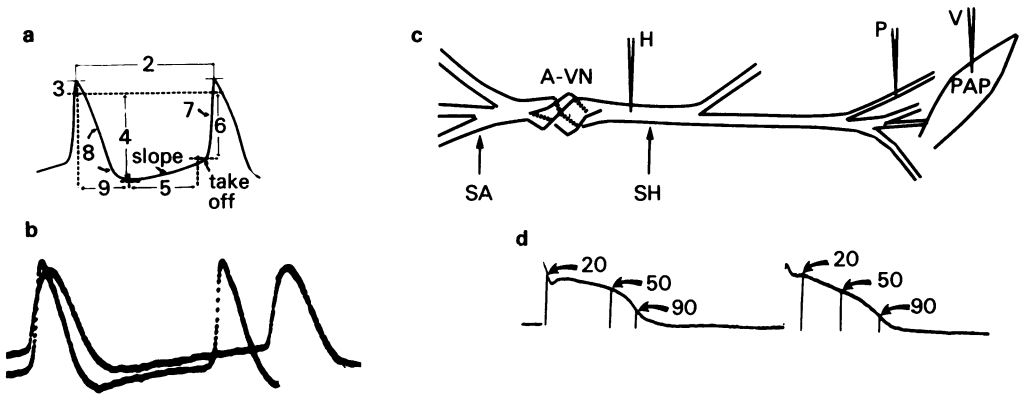

Figure 1 Stimulation and recording. (a) Diagram illustrating the measurements made by computer from tape recordings of intracellular sino-atrial node (SAN) potentials. The numbers correspond to the rows in Table 1 in which the measurements are presented. (b) Control SAN potential superimposed at its peak on a potential recorded from the same preparation after exposure to UP 339-01 (2.63 μM). (c) Diagram to illustrate the position of stimulating electrodes in the atrium (SA) close to the A-V node, and on the bundle of His (SH). Intracellular records were taken from the bundle of His (H), terminal Purkinje cells (P), and papillary muscle (V). (d) Intracellular records illustrating the effect of UP on terminal Purkinje cells.

cated in Figure 1a, and, in addition, beat-to-beat intervals have been converted to heart-rates in row 1 for convenience. The experiments were undertaken at 37°C, and indicate that the bradycardia produced by UP was similar to that already seen at 32°C (5.26 μM reduced heart rate by 15.6% at 32°C and by 16.6% at 37°C).

There was no significant reduction in the slope of the slow diastolic depolarization (row 5), though there was a small 'rebound' increase in slope after 60 min recovery in drug-free solution (not statistically significant). Thus the bradycardia produced by UP could not be attributed to any interference with currents responsible for the slow diastolic depolarization.

The maximum diastolic potential (row 4) and the take-off potential (row 6) both moved a few millivolts in the positive direction, so that the slow diastolic potential was shifted upwards without change in slope.

There was a dose-related and highly significant increase in the total duration of the phase of repolarization, significantly reversed on wash-out, and comparison of row 9 with row 2 indicates that this accounted for about half the increase in beat-to-beat interval.

The remainder of the bradycardic effect was accounted for by a small reduction in the maximum rate of depolarization (row 7) and of peak amplitude (row 3), both persisting on wash-out, so that the heart rate did not return to the pre-drug value during the recovery period. These effects would be consistent with a restriction by UP of slow inward current, which is responsible for the upstroke of the action potential in the sinus node. The peak itself was less sharp, i.e. the mean rate of depolarization was depressed even more than the maximum rate, adding a few more milliseconds to the peak-to-peak interval. Representative records from one of the experiments are depicted in Figure 1b.

Atrial myocardium

No significant change in electrical threshold was produced by concentrations of UP up to 5.26 μM (+5%, $P = 0.17$). In one experiment a concentration of 10.52 μM UP was used, which caused an increase in threshold of 40%, but contractions were depressed by 68%, and further experiments were not undertaken at such a high concentration. It was concluded that at clinically employable concentrations, UP would have a negligible effect on the electrical threshold of atrial muscle.

Concentrations of 1.32, 2.63 and 5.26 μM of UP reduced action potential amplitudes (APA), maximum rate of depolarization (MRD), conduction velocity and the magnitude and the time from start to peak of contractions in a dose-related manner. The higher concentration reduced APA from 86.3 to 76.9 mV, and MRD from 111 to 32 V/s, indicating that UP had a very powerful class 1 antiarrhythmic action, restricting fast inward current. There was a small fall in resting potential, reversed on wash-out. Though not statistically significant, attention is drawn to it because of the similar (and statistically significant) reduction in maximum diastolic potential in the SAN.

The largest effect of UP, significant at the lowest

concentration, was the reduction of MRD. Mean overshoot potentials were 18.4 mV (controls) and 18.21, 16.33 and 12.28 mV in the increasing concentrations of UP. Conduction velocity was also depressed.

UP had a profound and long-lasting negative inotropic action, only partially reversed on wash-out, and the time to peak contraction was just significantly prolonged in the highest concentration.

As found in the SAN, action potential duration (APD) was prolonged in the atrium, but only in its terminal phase. Measurement of the time from the peak of the potential to 20% (APD_{20}) and 50% (APD_{50}) repolarization was unchanged even by 5.26 μM UP. However, APD_{90} was prolonged in a dose-related manner, by 5.9 ($P=0.10$), 13.6 ($P=0.003$) and 15.0 ($P=0.0008$) ms in the three concentrations respectively. This effect on APD was significantly, but not fully reversed (by 7 ms) after 60 min recovery in drug-free solution. It was concluded that UP had a minor but significant class 3 action in atrial muscle.

Calcium antagonism (class 4 action)

Over a range of concentrations from about half to twice normal, there is a linear relation between the log of external calcium concentration and the force of contraction in cardiac muscles. There is no hysteresis in this relation; returning to a given concentration from another, the same force of contraction is observed as before, irrespective of the order of exposure to different concentrations (high before low or *vice versa*) (Millar & Vaughan Williams, 1981a, b). UP 2.63 μM significantly depressed this relation, as does verapamil and several other drugs loosely termed 'calcium antagonists' (Fleckenstein, 1980), but the potency of UP was less than one-fifth that of verapamil on a molar basis (cf. Figure 8, Salako, Vaughan Williams & Wittig, 1976).

A–V node and ventricular conduction system

The positions of the stimulating and recording electrodes for the study of the A–V node and ventricular conducting system are depicted in Figure 1c.

UP at concentrations up to 5.26 μM had no statistically significant effect on electrical threshold in the atrium (SA) or His bundle (SH) although at 5.26 μM small increases were observed (+8% and +11.5% respectively).

UP at all concentrations, as can be seen from Table 2, caused a significant prolongation of conduction time in all parts of the conduction system, A–V node (A–H, row 1), His bundle to terminal Purkinje fibres of the right bundle branch, RBB, (H–P, row 2), and RBB to papillary muscle (P–V, row 3). For simplicity, only the increases in conduction times above

Table 2 Conduction times and refractory periods in ventricular preparations.

UP 339-01 concentration	0.0	1.32 μM		2.63 μM		5.26 μM	
Conduction times (ms)	Mean (s.e.mean)	*Increase* Δ%	Δms	*Increase* Δ%	Δms	*Increase* Δ%	Δms
1 Atrio-Hisian A-H	40.6 (1.04)	(15.2)	6.16**	(38.4)	15.6***	(25.1)	10.2***
2 His-Purkinje H-P	14.71 (0.32)	(14.75)	2.17*	(14.2)	2.10*	(51.5)	7.57***
3 Purkinje-Ventricular P-V	14.83 (0.59)	(43.48)	6.45***	(74.1)	10.89***	(85.0)	12.61***
Effective refractory periods (S_1–S_2)							
4 A-H	137.4	(4.2)	5.8	(13.7)	18.8	(21.8)	30
5 H-V	145.2	(16.9)	24.6*	(22)	31.9*	(29)	42.1*
Functional refractory periods (AP_1–AP_2)							
6 A-H	218.2	(10)	22.2***	(20.9)	45.6***	(25.1)	54.8***
7 H-V	214.1	(13.6)	29.2	(24.2)	51.8	(39.8)	85.3

For simplicity, most s.e.means have been omitted. Means are from 9 experiments. Symbols as in Table 1.

control values (column 1) have been presented, in columns 2, 3 and 4. The delay of conduction was relatively most marked in the P–V pathway, as can be seen from the changes expressed as percentage increases (figures in parentheses).

As could be anticipated from the class 1 and class 4 actions already demonstrated in the atrium, the effective refractory periods in the A–V node and bundle of His were increased by UP in a dose-related manner. The conduction velocity of a very premature action potential is always slower than that of responses after a full diastolic interval, because the premature potential takes off from partially repolarized tissue. This is reflected in the difference between the functional refractory period (AP_1–AP_2) and the effective refractory period (S_1–S_2), here revealed by the difference between rows 6 and 4, and between rows 7 and 5. UP exaggerated this slowing of premature action potentials in a dose-related manner, the FRP-ERP differences being 80.8 ms (control), and 97.2, 107.6 and 105.6 ms in the three drug concentrations respectively, for A–H; and 68.9 ms (control) 73.5, 88.8 and 112.1 ms, for H–V. This suggests that UP would be very effective in suppressing or delaying the conduction of premature responses in the ventricular conduction system. Whether or not this could be an arrhythmogenic factor is discussed later.

Intracellular potentials

The resting potential was not altered in terminal Purkinje cells or papillary muscles by any concentration of UP, nor in the bundle of His by concentrations up to 2.63 μM. The highest concentration of UP (5.26 μM) caused a small reduction of resting potential in the bundle of His from −77.6 to −73 mV ($P = 0.004$).

The overshoot potential and maximum rate of depolarization were reduced by UP in all tissues, the effect being relatively larger on the Purkinje cells of the His bundle and terminal region, than in the ventricular muscle (Table 3). MRD was reduced to a similar extent in atrial muscle, His and terminal Purkinje cells, but in the papillary muscle the effect was about half as great at each concentration.

The effects of UP on action potential duration (APD) were markedly different in the various parts of the ventricular conduction pathway (Table 4). In the terminal Purkinje fibres, APD_{50} and APD_{90} were not significantly altered at any concentration but the APD_{20} was lengthened in a dose-dependent manner. This reflects the abolition of the 'transient outward current' or 'notch', which is normally seen in terminal Purkinje cells (Figure 1d), in association with the peak of the fast phase of depolarization being greatly depressed. In contrast, in ventricular muscle, all phases of repolarization were significantly prolonged in a dose-dependent manner. In the bundle of His, APD was lengthened but not so much as in the ventricular muscle and by the higher concentrations only. The prolongation of APD_{90} in the ventricular muscle was double that already noted in atrial muscle, and would constitute a highly significant class 3 action.

Hypoxia

In Langendorff-perfused guinea-pig hearts, when periods of hypoxia or reduced flow were alternated with periods of fully oxygenated reperfusion, it was found that the hypoxic shortening of APD was accelerated and exacerbated during successive periods of hypoxia, even though recovery during the intervening periods of reperfusion appeared to be complete (Cowan & Vaughan Williams, 1977; 1980). In rabbit isolated atria a similar phenomenon, though less marked than in Langendorff-perfused ventricles, was observed (Millar & Vaughan Williams, 1982) which

Table 3 Action potential amplitude and maximum rate of depolarization in ventricular preparations

	Bundle of His			*Terminal Purkinje cell*			*Papillary muscle*		
	n	MRD (V/s)		*n*	MRD (V/s)		*n*	MRD (V/s)	
UP 339-01 concentration (μM)		Mean (s.e.mean)	Δ		Mean (s.e.mean)	Δ		Mean (s.e.mean)	Δ
0.0	88	160.4 (6.62)		76	307.6 (12.72)		81	128.1 (3.57)	
1.63	78	114.0 (5.29)	−46.4***	60	211.8 (9.04)	−95.76***	81	115.8 (3.23)	−12.3
2.56	44	62.7 (4.87)	−97.7***	31	158.7 (14.3)	−148.9***	74	86.1 (3.76)	−41.9***
5.62	57	56.0 (5.18)	−104.4***	47	135.2 (8.78)	−172.3***	74	81.5 (3.47)	−46.6***

Symbols as in Table 1.

Table 4 Action potential duration from peak to 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) repolarization (ms)

	Bundle of His		*Terminal Purkinje cell*		*Papillary muscle*	
	APD_{20}	APD_{90}	APD_{20}	APD_{90}	APD_{20}	APD_{90}
UP 339-01 concentration (μM)	Mean (s.e.mean)	Mean (s.e.mean)	Mean (s.e.mean)	Mean (s.e.mean)	Mean (s.e.mean)	Mean (s.e.mean)
0.0	84.0 (2.62)	192.2 (2.53)	7.8 (0.32)	201.5 (2.28)	56.7 (2.06)	122.0 (2.17)
	Δ	Δ	Δ	Δ	Δ	Δ
1.32	4.0	0.0	3.4 $P=0.12$	−7.5 $P=0.12$	−0.8	12.7**
2.63	8.1 $P=0.16$	9.0 $P=0.09$	9.1*	−3.2	8.9**	29.2***
5.62	15.8**	11.8**	28.0***	8.2 $P=0.11$	9.9***	30.1***

Symbols as in Table 1

made it impossible to study the effects of a drug on the reactions to hypoxia in the same preparations as were used for controls. The protocol adopted, therefore was to expose atria for 30 min to fully oxygenated drug-free solution, followed by 30 min in normoxic solution (N_1) with or without drug: the solution, either drug-free (controls) or with drug (treated), was changed to an hypoxic solution (H_1) for 15 min, followed by normoxic solution for 25 min (N_2). The procedure was repeated until measurements had been obtained in three normoxic and three hypoxic solutions.

Successive periods of hypoxia and re-oxygenation in untreated isolated atria of rabbit caused the contractions to diminish and the time from start to peak of the contraction to shorten (Figure 2a). Full recovery occurred in both parameters during the intervening normoxic periods, and there was no progressive trend in either the hypoxic or recovery periods. The effect of UP (2.63 μM) was to reduce contractions and to lengthen the time to peak. However, the reduction of contractions and shortening of the time to peak was as great in UP as before, so that no protection was given against hypoxic depression of contractions. There was a progressive tendency to 'rebound' during recovery periods, especially in the time to peak.

Hypoxia alone progressively reduced action potential amplitude and rate of depolarization (Millar & Vaughan Williams, 1982) and exacerbated the effects of class 1 drugs on fast inward current (Vaughan Williams, 1980). UP 2.63 μM reduced APA from a mean pre-drug value of 86.6 mV to 82.1 mV, and the first period of hypoxia reduced it further to 78.5 mV. There was no progressive fall during the succeeding periods of hypoxia or recovery. MRD was reduced by UP to 35.7 V/s in the first normoxic period (N_1) and to 29.4 in hypoxia (H_1). Thereafter MRD was 37.1 and 32.1 during N_2 and N_3 respectively, and 29.5 and 19.4 during H_2 and H_3. Thus the effect of UP on MRD, in common with that of other class 1 drugs, was increased by hypoxia.

The most striking effect of UP was the protection afforded against APD shortening in hypoxia, as demonstrated in Figure 3. In the absence of the drug, hypoxia shortened APD_{90}, APD_{50} and APD_{20}, with a small, though not statistically significant trend towards exacerbation of the effect during successive periods of hypoxia. UP 2.63 μM itself slightly prolonged APD_{90}, as already observed, and completely prevented the shortening induced by hypoxia. The shortening of APD_{50} and APD_{20} was much attenuated, and the trend during successive periods of hypoxia was in the opposite direction, towards amelioration of the hypoxic shortening.

Discussion

Some antiarrhythmic drugs which may be classed together on the basis of their electrophysiological actions on cardiac tissues, differ considerably in their overall clinical effects. Such differences have been explained in various ways as due to subsidiary additional actions, inequalities of distribution or rates of metabolism, extra-cardiac factors etc., which have been discussed elsewhere (Vaughan Williams, 1980). However, it has become evident that even within the heart, sensitivity to a drug in different regions may vary considerably, so that to characterize fully the cardiac actions of a compound, it is necessary to prepare a sort of 'pharmacological map', quantitative responses to its presence being measured tissue by tissue (Millar & Vaughan Williams, 1981c).

UP 339.01 is a new antiarrhythmic drug which caused bradycardia *in vitro*. It was not a β-adrenoceptor antagonist, and had no effect on the slope of the slow diastolic depolarization of sino-atrial cells. The maximum diastolic potential and

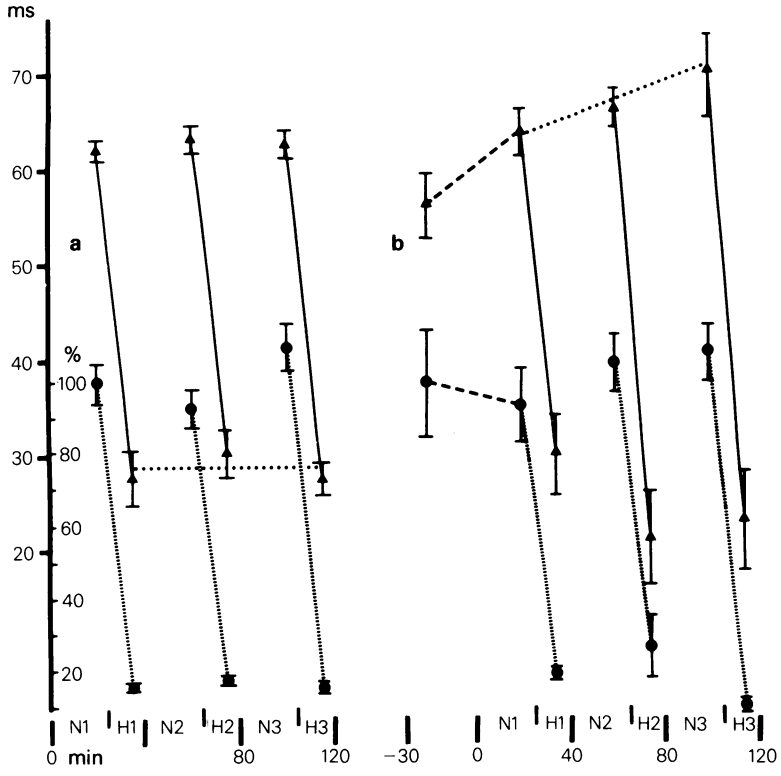

Figure 2 Effects of hypoxia on contractions of isolated rabbit atria. Ordinate scale: time from start to peak of contraction (ms), and contraction peak force expressed as a percentage of that observed in the initial normoxic period. Abscissa scale: time in min. Twenty five min periods of normoxia (N_1, N_2, N_3) were alternated with 15 min periods of hypoxia (H_1, H_2, H_3). UP 339-01 (2.63 μM) (b) did not protect the atria against hypoxic depression of contractions, in comparison with controls (a). The bars represent s.e.mean.

take-off potential were both shifted by a few millivolts in a positive direction, but the slope of the slow diastolic depolarization remained parallel to that of the controls. The bradycardia was due partly to a slowing of the maximum rate of depolarization (MRD), which suggested that UP restricted current through slow inward channels. There was a delay in repolarization, which also contributed to the bradycardia. Taken with the reduction of maximum diastolic potential, this implied some restriction by the drug of outward potassium current.

In the atrial myocardium the primary effect of UP was a large reduction of MRD, and a diminution of overshoot potential, which implied restriction of fast inward current. Other effects in keeping with this were a slowing of conduction velocity and a dose-related reduction of the maximum frequency at which a stimulus could be followed. UP also reduced resting potential by a few millivolts (an effect reversed on washout and consistent with the fall of maximum diastolic potential observed in the SAN). As in the SAN, again, the APD_{90} of the atrial myocardial cells was significantly prolonged.

UP had a strongly negative inotropic action, and shifted the relation between log $[Ca^{2+}]_o$ and contractions to the right, both effects implying restriction of slow inward current.

In the A–V node and His Purkinje system, conduction times were greatly prolonged, as were the effective and functional refractory periods of the A–H and H–V pathways. APD was greatly prolonged in ventricular muscle, the effect being twice as large as in the atrium. In contrast, APD was hardly altered in the terminal Purkinje cells but moderately increased in His bundle Purkinje cells.

All these actions can be explained by attributing three actions to the drug: (1) restriction of fast inward current as its most potent effect (class 1); (2) restriction of slow inward current, to account for effects on the SAN and AV nodes, and for the negative inotropic action (class 4); (3) moderate restriction of outward potassium current, to account for the delayed repolarization in the SAN and in atrial and ventricular muscle, and for the small reductions of

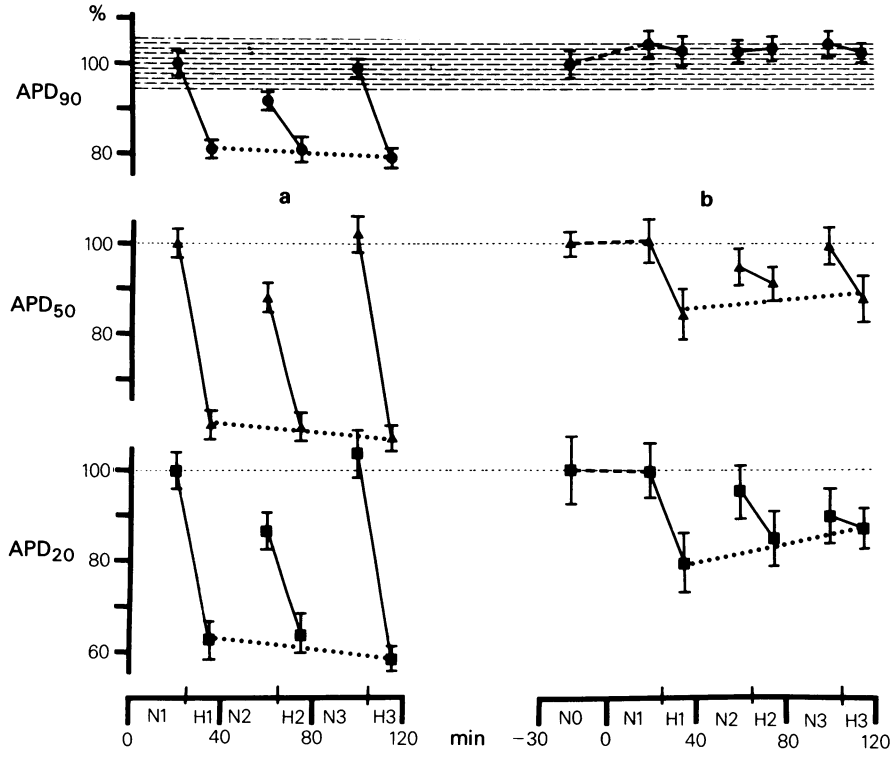

Figure 3 Effects of hypoxia on action potential duration from the peak to 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) repolarization. Ordinate scale: APD as percentage of that observed in the initial normoxic period. Abscissa scale: as for Figure 2. The width of the hatched area is equal to one standard deviation from the mean APD_{90} observed in the initial normoxic period (b) before addition of the drug. The bars represent s.e.mean. UP 339-01 protected the atria against hypoxically-induced shortening of APD.

diastolic potential in the SAN and of resting potential in the atrium.

Perhaps the most remarkable effect of UP was the complete abolition of hypoxically induced shortening of APD_{90} in atrial muscle. Hypoxic shortening of APD_{50} and APD_{20} were greatly attenuated. This suggests that UP could be a useful drug for the treatment of arrhythmias associated with ischaemia, if APD-shortening is an important arrhythmogenic factor in such circumstances. In contrast, another recently studied drug, Melperone, which lengthened APD acutely, did not alter the shortening of APD in hypoxia, but because the pre-hypoxic APD was long, the APD_{90} (time from action potential peak to 90% repolarization) was still close to normal during hypoxia (Millar & Vaughan Williams, 1982).

Various hypotheses have been advanced to explain the high incidence of arrhythmias after myocardial infarction. One is that slow conduction of impulses through ischaemic areas permits the development of re-entry circuits, and it has been suggested also that slow conduction of premature action potentials taking off from partially repolarized Purkinje cells may play a similar role (Wittig, Harrison & Wallace, 1973; Cranefield, 1975). UP greatly reduced conduction velocity in the ventricular pathway, and had an especially marked additional effect in slowing the conduction of premature action potentials more than of those occurring after a full diastolic interval. In the presence of an unchanged or hypoxically shortened refractory period, this slowing might be considered arrhythmogenic. Even if the explanation for ischaemic arrhythmias outlined above is correct (which is far from proven) UP would be unlikely to be arrhythmogenic because, in addition to slowing conduction velocity, the drug prolonged refractory periods and APD, and prevented the shortening of APD normally associated with hypoxia.

The action of UP was very persistent; several effects of the highest concentration did not fully decline during one hour's washing with drug-free solution. It is possible, therefore, that in the clinic the effect of UP on cardiac muscle could outlast its life in the plasma. The lowest concentration studied *in vitro* (1.32 μM) would be unlikely to be exceeded in the plasma of patients for any considerable period, and

the predominant effect of the drug at this concentration was its class 1 action.

In conclusion, UP 339.01 had a powerful class 1 antiarrhythmic action, and was a local anaesthetic on nerve with a potency equal to that of procaine. It had some class 3 and 4 effects as well, delaying repolarization and antagonizing the positive inotropic action of calcium. The drug was not, however, a β-adrenoceptor antagonist, so that the bradycardic and negative inotropic actions observed *in vitro* could probably be largely compensated *in vivo*, like those of verapamil, by sympathetic reflexes.

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