DIFFERENTIAL ACTIONS ON RABBIT NODAL, ATRIAL, PURKINJE CELL AND VENTRICULAR POTENTIALS OF MELPERONE, A BRADYCARDIC AGENT DELAYING REPOLARIZATION: EFFECTS OF HYPOXIA

J.S. MILLAR & E.M. VAUGHAN WILLIAMS

University Department of Pharmacology, Oxford

1 Melperone, a butyrophenone tranquillizer, caused bradycardia *in vivo* and *in vitro*.

2 Although Melperone had α-adrenoceptor antagonist activity in the pithed rat, it was not a β-adrenoceptor antagonist, nor was it a cholinoceptor agonist.

3 The bradycardic action could be attributed almost entirely to a prolongation by Melperone of action potential duration (APD) in sinus node cells, with little effect on the slow diastolic depolarization.

4 APD was prolonged by Melperone in atrial and ventricular muscle, and most of all in the bundle of His, but only moderately in the terminal Purkinje cells.

5 In all cardiac tissues depolarized by fast inward current, Melperone caused a dose-related reduction in the maximum rate of depolarization and conduction velocity. On desheathed frog nerve Melperone had a local anaesthetic potency equal to that of procaine.

6 There was no negative inotropic effect in cardiac muscle, nor interference with A-V nodal conduction, from which it was inferred that Melperone did not restrict slow inward current.

7 Melperone did not reduce hypoxic shortening of APD relative to the initial value at the start of hypoxia, but because APD was already lengthened by Melperone in normoxic conditions, APD_{90} during hypoxia remained close to normal values. There was no protection against hypoxic depression of contractions.

8 It was concluded that Melperone had class 1 and class 3 antiarrythmic actions and merited trial as an antiarrhythmic drug.

Introduction

The butyrophenone, Melperone, prolonged barbiturate sleeping time in mice, reduced body temperature, and had some analgesic, anticonvulsant and antitremor activity (Christensen, Hernestam, Lassen & Sterner, 1965). In consequence of these and other studies, the compound was tested for tranquillizing properties in man (Ambrozi, Birkmayer & Danielczyk, 1969; Molander & Duvhök, 1976). It was noted that Melperone prolonged action potential duration (APD) in guinea-pig isolated papillary muscle (Olsson & Arlock, 1976), and it was found to have an antiarrhythmic effect in animals (Petersen, 1978). Melperone did not depress contractions in guinea-pig papillary muscles (Arlock, Gullberg & Olsson, 1978) or rat isolated atria, the spontaneous frequency of the latter being reduced (Refsum, Amilie, Platou, Owren & Landmark, 1978).

In rabbits, APD is lengthened by prolonged treatment with amiodarone (Singh & Vaughan Williams, 1970) or β-adrenoceptor blockers (Vaughan Wil-liams, Raine, Cabrera & Whyte, 1975) and in man monophasic action potentials are prolonged by amiodarone (Olsson, Brorson & Varnauskas, 1973) and Q-T interval lengthened by long-term β-adrenoceptor-blockade (Vaughan Williams, 1980). Melperone, in contrast, prolongs APD acutely, and it was of interest to study whether repolarization was uniformly delayed in all cardiac tissues, and whether there were any selective anti-sympathetic effects. The shortening of APD in ischaemic myocardium may be responsible, at least in part, for the increased incidence of dysrhythmias after myocardial infarction, and experiments were undertaken to determine whether Melperone could prevent the shortening of myocardial APD in hypoxia.

The mechanism of the bradycardic action of Melperone has been studied by recording intracellular potentials from the sinus node. The results indicate that the delay of repolarization in sino-atrial node cells can account for most of the bradycardic action.

Measurement of the relative efficacy of Melperone in several cardiac tissues represented a sort of 'pharmacological mapping' and indicated that there were marked variations in sensitivity of different regions to the drug. It was apparent, for example, that the Purkinje cells in the terminal portions of the ventricular conducting system, such as are customarily chosen for electrophysiological studies, were different in their responses from Purkinje cells in the bundle of His.

Methods

In vivo *experiments*

Wistar rats of either sex, weighing $250-350$ g, were anaesthetized with pentobarbitone 60 mg/kg and pithed. The left jugular vein and right common carotid artery were cannulated. Mean blood pressure was measured with a mercury manometer, and heart rate by ECG.

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. Various concentrations of procaine or Melperone 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).

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 (Szekeres & Vaughan Williams, 1962) to both surfaces. The oxygenation was external to the bath, to avoid disturbance of microelectrodes by oxygen bubbles.

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. 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, together with a small portion of the right ventricular free wall. The preparation was then transferred to the organ bath, as described for atrial recording. The septum was anchored by threads tied to a rectangular grid to stabilize the origin of one of the papillary muscles, and the thread attached to the tendon was tied to the strain gauge. 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 1d) 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 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 His bundle and action potentials were recorded from the papillary muscle.

The physiological solution contained (mM): NaCl 125, KCl 5.6, NaHCO$_3$ 25, Na$_2$HPO$_4$ 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 microelec-

trodes, 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 Student's t test (Vaughan Williams, 1977).

Drugs

Melperone(γ-(4-methyl piperidino)-p-fluorobutyrophenone) was supplied by AB Ferrosan, Malmö, Sweden; other drugs used were isoprenaline sulphate (Burroughs Wellcome); phenylephrine HCl (Sigma); atropine sulphate (B.D.H.); procaine HCl (B.D.H.).

Results

Bradycardia

Antisympathetic effects in vivo. Peak rises in blood pressure in response to logarithmically increasing doses (range 0.5 to 20 µmol/kg) of phenylephrine (i.v.) were measured in pithed rats, and plotted against the logarithm of the dose of phenylephrine. Melperone 1 or 4 µmol/kg was injected intravenously and the dose-response curve to phenylephrine repeated. Provided that the post-Melperone measurements could be completed within about 15 min, the drug produced a parallel shift in the dose-response curve to the right, implying some competitive antagonism at α-adrenoceptors. However, this effect was short-lived. For example, 5 min after an injection of Melperone of 1 µmol/kg the hypertensive response to phenylephrine 5 µmol/kg was reduced to 30% of its pre-drug value, but 25 min later the response to the same dose of phenylephrine had recovered to 80% of control. The mean dose-ratio calculated from the phenylephrine log dose-response curves after Melperone 1 µmol/kg was 8 ± 1.8 (s.e.mean) ($n = 6$).

The injections of Melperone produced a dose-related fall in heart rate. Increases in heart rate in response to logarithmically increasing intravenous doses of isoprenaline were measured. The responses were attenuated by Melperone, but the threshold dose of isoprenaline for an increase in heart rate was not altered, and the dose of isoprenaline producing a maximal effect was also the same before and after Melperone. There was no evidence that Melperone produced bradycardia by a competitive antagonism at β-adrenoceptors.

Cholinomimetic effects The bradycardia induced by Melperone was studied in pairs of isolated atria and in isolated sinoatrial node preparations. The mean percentage depressions of frequency by concentrations of Melperone of 2.67, 5.34 and 10.68 µM were,

respectively, 12.48, 19.20 and 30.4 ($n = 10$). In the presence of atropine 1 µM, the same concentrations of Melperone depressed frequency by 12.50%, 22.56% and 29.8% respectively. It had previously been determined that this concentration of atropine blocked the bradycardic response of rabbit isolated atria to acetycholine 3 µM, and it was concluded that Melperone did not produce bradycardia by an agonist action at cholinoceptors.

Sino-atrial cells The sinus-node preparation, mounted as described and superfused at 37°C, was explored with microelectrodes and records were made on tape of all potentials exhibiting a slow diastolic depolarization. The solution was then changed to one containing, successively, 2.67, 5.34 or 10.68 µM Melperone, and a further series of records was obtained at each drug concentration. A final set of records was made after washing for 1 h with drug-free solution ('recovery'). The tapes were subsequently played back in real time, and no record was accepted as coming from a sinus node cell unless the maximum rate of depolarization was less than 15 V/s. Acceptable records were analysed by computer and stored (Vaughan Williams, 1977). The programme made the measurements depicted in Figure 1a. The means and s.e.mean of these measurements are presented in Table 1, together with differences and statistical significances.

The first row of Table 1 represents the intervals between beats in milliseconds. The dose-related bradycardia produced by Melperone is apparent, with complete recovery on wash-out. Comparison of row 1 with row 8 shows that all but about 10 ms of the increases in cycle length can be accounted for by the increases in action potential duration. A control record, with a superimposed record showing the effect of a concentration of Melperone of 5.34 µM, is presented in Figure 1b. The 'take-off' potential (row 5) was not significantly altered, and the slope of the slow diastolic depolarization (row 4) was reduced only by the higher concentrations of Melperone. On wash-out the duration of the action potential, which had increased from 108 to 264 ms (row 8), returned to within 10 ms of control. The slope of the slow diastolic depolarization (row 4) did not return to control values, but the 'take-off' potential (row 5) was significantly more negative on wash-out, so that there was no net influence of these two parameters on heart rate. There was a small decrease in maximum diastolic potential (row 3), completely reversed on wash-out. Although the effect was not statistically significant, attention is drawn to it because such a decrease would be expected if the action of Melperone involved a reduction in the outward potassium currents responsible for repolarization. There was a significant increase in peak potential (row 2) reversed on

Table 1 Effect of Melperone on heart rate and sino-atrial intracellular potentials

(n)	Control 0.0 Mean	(96) Diff. from control (P value)	2.67 µM Mean	(85) Diff. from control (P value)	5.34 µM Mean	(76) Diff. from control (P value)	10.68 µM Mean	(83) Diff. from control (P value)	Recovery 0.0 Mean	(93) Diff. from 10.7 µM (P value)
1 Cycle length (ms)	303.80 ±9.00		369.80 ±5.66	+66.00 ***	411.10 ±6.11	+107.30 ***	476.70 ±5.29	+173.00 ***	310.80 ±2.83	−166.00 ***
2 Peak potential (mV)	−0.18 ±0.67		2.70 ±0.65	+2.88 *	1.31 ±0.51	+1.49 (0.22)	5.61 ±0.65	+5.79 ***	−0.67 ±0.49	−6.27 ***
3 Maximum diastolic potential (mV)	−64.84 ±0.99		−62.35 ±0.97	+2.49 (0.21)	−60.49 ±1.27	+4.35 (0.06)	−61.43 ±1.12	+3.41 (0.11)	−64.82 ±1.35	−3.39 (0.18)
4 Rate of slow diastolic depolarization (mV/s)	60.36 ±2.32		60.41 ±4.47	0.08	53.15 ±2.98	−7.22 (0.17)	42.39 ±2.80	−17.97 ***	48.17 ±1.88	+5.78 (0.22)
5 Take-off potential (mV)	−44.75 ±0.95		−42.03 ±0.99	+2.71 (0.16)	−43.65 ±1.27	+1.10	−42.09 ±1.23	+2.66 (0.23)	−53.14 ±0.75	−11.05 ***
6 Maximum rate of depolarization (V/s)	8.14 ±0.35		6.49 ±0.36	−1.65 *	5.02 ±0.34	−3.12 ***	5.79 ±0.40	−2.35 ***	9.01 ±0.33	+3.22 ***
7 Mean rate of repolarization (V/s)	0.60 ±0.01		0.48 ±0.08	−0.12	0.32 ±0.01	−0.28 ***	0.26 ±0.01	−0.34 ***	0.56 ±0.01	+0.30 ***
8 Total duration of repolarization (ms)	108.10 ±1.23		165.80 ±3.43	+57.70 ***	204.00 ±5.19	+95.91 ***	263.90 ±3.86	+155.80 ***	118.84 ±2.01	−145.00 ***

Mean results of 6 experiments are shown ± s.e.mean. n (in parentheses) = number of observations.
Each row presents the measurements depicted in Figure 1a and described in the text. In this, and subsequent tables, the statistical significance of differences is presented thus: $*P<0.05$; $**P<0.01$; $***P<0.001$; when $P>0.05$ but approaches significance, the actual value is given in parentheses.

wash-out, which would be consistent with a delay in the activation of outward repolarizing current.

Finally, the maximum rate of depolarization (MRD) was significantly decreased, but totally restored on wash-out (row 6).

Atrial myocardium

Pairs of rabbit atria were set up for recording contractions and myocardial intracellular potentials at a lower temperature (32°C) than was used for the study of the sinus node. The mean spontaneous frequency was 131.3 ± 5.1 beats/min, and Melperone 2.67, 5.34 and 10.68 µM produced significant and dose-related mean decreases in heart rate of 5.5, 15.5 and 28.5 beats/min respectively. On wash-out the heart-rate returned to within 11% of the initial control.

Melperone did not alter the stimulus threshold for pacing significantly at any concentration. There was, however, a significant and dose-related reduction in the maximum frequency at which the atria would follow a pacing stimulus (of twice threshold strength) from 507 ± 16, to 418, 368 and 334 beats/min at the three concentrations of Melperone studied.

Melperone had no negative inotropic effect on the atrial myocardium. On the contrary, a concentration of 5.34 µM produced a significant *increase* in contractions. From the mean control value of 265 ± 22 mg, the three concentrations caused respective increases of 19.7% (NS), 42.4% ($P=0.001$) and 13.6% (NS). During the recovery period contractions were still 11% (NS) above control. There was no significant change in the time from the start to the peak of contractions.

The most striking effects of Melperone were electrophysiological and are presented in Table 2. There was a small dose-related increase in resting potential (row 1), not quite significant ($P=0.06$) in Melperone 5.34 µM but significant ($P=0.01$) in 10.68 µM. This effect is the opposite of that on the maximum diastolic potential in the SAN, and may be secondary to a reduction in Na permeability (see below) in atrial myocardium (in the SAN most Na channels are inactivated already at the maximum diastolic potential). The action potential amplitude (row 2) and maximum rate of depolarization, MRD (row 3) were increased at the lower concentrations of Melperone but were both depressed by the higher concentration, especially MRD. Taken with the dose-related depression of conduction velocity (row 4), and of the maximum follow frequency already noted, the implication is that Melperone restricts fast inward (sodium) current, the effect becoming highly significant at the highest concentrations studied.

Even at the lowest concentration of Melperone, 2.67 µM, there was a large and highly significant prolongation of APD_{90}, of 28.5 ms. Thus, from the
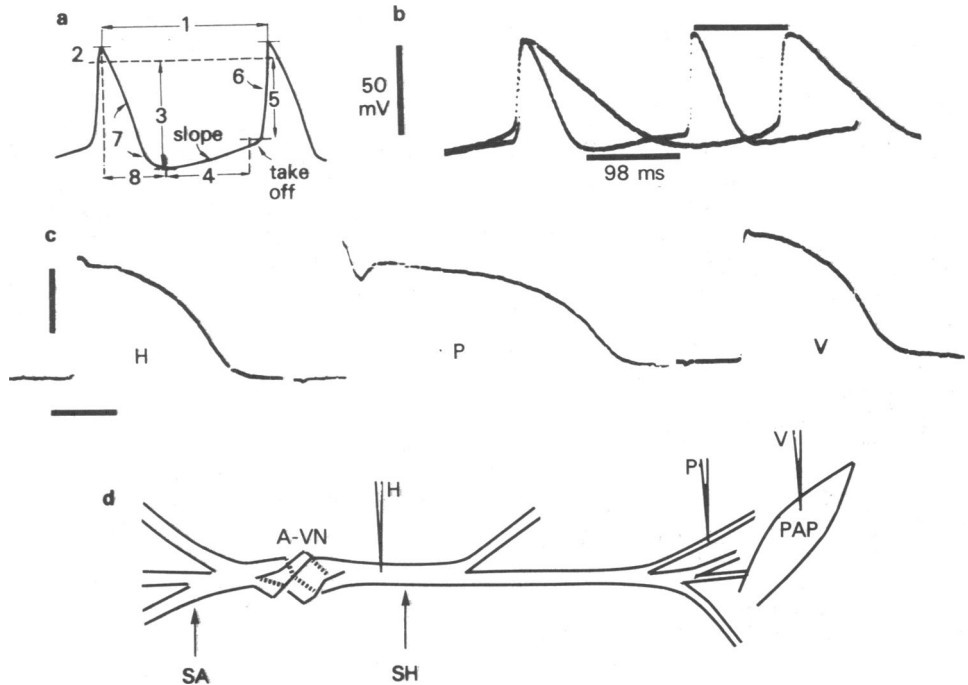
Figure 1   (a) Diagram of sinus node action potentials, to illustrate the measurements (made by computer) presented in correspondingly numbered rows in Table 1. (b) Actual sinus node cell intracellular records, a control record being superimposed, and coinciding at the peaks of the first action potential, on one taken from the same node in the presence of Melperone 34 μM. A bar equal to the prolongation of cycle-length is placed above the second peaks, and a bar of equal length is placed below the troughs of the action potentials to illustrate that the bradycardia is entirely accounted for by the prolongation of action potential duration. The slopes of the slow diastolic depolarization are unchanged. (c) Representative intracellular potentials from the bundle of His (H), a terminal Purkinje cell (P) and a papillary muscle cell (V). The vertical and horizontal bars represent 50 mV and 50 ms respectively. (d) Diagram to indicate the position at which stimulating and recording electrodes were placed.

atrial records, Melperone may be considered to possess a class 3 antiarrhythmic action even at low concentrations, and a class 1 action in addition at higher concentration (Vaughan Williams, 1980).

In view of the absence of universal agreement concerning the mechanism by which repolarizing currents are activated in different cardiac tissues, it would be inappropriate to speculate which ionic channels might be affected by melperone, in relation to the prolongation of APD and the failure of the highest dose to prolong it further in the atrium.

Local anaesthetic activity

Most class 1 antiarrhythmic drugs have, incidentally, local anaesthetic activity on nerve at higher concentrations than occur clinically. In view of the effect of Melperone on MRD and overshoot potential observed in the atrium, it was of interest to measure the local anaesthetic potency of the drug. The result of an

assay on frog nerve deomonstrated that the log dose-response curve for Melperone was steeper than that for procaine but the mean effect of the 0.5 μM concentration of both drugs was identical at exactly 50% depression of the action potential amplitude. Melperone may thus be considered equal to procaine in potency as a local anaesthetic, but its duration of action was more prolonged.

Ventricular conduction pathway

The positions of stimulating electrodes and of intracellular microelectrodes for the study of the A-V node and ventricular conduction pathway, are depicted diagrammatically in Figure 1d. Melperone did not alter the stimulation threshold at either site, atrial or His bundle.

Conduction times   For all calculations of conduction times, the ventricular preparation was stimulated at

Table 2 Electrophysiological effects of Melperone on atrial myocardium

(n)	Control 0.0 (27) Mean	2.67 µM (24) Mean	(24) Diff. from control (P value)	5.34 µM (24) Mean	(24) Diff. from control (P value)	10.68 µM Mean	(28) Diff. from control (P value)
1 Resting potential (mV)	−65.60 ±1.49	−67.43 ±1.82	−1.86	−70.43 ±2.07	−4.76 (0.06)	−70.95 ±1.44	−5.38 **
2 Action potential (mV)	82.30 ±1.59	86.21 ±1.57	+3.90 −(0.08)	84.28 ±2.17	+1.97	78.99 ±1.54	−3.30 −(0.13)
3 Maximum rate of depolarization (V/s)	82.00 ±6.05	92.13 ±6.65	+10.13	75.01 ±6.00	−7.00	50.42 ±4.47	−31.58 ***
4 Conduction velocity (m/s)	0.80 ±0.045	0.66 ±0.044	−0.136 *	0.57 ±0.031	−0.23 ***	0.45 ±0.027	−0.35 ***
5 From peak to 20% repolarization APD_{20} (ms)	21.00 ±1.54	23.37 ±1.13	+2.35 (0.09)	22.67 ±0.92	+1.65	19.93 ±0.89	−1.09
6 APD_{50} (ms)	41.82 ±1.54	50.69 ±1.72	+8.88 ***	48.54 ±1.30	+6.73 ***	43.82 ±1.48	+2.01
7 APD_{90} (ms)	74.48 ±2.19	102.90 ±2.06	+28.46 ***	108.00 ±2.61	+33.48 ***	105.77 ±1.75	+31.29 ***

The results of 5 experiments are presented ± s.e.mean.

the atrial remnant, the electrode, SA, being placed close to the A-V node. The mean results are presented in Table 3. A-H represents the time from the stimulus to the His bundle potential; H-P from the His potential to the terminal Purkinje potential; P-V from the Purkinje potential to the ventricular potential (papillary muscle). Typical records from the three regions are illustrated in Figure 1c. The lowest concentration of Melperone did not significantly alter any of the intervals, but the higher concentrations increased both A-H and H-P times, the effect being *relatively* much greater in the latter (+ 79% for H-P interval at 10.68 µM Melperone, compared with + 26% for A-H). The small decrease in the P-V interval observed at 5.34 µM Melperone, although statistically significant, was not dose-related.

Intracellular potentials of ventricular pathway The effects of Melperone in the ventricle were broadly similar to those observed in the atrium, but there were some interesting quantitative differences. The resting potential was not significantly altered in any tissue but the maximum rate of depolarization (MRD) was reduced in the terminal Purkinje fibres even by the lowest concentration (Table 4). MRD was depressed in the His bundle and ventricle also, but the most prominent dose-related effect was observed in the bundle of His. Action potential height was significantly depressed in the His bundle from a control value of 94.2 mV to 86.7 mV after 2.6 µM Melperone, but no subsequent reductions occurred after higher doses of drug. In terminal P fibres and ventricular muscle the control heights were 105.7 mV and 99.4 mV respectively before drug, and 103.3 mV and 100.6 mV after the highest dose of Melperone.

The most striking quantitative differences in the effects of Melperone in different parts of the ventricular conducting pathway concerned the action potential duration, which was greatly prolonged in the His bundle (+ 54%) and ventricle (+ 40%) but hardly altered (+ 15.6%) in the terminal Purkinje cells (Table 5) even by the highest concentration of the drug. The prolongation of APD_{20}, APD_{50} and APD_{90} by concentrations of 5.34 µM and above was uniform and dose-related in the bundle of His and ventricle, but APD_{20} and APD_{50} were unchanged in terminal Purkinje cells.

Papillary muscle contractions

In order to pace the ventricular preparation at a constant frequency it was necessary to overdrive spontaneous pacemakers, the required stimulation frequency being from 1.5–1.8 Hz. Contractions of untreated papillary muscles began to decline after 1 h, but within this time Melperone 2.67 µM had no

Table 3 Effect of Melperone on conduction times in the ventricular pathway

Melperone (μM)	A-H interval (ms)		H-P interval (ms)		P-V interval (ms)	
	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)
0.00	49.23 ±1.34		13.22 ±0.44		15.14 ±0.98	
2.67	49.16 ±1.56	−0.075	14.57 ±0.79	+1.35	12.48 ±1.06	−2.67 (0.18)
5.34	58.25 ±1.51	+9.02 ***	18.35 ±0.51	+5.13 ***	8.29 ±0.47	−6.85 ***
10.68	61.85 ±0.75	+12.62 ***	23.63 ±1.12	+10.40 ***	12.30 ±0.61	−2.85 (0.07)

In this and subsequent tables the mean results of nine experiments are presented ± s.e.mean.

significant negative inotropic effect. There was no change in the time from the start to the peak of contractions at any concentration of Melperone during 4 h of measurements. From a control value of 129 ± 5 mg, and after exposure for 1 h to 2.67 μM, followed by 1 h in 5.34 μM Melperone, the mean peak tension had declined to 103 ± 5 mg.

Refractory periods

The effective refractory period (ERP), measured by programmed premature stimuli applied to the bundle of His (electrode SH, Figure 1d) was prolonged in a dose-related manner by Melperone, the results being presented in Table 6, column 5. The maximum frequency at which the His bundle would follow a stimulus was also determined, and the shortest interstimulus intervals that could be followed are presented in column 4. For comparison, the APD_{50} of the His bundle has been printed in column 6. The similarity in the increases in all three parameters at

each drug concentration is striking and implies that in the bundle of His the prolongation of ERP by Melperone is determined almost entirely by its effect in lengthening APD (Class 3), and that the restriction of fast inward current (Class 1) makes little contribution at the concentrations studied. The prolongation of the functional refractory period (FRP) of the H-V pathway exactly paralleled the prolongation of ERP, implying that Melperone was not causing any delay in activation of ventricular muscle. This is consistent with the lack of effect on P-V conduction time (Table 3, column 3).

The fact that Melperone had little negative inotropic action and caused only a small reduction of MRD in the SAN cells, suggested that it did not restrict current through slow inward channels to any great extent. It would not, therefore, be anticipated that Melperone would depress A-V nodal conduction or increase refractoriness. A-V nodal responses cannot be measured directly, but can only be inferred from A-H conduction times, alterations in which

Table 4 Effect of Melperone on depolarization in the ventricular pathway

Melperone (μM)	n	Bundle of His Max rate of depolarization (V/s)		n	Purkinje cell Max. rate of depolarization (V/s)		n	Ventricle Max. rate of depolarization (V/s)	
		Mean	Diff. from control (Pvalue)		Mean	Diff. from control (Pvalue)		Mean	Diff. from control (Pvalue)
0.00	94	151.30 ±7.16		73	317.20 ±9.00		90	148.50 ±5.12	
2.67	44	132.20 ±7.10	−19.1 (0.11)	58	236.00 ±10.86	−81.2 ***	59	125.60 ±0.05	−22.9 *
5.34	32	125.10 ±5.79	−26.2 *	46	253.60 ±12.90	−63.6 ***	48	116.40 ±6.31	−32.1 **
10.68	40	96.90 ±5.33	−54.4 ***	43	253.20 ±16.50	−64.0 **	61	129.85 ±6.00	−18.7 (0.09)

Table 5   Effects of Melperone on action potential duration, from peak to 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) repolarization (ms)

Melperone (μM)	Bundle of His								
	APD_{20}		APD_{50}		APD_{90}			APD_{20}	
	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)		Mean	Diff. from control (Pvalue)
0.00	76.70 ±2.40		126.40 ±2.84		162.00 ±3.32			8.10 ±1.19	
2.67	65.70 ±6.28	−10.90 (0.21)	155.90 ±4.85	+29.50 ***	211.10 ±5.89	+49.10 ***		10.62 ±1.50	+2.55
5.34	99.00 ±5.94	+22.30 **	162.30 ±7.02	+35.90 ***	220.30 ±7.49	+58.30 ***		8.13 ±1.37	+0.06
10.68	118.50 ±3.32	+41.90 ***	187.70 ±4.45	+61.30 ***	249.00 ±5.43	+86.90 ***		8.77 ±1.08	+0.70

Numbers as in Table 4.

could be due in part to changes produced in the atrium rather than to effects on the node itself.

The smallest intervals between stimuli which the atria would follow at increasing concentrations of Melperone have been presented in Table 6, column 1, and the values for atrial APD_{90} are shown in column 3. APD_{90}, rather than APD_{50}, has been presented because absolute refractory period in the bundle of His ends at about APD_{50}, but is closer to APD_{90} in atrium. In contrast to the observations in the bundle of His, the prolongation of the APD of the atrium by the higher concentrations of Melperone cannot wholly account for the lengthening of the interval between stimuli, so that it may be concluded that the class 1 action of Melperone makes a substantial contribution to atrial refractoriness. The effective refractory period of the A-V node measured by programmed atrial premature stimuli producing a response in the His bundle, have been presented in column 2, and the increases in ERP produced by Melperone closely approximate the increases presented in column 1. It may be inferred, therefore, that the increases of ERP in the A-V node can be attributed wholly to atrial effects, and that Melperone has little action in the A-V node itself.

Effects of hypoxia

In Langendorff-perfused guinea-pig hearts, the APD-shortening induced by hypoxia or by reduced coronary flow was accelerated and exacerbated when successive periods of hypoxia were alternated with periods of normoxic reperfusion (Cowan & Vaughan Williams, 1977; 1980). The effect was maximal at the third hypoxic period. Langendorff preparations may be mildly hypoxic even during perfusion with fully oxygenated fluid, and it was thought advisable to employ isolated atria to study the effects of Mel-

perone in hypoxia. The protocol adopted was similar to that used previously, three hypoxic periods of 15 min (H_1, H_2, H_3) following three 25 min periods of normoxia (N_1, N_2, N_3). In view of the fact that the response to a second hypoxic period was not the same as the response to the first, it was not possible to study the effects of Melperone in the same preparations as were used for control studies, but exactly similar protocols were used for the control and Melperone experiments.

The effects of successive periods of hypoxia in drug-free solution on action potential amplitude APA (interrupted lines) and MRD (solid lines) are shown in Figure 2a. An increasing depression, especially of MRD, by successive hypoxic periods is apparent. It is also noteworthy that both measurements returned to normal values during the intervening normoxic periods. Melperone 5.34 μM itself depressed MRD significantly, but not APA (Figure 2b, dashed lines). The combination of Melperone and hypoxia not only greatly exaggerated the depression of MRD, but now reduced APA also to well below normal limits, emphasizing the increase by hypoxia of class 1 antiarrhythmic action (Vaughan Williams, 1980). The exacerbation of these depressions by successive periods of hypoxia is also apparent (dotted lines).

The shortening of action potential duration by hypoxia in drug-free solution is shown in Figure 3a. The shortening was uniform, APD_{90}, APD_{50} and APD_{20} being reduced in similar proportions; there was little exacerbation of the effect by successive periods of hypoxia. Melperone 5.34 μM increased APD (Figure 3b, dashed lines), especially APD_{90}, which was well above normal values. Melperone did not alter the shortening of APD by hypoxia, relative to the initial value at the start of the hypoxic period, but the APD_{90}, since it started longer, was still within

| Purkinje cell | | | | Ventricle | | | | | |
| APD_{50} | | APD_{90} | | APD_{20} | | APD_{50} | | APD_{90} | |
Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)	Mean	Diff. from control (Pvalue)
129.60 ±4.94		179.10 ±5.81		39.00 ±1.39		70.20 ±1.82		105.90 ±2.24	
148.50 ±9.38	+18.90 (0.20)	214.50 ±11.44	+35.40 *	41.50 ±1.87	+2.46	89.40 ±3.17	+19.25 ***	131.90 ±3.36	+26.0 ***
112.00 ±1.70	−17.60	180.20 ±6.84	+1.10	42.60 ±2.02	+3.51	96.70 ±4.08	+26.50 ***	141.10 ±4.15	+35.2 ***
130.30 ±5.33	+0.77	207.10 ±8.06	+28.10 *	44.80 ±1.62	+5.80	100.60 ±2.50	+30.40 ***	147.90 ±2.70	+35.2 ***

normal limits in the presence of Melperone during the first hypoxic period. It is noteworthy that in both the control and Melperone experiments, APD recovered completely during the intervening nor-moxic periods.

The effects of hypoxia in drug-free solution in depressing contractions (% control, interrupted lines) and in shortening the time from start to peak
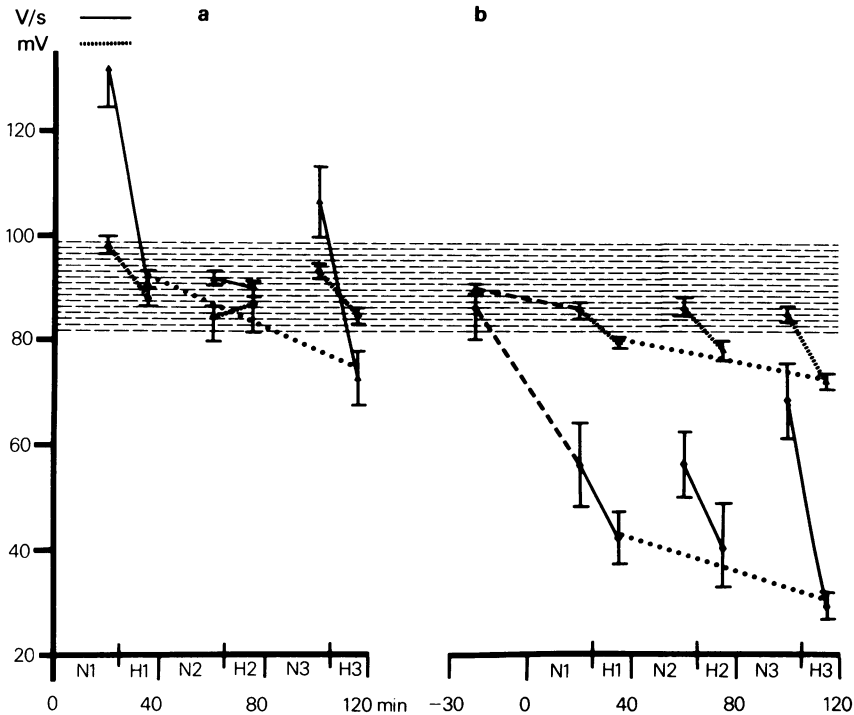

Figure 2 Effects of hypoxia on atrial depolarization. Maximum rate of depolarization (continuous lines), and action potential amplitude (interrupted lines) in normoxia and hypoxia in drug-free solution (a) and in the presence of Melperone 5.34 μM (b). Dashed lines; effect of Melperone in normoxia. The dotted lines illustrate the exacerbated depressions observed during successive periods of hypoxia. The observations at −30 min in (b) were made just before the exposure to Melperone. Ordinate scale: V/s (for MRD) or mV (for APA). Abscissa scale: time in min. The width of the dashed area is one standard deviation (½ s.d. in each direction) from the mean of the predrug APA.

Table 6 Effect of Melperone on refractoriness in the ventricular conduction pathway

Melperone (μM)	Atrio-Hissian refractoriness								His-Ventricular refractoriness			
	Maximum follow freq. atrial stimuli 1		*Programmed pacing atrial stimuli* 2		*Atrial potentials* 3		*Maximum follow freq. for His stimuli* 4		*Programmed pacing His bundle stimuli* 5		*His bundle potentials* 6	
	Smallest interval between stimuli for atrial follow	Δ	*S₁–S₂ interval of premature stimulus for His bundle follow*	Δ	APD_{90}	Δ	*Smallest interval between stimuli*	Δ	*S₁–S₂ interval of premature stimulus for ventricular follow*	Δ	APD_{50}	Δ
0.00	118.50 ±3.89		123.9		75		125.2		102.1		126.4	
2.67	144.00 ±1.50	+25.5	156.2	+32.3	103	+28	132.6	+7.4	122.4	+20.3	155.9	+29.4
5.34	163.50 ±5.17	+45.0	163.6	+39.4	108	+33	148.8	+23.6	125.4	+23.3	162.3	+35.9
10.68	181.50 ±10.50	+63.0	179.9	+56.0	106	+31	184.2	+59.0	172.1	+70.0	187.7	+61.3

For clarity, s.e.means have been omitted except in column 1; Δ = difference from control; all measurements in ms.

(solid lines) are shown in Figure 4a. The depressions were not exacerbated in successive hypoxic periods, and recovery was complete in the intervening normoxic periods. Melperone 5.34 μM had no negative inotropic effect, but afforded no protection against the effects of hypoxia. An exacerbation by successive periods of hypoxia became apparent in the presence of Melperone but recovery during the normoxic periods was still complete.

Discussion

Several currently available antiarrythmic drugs were originally introduced for other purposes; amiodarone, verapamil and perhexiline as antianginal remedies, mexiletine as an anti-epileptic. Melperone, a butyrophenone, was first presented as a neuroleptic drug (Christensen *et al.*, 1965) but a decade later was found to prolong action potential duration (APD) in papillary muscle (Olsson & Arlock, 1976). We have confirmed that Melperone prolongs APD, not only in ventricular muscle but in atrial muscle also, and in the ventricular conduction pathway. In the bundle of His, APD is normally about 20 ms shorter than in terminal Purkinje fibres in the rabbit and ventricular potentials are briefer still, by more than 70 ms. Lignocaine shortens APD throughout the ventricular conducting system but the effect is much greater in the terminal Purkinje cells, in which APD is normally longest, than in ventricular muscle or the bundle of His (Wittig, Harrison & Wallace, 1973). The action of Melperone was opposite to that of lignocaine. APD was lengthened throughout, but the effect was greater in the bundle of His (+54%) and ventricle (+40%) than in Purkinje fibres (+15.6%).

Shortening of APD by hypoxia or ischaemia may be one arrhythmogenic factor contributing to the high incidence of arrythmias observed in ischaemia, especially after myocardial infarction. A drug which could prevent hypoxic APD-shortening might, therefore, be a useful antiarrhythmic agent in such circumstances. The effects of Melperone in hypoxia were studied, and although APD_{90} was prolonged initially, the drug did not prevent the hypoxic shortening. The relative reduction in APD measured from the initial value at the start of each hypoxic period, was the same in the presence and absence of the drug. In absolute terms, however, because APD_{90} was prolonged initially by Melperone, the hypoxically-shortened APD_{90} in the presence of Melperone was still very close to the normal pre-drug duration.

Melperone reduced the maximum rate of depolarization in a dose-related manner in all cardiac tissues depolarized by fast inward current. For example, 5.34 μM Melperone depressed MRD by 9% in at-

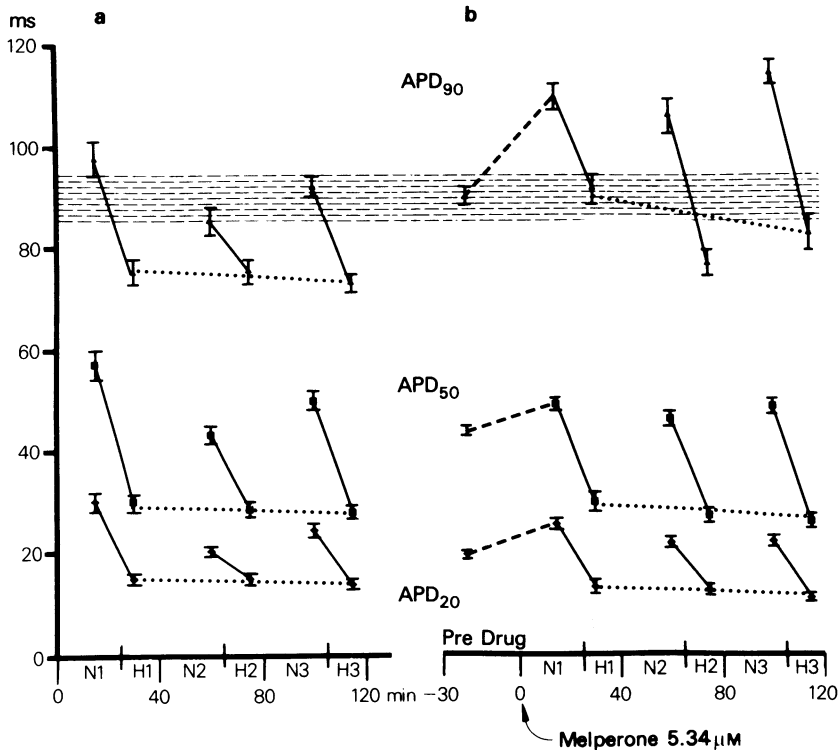**Figure 3** Effects of hypoxia on action potential duration (APD). APD was measured from the peak to 20% (APD_{20}), 50% (APD_{50}) and 90% (APD_{90}) of full repolarization. Protocol as for Figure 2. The bars give one standard error of the mean in each direction. The width of the striped area is one standard deviation ($\frac{1}{2}$ s.d. in each direction), from the mean of the pre-drug APD_{90}.

rium, 17% in the bundle of His, 20% in terminal Purkinje cells and 22% in ventricle. The atrial action potential amplitude was slightly reduced by the higher concentration of Melperone in normoxic solution, and significantly reduced in hypoxia. There were dose-related decreases in the maximum frequency at which a stimulus could be followed and on desheathed frog nerve Melperone had the same local anaesthetic potency as procaine. It was concluded that Melperone restricted fast inward current, i.e. had a class 1 antiarrythmic action in addition to its class 3 effect.

In the dog heart *in situ*, an increase in both effective and functional refractory periods of the right atrium and ventricle was observed in conjunction with a decrease in A-V node conduction time and refractoriness (Refsum, Amilie, Platou, Owren & Landmark, 1981). Combination of both adrenergic and cholinergic blockade abolished the effect on A-V node refractoriness whereas the decrease in conduction time persisted implying the latter to be a direct effect of Melperone on the A-V node (Platou, Refsum,

Amilie & Landmark, 1981). In the present study, Melperone had no negative inotropic action at concentrations up to 10 μM, and studies of A-H conduction time and of refractory periods by programmed stimulation led to the conclusion that it did not depress conduction in the A-V node. It was inferred that Melperone did not restrict current through slow inward channels.

Perhaps the most interesting effect of Melperone was the slowing of heart rate *in vitro*, because the bradycardia was achieved by a mechanism so far unique. Although Melperone was found to block α-receptors in the vasculature of the pithed rat, it exerted no competitive β-adrenoceptor blockade, nor was it a cholinoceptor agonist. Intracellular records from sinus node cells showed that at low concentrations of Melperone, the slow diastolic depolarization was not retarded, and even high concentrations had a comparatively minor effect on the slope of the slow depolarization. There was no significant change in maximum diastolic potential or 'take off' potential at any concentration. The bradycardia was
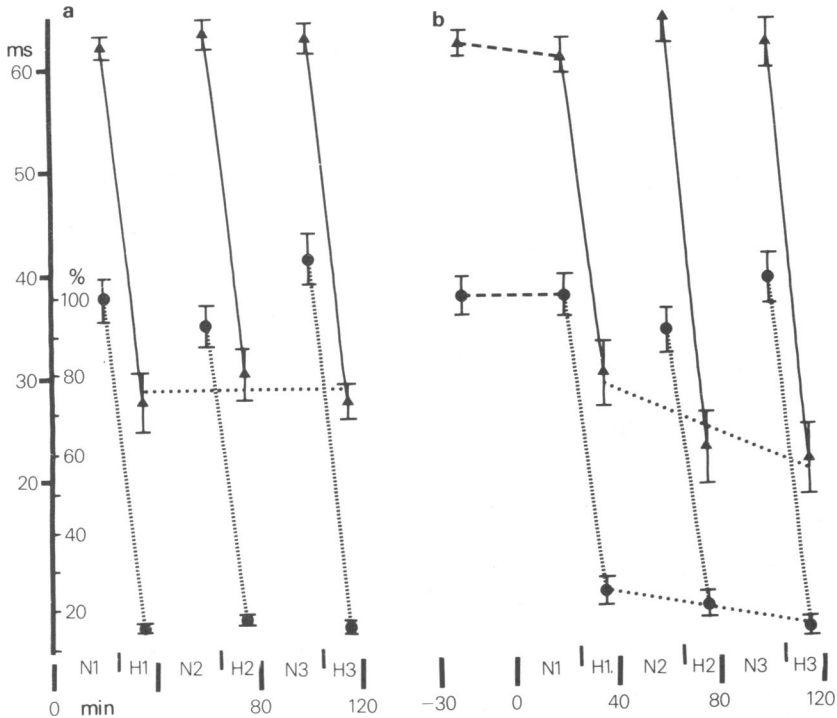
Figure 4   effects of hypoxia on contractions. Interrupted lines: contractions as % of control (right hand ordinate scale). Continuous lines: time from start to peak of contractions (left hand scale, ms). Protocol as for Figure 2.

almost entirely attributable to the prolongation of the SAN action potential. For example, in the sinus node preparation at 37°C, Melperone 2.67 µM increased the cycle length by a mean of 66 ms and prolonged APD in nodal cells by 58 ms. This effect is in complete contrast to that of another bradycardic drug, alinidine, which, like Melperone, is not an adrenoceptor antagonist, a cholinoceptor agonist nor does it restrict slow inward current. A concentration of alinidine of 1.0 µM which increased cycle length to a comparable extent (58 ms), had only a minor effect on sinus node APD (+ 18 ms), but greatly reduced the slope of the slow diastolic depolarization (− 34%) (Millar & Vaughan Williams, 1981). Possibilities that might be entertained as explanations for these very different means of producing an equal bradycardia are that alinidine restricts current through anion-selective channels, and that Melperone restricts, or delays the activation of, outward

potassium repolarizing current. The small additional effect of Melperone on the slow diastolic depolarization could be attributed to a reduction of background sodium current, in view of the demonstrated class 1 action of the drug.

In conclusion, the electrophysiological effects of Melperone are such as would lead to an expectation that it could be effective as an antiarrhythmic agent. A study of the antiarrhythmic action of Melperone in supraventricular tachycardias of 'nodal' origin would be especially interesting, in the light of the selectivity of the drug in prolonging APD in the bundle of His. The augmented class 1 action of Melperone in hypoxia and the maintenance of the hypoxic APD_{90} near normal levels, suggest that the effect of the drug in post-infarction arrhythmias could also merit examination.

References

AMBROZI, L., BIRKMAYER, W. & DANIELCZYK, W. (1969). Die objektievbarkeit psychopharmakologischer drogen am beispel von methylperone. *Int. Pharmacopsychiat.*, **2**, 48–58.

ARLOCK, P., GULLBERG, B. & OLSSON, S.-O.R. (1978). Cardiac electrophysiology of four neuroleptics: melperone, haloperidol, thioridazine and chlorpromazine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **304**, 27–36.

CHRISTENSEN, J.A., HERNESTAM, S., LASSEN, J.B. & STERNER, N. (1965). Pharmacological and toxicological studies on γ-(4-methyl piperidino)-p-fluorobutyrophenone (FG 5111) – a new neuroleptic agent. *Acta pharmac. tox.*, **23**, 109–132.

COWAN, J.C. & VAUGHAN WILLIAMS, E.M. (1977). The effects of palmitate on intracellular potentials recorded from Langendorff-perfused guinea-pig hearts in normoxia and hypoxia, and during perfusion at reduced rate of flow. *J. molec. cell. Cardiol.*, **9**, 327–342.

COWAN, J.C. & VAUGHAN WILLIAMS, E.M. (1980). The effects of various fatty acids on action potential shortening during sequential periods of ischaemia and reperfusion. *J. molec. cell. Cardiol.*, **12**, 347–369.

DOHADWALLA, A.N., FREEDBERG, A.S. & VAUGHAN WILLIAMS, E.M. (1969). The relevance of β receptor blockade to ouabain-induced cardiac arrhythmias. *Br. J. Pharmac.*, **36**, 257–267.

MILLAR, J.S. & VAUGHAN WILLIAMS, E.M. (1981). Pacemaker selectivity. Influence on rabbit atria of ionic environment and of alinidine, a possible anion antagonist. *Cardiovasc. Res.*, **15**, 335–350.

MOLANDER, L. & DUVHÖK, C. (1976). Acute effects of oxazepam, diazepam and methylperone, alone and in combination with alcohol on sedation, co-ordination and mood. *Acta pharmac. tox.*, **38**, 145–160.

OLSSON, S.-O.R. (1976). Antiarrhythmic effect of a neuroleptic compound, melperone. Electrophysiological study. *7th Eur. Congr. Cardiol., Abt. II*, 376.

OLSSON, S.B., BRORSON, L. & VARNAUSKAS, E. (1973). Class 3 antiarrhythmic action in man. Observations from monophasic action potential recordings and amiodarone treatment. *Br. Heart J.*, **35**, 125–129.

PETERSEN, E.N. (1978). Experimental anti-arrhythmic properties of melperone, a neuroleptic butyrophenone. *Acta pharmac. tox.*, **42**, 388–394.

PLATOU, E.S., REFSUM, H., AMLIE, J.P. & LANDMARK, K. (1981). Influence of β- adrenergic and cholinergic blockade on the electrophysiological effects of melperone in the dog heart in situ. *Cardiovasc. Res.*, **15**, 137–143.

REFSUM, H., AMLIE, J.P., PLATOU, E.S., OWREN, T. & LANDMARK, L. (1981). Electrophysiological effects of melperone in the dog heart in situ – a new antiarrhythmic drug. *Cardiovasc. Res.*, **15**, 131–136.

REFSUM, H., PASSWAL, M. & OLSSON, S.-O.R. (1978). Comparison of the electrophysiological effects of two neuroleptics, melperone and thioridazine, on isolated rat atria. *Eur. J. Pharmac.*, **49**, 285–293.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1970). The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. *Br. J. Pharmac.*, **39**, 657–668.

SZEKERES, L. & VAUGHAN WILLIAMS, E.M. (1962). Antifibrillatory action. *J. Physiol.*, **160**, 470–482.

VAUGHAN WILLIAMS, E.M. (1977). Automated measurement of potentials. *J. Physiol.*, **272**, 1P.

VAUGHAN WILLIAMS, E.M. (1980). *Antiarrhythmic Action*. London: Academic Press.

VAUGHAN WILLIAMS, E.M., RAINE, A.E.G., CABRERA, A.A. & WHYTE, J.M. (1975). The effects of prolonged β-adrenoceptor blockade on heart weight and cardiac intracellular potentials in rabbits. *Cardiovasc. Res.*, **9**, 579–592.

WITTIG, J.H., HARRISON, L.A. & WALLACE, A.G. (1973). Electrophysiological effects of lidocaine on distal Purkinje fibers of canine heart. *Am. Heart J.*, **86**, 69–78.