Br. J. Pharmac. (1970), **38**, 749–757.

Local anaesthetic and anti-arrhythmic actions of alprenolol relative to its effect on intracellular potentials and other properties of isolated cardiac muscle

B. N. SINGH* AND E. M. VAUGHAN WILLIAMS

Department of Pharmacology, Oxford University

Summary

1. Alprenolol, a β-adrenoceptor blocking drug reported to have the same potency as propranolol *in vitro* and *in vivo*, was found to be four times more active than procaine as a local anaesthetic on frog sciatic nerve.

2. At doses of 0·125 mg/kg and above alprenolol protected anaesthetized guinea-pigs against ouabain-induced ventricular fibrillation.

3. In isolated rabbit atria, resting potentials were unchanged, and the duration of the action potential was not prolonged, by concentrations of alprenolol up to $10{\cdot}5 \times 10^{-6}$M. The maximum rate of depolarization (MRD), however, was reduced by 30% at a concentration of $0{\cdot}525 \times 10^{-6}$M. This was 214 times less than the concentration which reduced the frog action potential height by 25%.

4. The concentration of alprenolol required to produce more than 15% decrease in contraction or maximum driven frequency was $3{\cdot}5 \times 10^{-6}$M.

5. As a test of the direct action of alprenolol on isolated cardiac muscle, MRD was also a more sensitive test than the measurement of electrical threshold or conduction velocity.

Introduction

Most drugs with cardiac anti-arrhythmic properties (including quinidine, pronethalol, propranolol, oxprenolol and lignocaine) also have a local anaesthetic action on nerve, though there are differences in the relative specificity of the respective effects. When a new anti-arrhythmic drug is introduced, therefore, it is of interest to determine the extent to which its action may be attributed to non-specific or quinidine-like properties, or to other effects, including β-adrenoceptor blockade.

Excitable membranes behave as non-linear semiconductor devices, and have the property of changing their resistance in response to alterations in applied voltage. The changes are not always instantaneous, and the time required after a step change in voltage for the resistance to alter depends, *inter alia*, on the species of ion which is carrying the current. In nerve, depolarization initiates within a fraction of a millisecond an increase in sodium conductance which is inactivated almost

* Nuffield Dominions (New Zealand) demonstrator, Oxford University.

immediately. Repolarization occurs by the activation in 1–2 ms of an outward potassium current, but in cardiac muscle repolarization occurs at least a hundred times more slowly. Although views have changed in the past few years concerning the modifications which must be made to the Hodgkin-Huxley equations for nerve in order to describe the repolarization process in cardiac muscle (Noble, 1962; McAllister & Noble, 1967; Noble & Tsien, 1969) there seems little doubt that such equations can describe depolarization with only minor alterations (Noble, 1966). Depolarizing current in nerve is carried almost exclusively by sodium ions, whereas in smooth muscle a substantial inward current is carried by calcium ions (Brading, Bülbring & Tomita, 1969). Attempts to fractionate inward depolarizing current in cardiac muscle (Reuter, 1967; Besseau & Gargouïl, 1969) indicate that inward calcium current is slowly activated and so would contribute little to the initial depolarization, but there is doubt whether residual currents remaining in the presence of procedures adopted to limit sodium current (low external Na, tetrodotoxin) are the same as they would normally be in their absence.

Tetrodotoxin acts specifically on nerve and blocks conduction at a concentration of 0·01 mg/l., but concentrations a hundred times greater have no effect on the rate of rise or duration of the cardiac action potential (Dudel, Peper, Rudel & Trautwein, 1967). The concentration has to be raised to 10–30 mg/l. before the rate of depolarization of Purkinje fibres is reduced by tetrodotoxin. In contrast, propranolol has a converse specificity for cardiac muscle, depressing the rate of rise of the action potential by 30% at a concentration 260 times less than that required to cause a comparable reduction in the height of the action potential in frog nerve (Dohadwalla, Freedberg & Vaughan Williams, 1969).

The present experiments were undertaken to compare the concentration of alprenolol required to depress depolarization in frog nerve and isolated cardiac muscle, and also to determine what concentration would produce changes in contractions, spontaneous and maximum driven frequencies, conduction velocity and electrical threshold in isolated rabbit atria. Finally, the efficacy of alprenolol *in vivo* was tested in protecting anaesthetized guinea-pigs against ouabain-induced cardiac arrhythmias in relation to that of propranolol.

Methods

Local anaesthesia

Action potentials of a frog sciatic nerve stimulated at one end were recorded at the other, as previously described (Papp & Vaughan Williams, 1969). The central portion was bathed in frog Ringer, mM: NaCl, 120; KCl, 1·88; $CaCl_2$, 1·08; $NaHCO_3$, 2·38; with or without drug, and buffered at pH 7·5 by Tris (Sigma), 10 ml/litre. The height of the fastest wave of action potentials was measured before and after 30 min exposure to each concentration of the drug used.

Protection against ouabain-induced arrhythmias

The method was similar to that described by Vaughan Williams & Sekiya (1963) and subsequently automated (Dohadwalla *et al.*, 1969). Guinea-pigs of either sex were given 1·6 g/kg urethane intraperitoneally, and were artificially respired, body temperatures being maintained at 37° C by an intrarectal thermistor operating a heated plate. The electrocardiogram (lead II) was recorded for 5 s every 2 min. Ouabain, 3·6 μg, was infused during 30 s from a motor-driven syringe every 2 min.

Intracellular potentials, conduction velocity, contractions

Intracellular potentials were recorded (Vaughan Williams, 1958; Szekeres & Vaughan Williams, 1962) from the inner surface of isolated rabbit atria suspended horizontally in a bath through which nutrient fluid was recirculated rapidly at 32° C by an oxygenator.

Mean values were calculated from all records except those rejected according to the criteria of Vaughan Williams (1959): if the resting potential was not the same at the beginning and end of the recording run, or if there were gross irregularities in the repolarization record, implying movement artefact. No records were rejected on the grounds of low values of any parameter.

Contractions were recorded with an RCA 5734 transducer, and conduction velocity was calculated from the interval between a stimulus (1 ms square-wave, strength at least twice threshold) to the left atrium and an action potential recorded from the external surface of the right atrium. The solution contained (mM): NaCl, 125; KCl, 5·6; $CaCl_2$, 2·16; $NaHCO_3$, 25; glucose, 11; and was gassed with 95% oxygen and 5% carbon dioxide to pH 7·4.

Drugs used

Alprenolol (Astra H 56/28), 1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol. Propranolol HCl (I.C.I.). Procaine HCl (B.D.H.). Strophanthin G (ouabain) (B.D.H.). Urethane (Hopkin and Williams). Weights are expressed in terms of the salt. Statistical significance was calculated by Student's *t* test, and by χ^2 test (for incidence of fibrillation).

Results

Local anaesthesia

Alprenolol was found to be a powerful local anaesthetic on frog nerve, with four times the potency of procaine on a molar basis (Table 1).

Protection against ouabain-induced arrhythmias

In the anaesthetized guinea-pig the first sign of arrhythmia induced by an intermittent infusion of ouabain is an irregularity of sinus rhythm, associated with lengthening of the P-R interval on the e.c.g., and the mean dose of ouabain required to produce this effect is given in column 1 of Table 2. The succeeding columns indicate the mean doses of ouabain required to induce ventricular extrasystoles; a purely ventricular rhythm (when ventricular complexes were no longer preceded by P waves); ventricular fibrillation; and cardiac arrest for 20 s or longer. The figures in brackets give the incidence of a particular effect when this was less than 100%, and the figure above indicates the mean dose of ouabain required to produce the effect in those animals in which it occurred.

TABLE 1. *Local anaesthetic effect of alprenolol on desheathed frog sciatic nerve*

% reduction of action potential	Alprenolol concentration (mM)	Procaine concentration (mM)	Potency ratio $\frac{\text{Alprenolol}}{\text{Procaine}}$
25 (n=10)	0·112±0·009	0·399±0·034	3·7
50 (n=10)	0·133±0·008	0·564±0·028	4·3
75 (n=10)	0·181±0·034	0·824±0·026	4·5

The results of pretreatment with various doses of alprenolol 5 min before the start of the ouabain infusion are presented in Table 2, together with the effect of pretreatment with 1 mg/kg propranolol (racemic). A comparison of these results with those presented in previous investigations (Dohadwalla *et al.*, 1969; Papp & Vaughan Williams, 1969) indicates that alprenolol is approximately three times more effective than propranolol in protecting anaesthetized guinea-pigs against ventricular fibrillation induced by ouabain.

Effects on conduction velocity, spontaneous and maximum driven frequency, contractions and electrical threshold of isolated rabbit atria

Although procaine exerts a maximal effect in about 20 min, previous experience has indicated that quinidine, propranolol and some other cardio-active drugs continue to exert an increasing effect for at least an hour *in vitro*. In the present experiments, therefore, measurements of the functions enumerated above were made after 10, 30, 60 and 120 min respectively, and the results have been presented in Table 3. It is clear that the effect of a given concentration of alprenolol was not maximal in several instances even at the end of an hour, and that if measurements had been made after an exposure to the drug of half an hour or less, some effects would have been missed. The weak positive inotropic and chronotropic actions of alprenolol already reported (Åblad, 1967) were apparent at low concentrations, and it was not until after exposure for 1 h to a concentration of $3{\cdot}5 \times 10^{-6}$M that a depression of any importance (more than 15%) of contractions or maximum driven frequency was observed. A comparable increase in electrical threshold and

TABLE 2. *Effects of alprenolol on ouabain-induced cardiac arrhythmias in the guinea-pig*

	Amount of ouabain (μg/kg intravenously) required to produce				
	Unequal intervals	Extra-systoles	Ventricular rhythm	Ventricular flutter-fibrillation	Cardiac arrest
Control $n=30$	88·5±4·8	204·7±10·6	222·6±11·8	240·7±11·7 (29/30)	311·2±12·7
0·125 mg/kg alprenolol $n=10$	107·2±29·1	184·7±15·8	200·8±12·4	233·9±9·3 (8/10*)	304·5±14·7
0·25 mg/kg alprenolol $n=10$	87·1±4·1	203·3±17·3	226·9±12·6	235·3±12·1 (4/10***)	303·9±21·9
0·5 mg/kg alprenolol $n=10$	107·6±14·4	204·5±9·8	227·3±5·8	298·0±28·4 (2/10***)	286·2±21·4
1·0 mg/kg $n=10$	104·7±10·0	241·6±46·4	260·0±16·6	None	300·5±26·0
1·0 mg/kg propranolol $n=10$	94·9±8·6	186·0±15·6	211·4±17·7 (4/10***)	228·1±25·9 (2/10***)	300·0±19·6
3 mg/kg alprenolol $n=10$	101·9±14·3	272·5±20·5 (2/10***)	None	None	297·2±17·6
6 mg/kg alprenolol $n=5$	101·8±9·8	253·4 (1/10***)	None	None	356·6±16·2*

Molar ratio $\frac{\text{Alprenolol HCl}}{\text{Propranolol HCl}} = 0{\cdot}96$.

Statistical significance of difference from control: * $P<0{\cdot}05$; ** $P<0{\cdot}01$; *** $P<0{\cdot}001$.

slowing of conduction velocity occurred at about one-third this concentration, but spontaneous frequency was less susceptible to depression. Taken as a whole, the table indicates that a concentration of alprenolol less than three micromolar does not have any significant "non-specific" depressant effect on isolated atria as measured by these tests. This concentration is 200 times greater than that required to double the ED50 of isoprenaline in augmenting the contractions of isolated rabbit papillary muscle (Åblad, Brogård & Ek, 1967).

Cardiac intracellular potentials

Alprenolol had no effect on the resting potential of isolated rabbit atria, even at the highest concentration used. It was found in the previous section that a threshold concentration of 1·0 mg/l. ($3{\cdot}5 \times 10^{-6}$M) was required to produce any depression greater than 15% of the functions measured. The effect of this concentration of alprenolol on intracellular potentials is shown in Fig. 1. The overshoot potential was significantly reduced by a concentration of 10^{-6}M and above. Repolarization was not delayed by any concentration, and at concentrations of 3·5 and $10{\cdot}5 \times 10^{-6}$M it was accelerated by a few milliseconds. The most striking effect of alprenolol on intracellular potentials, as with other anti-arrhythmic drugs, was to reduce the rate of rise of the action potential. Even a concentration of $0{\cdot}525 \times 10^{-6}$M caused a highly significant ($P<0{\cdot}001$) fall in the maximum rate of rise from 91 to 64 V/s. Measurement of the maximum rate of rise was thus a more sensitive test of the direct action of alprenolol on atrial muscle than any of

TABLE 3. *Effects of various concentrations of alprenolol on contraction height, maximum driven frequency, spontaneous rate, electrical threshold, and conduction velocity of rabbit atria in relation to duration of exposure to the drug*

	Concentration mg/l. (Molar conc.)	10 min	30 min	60 min	120 min
1. Contraction	0·15 ($0{\cdot}525 \times 10^{-6}$M)	+11·2±1·2(3)	+ 6·0±2·1(2)	+ 4·4±1·6(3)	+ 4·2±1·3(2)
	0·3 ($1{\cdot}05 \times 10^{-6}$M)	+ 2·4±0·6(3)	+ 2·2±0·8(2)	− 3·1±1·2(3)	− 3·6(1)
	1·0 ($3{\cdot}5 \times 10^{-6}$M)	+ 2·8±0·9(3)	− 7·6±0·8(3)	−16·7±3·9(3)	−22·2±2·2(3)
	3·0 ($10{\cdot}5 \times 10^{-6}$M)	−10·1±2·6(2)	−29·6±3·4(2)	−42·6±2·7(3)	—
2. MDF	0·15	—	—	− 7·7±2·4(3)	—
	0·3	—	− 2·6±1·4(2)	− 8·9±2·5(3)	−12·5±1·7(2)
	1·0	− 2·6±0·6(3)	− 5·0±0·8(3)	−17·4±1·2(4)	−28·2±1·2(3)
	3·0		−14·3±0·5(3)	−28·0±2·0	—
3. Spontaneous rate	0·15	+ 9·8±1·2(3)	+ 8·5±1·4(3)	+ 6·7±1·4(3)	+ 4·4±0·9(3)
	0·3	+ 2·4±1·5(3)	+ 2·1±1·5(3)	+ 3·8±0·8(3)	—
	1·0	+ 2·1±1·0(3)	− 4·7±1·2(3)	− 8·9±1·2(3)	−13·0±1·6(3)
	3·0	− 5·0±0·7(3)	−12·8±0·5(3)	−23·2±1·1	—
4. Threshold	0·15	—	—	+ 8·2±2·6(3)	—
	0·3	—	+12·1(1)	+19·6±2·2(3)	—
	1·0	+10·9±0·7(3)	+29·7±5·0(3)	+47·9±8·4(3)	+63·2±6·9(3)
	3·0	+ 9·7±0·8(3)	+35·7±1·9(3)	+57·3±1·6(3)	—
5. Conduction velocity	0·15	—	− 7·0(1)	−10·6±0·5(3)	—
	0·3	—	−11·6±5·0(2)	−21·1±2·0(3)	−22·6±1·0(2)
	1·0	− 4·7±0·8(3)	−22·6±1·6(3)	−39·1±2·2(3)	−49·9±2·4(3)
	3·0	− 4·6±2·6(2)	−22·5±1·5(2)	−58·7±2·5(3)	— —

The figures indicate mean difference from control as percentages±S.E., with the number of experiments in brackets.

the measurements described in the previous section. Measurements have been presented in Table 4 of the various parameters of intracellularly recorded potentials, before (control, C) and after (effect, E) 60–120 min exposure to alprenolol, together with a second set of control measurements (recovery, R) made after washout of the drug for at least an hour.

Discussion

Alprenolol is a powerful local anaesthetic on frog nerve, four times more potent than procaine, and thus marginally (18%) more active than (±)-propranolol (Dohadwalla *et al.*, 1969). The mean concentration required to reduce the action potential of frog sciatic nerve by 25% was $0{\cdot}112 \times 10^{-3}$M. Alprenolol, like propranolol, had a much greater specificity for cardiac muscle than for nerve, for a concentration of $0{\cdot}525 \times 10^{-6}$M (that is, 214 times less) reduced the maximum rate of rise of the action potential by 30%. In these "non-specific" *in vitro* actions on

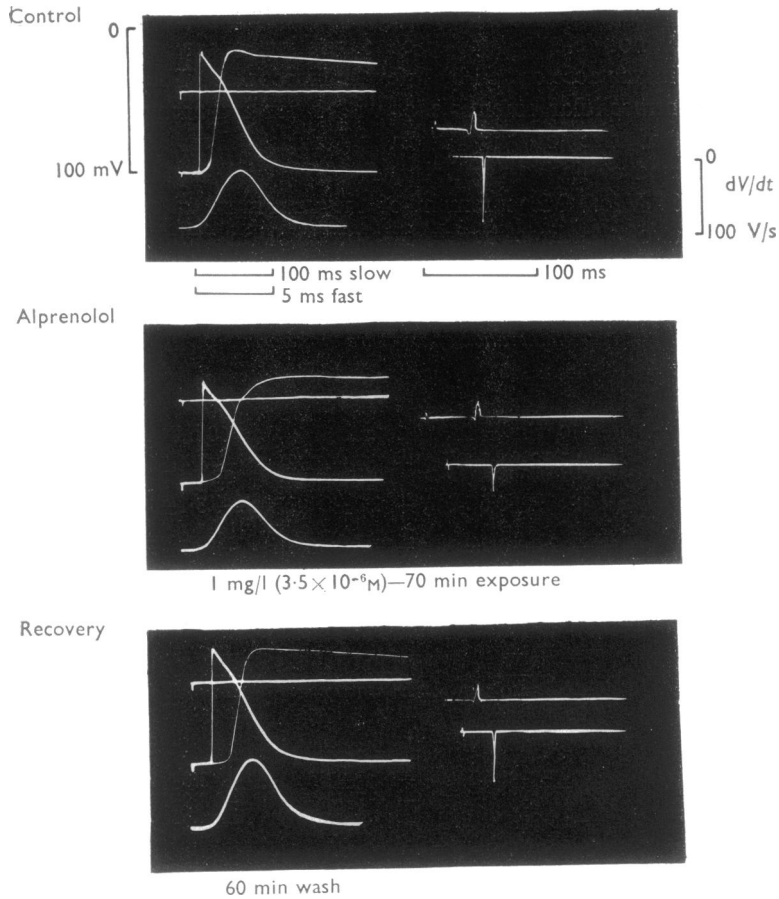

FIG. 1. Effect of alprenolol on atrial intracellular potentials. Left: In each frame the horizontal line indicates zero potential, and the superimposed traces show intracellular action potentials at slow and fast sweep speeds. The bottom trace records contractions. Right: The upper trace indicates the interval between a stimulus to the left atrium and an action potential recorded from the surface of the right atrium. The lower trace shows the rate of rise of the intracellular record. Alprenolol at a concentration of $3{\cdot}5 \times 10^{-6}$M reduced contractions by only 16%, but depressed the rate of rise of the action potential by 59%. The overshoot potential and conduction velocity were also reduced, but the resting potential and the duration of the action potential were not affected.

TABLE 4. *Effects of alprenolol on cardiac intracellular potentials*

Concentration mg/ml (Molar conc.)		Total No. of fibres	Resting potential (mV)	Action potential (mV)	Max. rate of rise (V/s)	Mean rate of rise (V/s)	50% repolarization time (ms)	90% repolarization time (ms)
0·075	C	22	61·2±0·4	89·8±0·7	88·3±2·8	50·7±1·5	49·5±1·0	104·8±1·4
(0·263×10^{-6}M)	E	17	62·4±0·6	89·7±0·9	86·6±3·1	51·4±1·9	50·3±1·1	105·2±1·5
2 h	E	21	63·0±0·5	89·6±0·7	86·8±2·7	50·8±1·7	49·8±1·1	105·6±1·5
0·15	C	29	64·6±0·6	90·3±0·8	91·1±2·8	59·1±1·8	55·1±0·6	110·7±0·8
(0·525×10^{-6}M)	E	28	63·6±0·6	89·7±0·9	64·0±1·3**	45·9±1·2**	56·1±0·6	110·7±0·8
	R	16	64·1±0·8	90·7±1·2	87·1±3·8	58·6±2·7	56·3±1·1	110·9±1·5
0·3	C	24	63·1±0·5	89·7±1·0	85·6±2·2	50·6±1·5	48·3±1·1	107·3±1·5
(1·05×10^{-6}M)	E	33	63·2±0·4	81·3±0·7**	59·9±1·2**	40·8±0·6**	47·4±0·8	105·0±1·2
	R	17	63·4±0·7	89·1±1·3	79·9±2·2	49·7±1·8	48·9±1·3	106·9±1·8
1·0	C	30	63·9±0·4	89·3±0·9	80·0±1·4	50·5±1·1	53·1±0·6	101·8±0·9
(3·5×10^{-6}M)	E	33	63·9±0·3	83·5±0·8*	47·6±1·4**	30·0±0·8**	47·3±0·3*	90·4±1·4*
	R	8	63·4±0·6	90·3±1·1	74·7±1·7	49·9±1·4	47·1±1·1	86·6±3·2
3·0	C	28	62·5±0·4	88·2±0·4	104·7±1·5	63·9±1·7	48·4±1·3	101·5±2·0
(10·5×10^{-6}M)	E	29	62·3±0·3	75·2±0·3**	26·3±0·6**	17·2±0·4**	43·5±1·1**	92·2±1·9*
	R	11	62·9±0·6	83·5±0·7	66·5±3·5	38·0±3·4	45·0±1·4	98·9±3·2

Statistical significance of difference from control: * $P<0·01$; ** $P<0·001$.

Figures are means ± S.E. of all measurements from two experiments at 0·263×10^{-6}M, and of three experiments at other concentrations (total fourteen rabbits).
C, Control. E, 1–2 h after exposure to alprenolol. R, 1 h after washout of drug.

nerve and cardiac muscle, therefore, alprenolol and propranolol were approximately equipotent. *In vivo,* however, in protecting guinea-pigs against ouabain-induced arrhythmias alprenolol had about three times the activity of propranolol. This calls to mind the even greater discrepancy between the *in vitro* and *in vivo* activities of propranolol and practolol (ICI 50172), the ratios of potencies in similar tests being 30 : 1 *in vitro,* and less than 3 : 1 *in vivo* (Papp & Vaughan Williams, 1969). On the other hand, propranolol and alprenolol were found to be equipotent as β-adrenoceptor blockers both *in vitro* and *in vivo* in animals (Åblad *et al.*, 1967) and *in vivo* in man (Johnsson, 1967 ; Forsberg & Johnsson, 1967 ; Johnsson, Norrby & Sölvell, 1967).

Alprenolol, even in high concentrations which caused large depressions of contractions, overshoot potentials and other properties of cardiac muscle, had no effect on intracellularly recorded resting potentials. Drugs sometimes produce effects which may be statistically significant, but which are so small as to be of doubtful physiological importance. For this reason it was arbitrarily decided to regard as above " threshold " only those concentrations of alprenolol which produced, after one hour's exposure, at least a 15% change in the functions measured. By this criterion the threshold concentration of alprenolol required to depress contractions and maximum driven frequency was $3{\cdot}5 \times 10^{-6}$M. The threshold concentration for increasing electrical threshold and slowing conduction velocity was one-third of this. At concentrations below $1{\cdot}0 \times 10^{-6}$M alprenolol had a weak positive inotropic and chronotropic action, as previously reported (Åblad, 1967).

In contrast, the maximum rate of rise of the action potential was reduced at lower concentrations, $0{\cdot}525 \times 10^{-6}$M alprenolol causing a 30% fall. This finding is in agreement with previous observations with propranolol and practolol, that measurement of the maximum rate of depolarization (MRD) was the most sensitive of the various tests employed to detect a direct depression of cardiac function (Dohadwalla *et al.*, 1969). It must be emphasized, however, that measurement of MRD is not a test of β-adrenoceptor blockade, since it did not distinguish between the effects of (+)- and (−)-propranolol. The lowest concentration of alprenolol which caused a significant fall in MRD was 30 times greater than that reported to double the ED50 of isoprenaline in augmenting contractions of isolated rabbit papillary muscle (Åblad *et al.*, 1967).

The question arises whether alprenolol administered clinically in amounts designed to produce β-adrenoceptor blockade would ever give rise to blood concentrations approaching those required *in vitro* to reduce MRD. A dose of 5 mg of alprenolol injected intravenously to human subjects was found partially to block the chronotropic effects of isoprenaline (0·09 μg/kg min^{-1} intravenously) during a period of 2 h (Johnsson *et al.*, 1967). Assuming an immediate even distribution in the plasma, an initial concentration of well over 1 mg/l. of alprenolol would be achieved, falling to about 0·4 mg/l. as the drug became distributed into the extracellular space. These concentrations are well above those observed to reduce MRD *in vitro.* The decline of concentration subsequently would, of course, depend on the rate of uptake by cells, and excretion in the urine.*

* *Note added in proof.* R. Johansson (personal communication) has found that the serum concentrations after oral administration in humans vary widely with the individual. One hour after ingestion, following overnight starvation, of 200 mg alprenolol, serum levels ranged from 0·32 to 0·022 mg/l. (mean 0·184). One hour after intravenous injection of 0·1 mg/kg alprenolol the mean serum concentration was 0·028 mg/l. (range 0·041 to 0·02).

Another point to be considered is that although a concentration of 0·15 mg/l. *in vitro* was required to reduce MRD by 30%, this does not mean that lower concentrations would necessarily be without effects on excitability. If alprenolol is interfering with the relation between membrane voltage and the time of return of excitability, the generation of abnormal impulses could well be prevented by concentrations lower than those required to achieve a reduction of MRD at a normal frequency of impulse formation.

REFERENCES

ÅBLAD, B. (1967). Pharmacological studies on H 56/28. *Acta pharmac. tox.*, **25**, suppl. 2, 5–8.

ÅBLAD, B., BROGÅRD, M. & EK, L. (1967). Pharmacological properties of H 56/28—a β-adrenergic receptor antagonist. *Acta pharmac. tox.*, **25**, suppl. 2, 9–40.

BESSEAU, A. & GARGOUÏL, Y. M. (1969). Ionic currents in rat ventricular heart fibres: voltage-clamp experiments using double sucrose-gap technique. *J. Physiol., Lond.*, **204**, 95–96*P*.

BRADING, A., BÜLBRING, E. & TOMITA, T. (1969). The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **200**, 637–654.

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.

DUDEL, J., PEPER, K., RUDEL, R. & TRAUTWEIN, W. (1967). Effect of tetrodotoxin on membrane currents in mammalian cardiac fibres. *Nature., Lond.*, **213**, 296.

FORSBERG, S. A. & JOHNSSON, G. (1967). Hemodynamic effects of propranolol and H 56/28 in man —a comparative study of two β-adrenergic receptor antagonists. *Acta pharmac. tox.*, **25**, suppl. 2, 75–83.

JOHNSSON, G. (1967). The effects of intra-arterially administered propranolol and H 56/28 on blood flow in the forearm—a comparative study of two β-adrenergic receptor antagonists. *Acta pharmac. tox.*, **25**, suppl. 2, 63–74.

JOHNSSON, G., NORRBY, A. & SÖLVELL, L. (1967). Potency and time-effect relationship in man of propranolol and H 56/28. *Acta pharmac. tox.*, **25**, suppl. 2, 95–105.

MCALLISTER, R. E. & NOBLE, D. (1967). The effect of subthreshold potentials on the membrane current in cardiac Purkinje fibres. *J. Physiol., Lond.*, **190**, 381–387.

NOBLE, D. (1966). Applications of Hodgkin-Huxley equations to excitable tissues. *Physiol. Rev.*, **46**, 1–50.

NOBLE, D. & TSIEN, R. W. (1969). Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol., Lond.*, **200**, 205–232.

NOBLE, D. (1962). A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pace-maker potentials. *J. Physiol., Lond.*, **160**, 317–352.

PAPP, J. Gy. & VAUGHAN WILLIAMS, E. M. (1969). A comparison of the effects of I.C.I. 50172 and L-propranolol on intracellular potentials and other features of cardiac function. *Br. J. Pharmac.*, **37**, 391–399.

REUTER, H. (1967). The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. *J. Physiol., Lond.*, **192**, 479–492.

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

VAUGHAN WILLIAMS, E. M. (1958). The mode of action of quinidine on isolated rabbit atria interpreted from intracellular records. *Br. J. Pharmac. Chemother.*, **13**, 276–287.

VAUGHAN WILLIAMS, E. M. (1959). The effect of changes in extracellular potassium concentration on the intracellular potentials of isolated rabbit atria. *J. Physiol., Lond.*, **146**, 411–427.

VAUGHAN WILLIAMS, E. M. & SEKIYA, A. (1963). Prevention of arrhythmias due to cardiac glycosides by block of β-sympathetic receptors. *Lancet*, **1**, 420–421.

(*Received January* 15, 1970)