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# Effects on cardiac muscle of the β-adrenoceptor blocking drugs INPEA and LB46 in relation to their local anaesthetic action on nerve

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## Summary

1. As a local anaesthetic on frog nerve, LB46 had 0·4 times the activity of procaine and 2 times that of INPEA.

2. In reducing the maximum rate of depolarization (MRD) of intracellularly recorded cardiac action potentials, LB46 was 20 times more potent than INPEA.

3. As an antagonist of the chronotropic effect on atrial muscle and of the inotropic effects on ventricular muscle of isoprenaline the $pA_2$ values for LB46 were $9{\cdot}05 \pm 0{\cdot}15$ and $9{\cdot}30 \pm 0{\cdot}06$ respectively, and for INPEA $6{\cdot}00 \pm 0{\cdot}16$ and $6{\cdot}10 \pm 0{\cdot}12$.

4. LB46 was 8 times more active than racemic propranolol in blocking the effects of isoprenaline on isolated cardiac muscle, and since its direct action on the cardiac membrane was only 0·1 times that of propranolol, there was a net gain of 80 times in the specificity of LB46 for class 2 (antisympathetic) over class 1 (depression of MRD) antidysrhythmic actions.

5. INPEA was 2·5 times less active as a β-adrenoceptor blocking drug on cardiac muscle than (+)-propranolol, and 1,300 times less active than LB46.

6. INPEA, but not LB46, prolonged the duration of both atrial and ventricular intracellular action potentials.

## Introduction

The β-adrenoceptor blocking drug pronethalol is a local anaesthetic which is about twice as effective as procaine (Gill & Vaughan Williams, 1964). Propranolol, which has an -O-$CH_2$-link between the naphthalene ring and the isopropylaminoethanol side chain, was 10 times more active than pronethalol in blocking β-adrenoceptors, but was only slightly more potent as a local anaesthetic (Morales-Aguilerá & Vaughan Williams, 1965) so that a considerable gain was achieved in specificity for antisympathetic (class 2, Vaughan Williams, 1970) over 'non-specific' (class 1) antidysrhythmic actions. The presence of electron-withdrawing groups in the aromatic ring reduces the potency of amide and ester local anaesthetics (Ritchie & Greengard, 1965). Several β-adrenoceptor blocking drugs with such groups have been introduced and although the local anaesthetic activity has been reduced in each case this has not necessarily been associated with a gain in specificity. Practolol, for example, which has the electrophilic acetamido group in the ring, had 0·012 times the activity of propranolol as a local anaesthetic, and

its class 1 action on isolated cardiac muscle was similarly reduced. Since, however, its β-adrenoceptor blocking activity *in vitro* was lost to approximately the same extent, there was little net gain in specificity (Papp & Vaughan Williams, 1969).

To improve specificity, an attempt must be made selectively either to reduce class 1 activity or to increase β-adrenoceptor blockade. The drugs studied here provide an example from each group. INPEA, which has the same side chain as isoprenaline and pronethalol, has less local anaesthetic activity by virtue of the electron-withdrawing $-NO_2$ in the ring. LB46, although it has the same side chain as propranolol, is more potent for β-adrenoceptor blockade (Hill & Turner, 1969). It was thought of interest, therefore, to compare the relative effects of these two drugs in their class 1 actions on cardiac intracellular potentials, in their local anaesthetic activity on nerve, and in their effects on other features of the function of isolated cardiac muscle, including responses to isoprenaline.

## Methods

### *Protection against ouabain-induced arrhythmias*

The method used was that described by Vaughan Williams & Sekiya (1963) as modified by Dohadwalla, Freedberg & Vaughan Williams (1969). Guinea-pigs of either sex were anaesthetized with 1·6 g/kg of urethane intraperitoneally and were respired artificially. Body temperature was maintained at 37° C by an intrarectal thermistor probe controlling a heated plate under the animal. The electrocardiogram was recorded for 5 s every 2 min and ouabain (3·6 μg) was infused over 30 s from a motor-driven syringe every 2 minutes.

### *Local anaesthesia of nerve*

Frog sciatic nerves were stripped of their sheaths under magnification and were placed in a three compartment chamber at room temperature. They were stimulated in moist air at one end, and action potentials were recorded from the other. The segment of nerve in the central compartment was bathed in frog Ringer containing various concentrations of procaine or other drugs. The solution contained (mM): NaCl, 120; KCl, 1·88; $CaCl_2$, 1·08; $NaHCO_3$, 2·38; Tris (Sigma) buffer, at pH 7·5, 10 ml/litre. The height of the fastest wave of the action potential was measured before and after exposure for 30 min to each concentration of the drug used.

### *Intracellular potentials*

The method was as previously described (Vaughan Williams 1958; Szekeres & Vaughan Williams, 1962). Single fibres were penetrated from the internal surface of isolated rabbit atria suspended horizontally in a bath through which modified Locke solution was recirculated at 32° C by an external oxygenator. Mean values of all parameters were measured according to defined criteria (Vaughan Williams, 1959). Contractions were recorded with an RCA 5734 transducer, and conduction velocity was calculated from the interval between a stimulus (1 ms, strength at least twice threshold) to an electrode on the left atrium and an action potential recorded from the surface of the right atrium with a bipolar-electrode.

TABLE 1. *Effects of INPEA and LB46 on ouabain-induced cardiac arrhythmias in anaesthetized guinea-pigs*

| | | | Amounts of ouabain (μg/kg i.v.) required to produce | | | | |
|---|---|---|---|---|---|---|---|
| | | *n* | Unequal R–R intervals | Ventricular ectopics | Persistent ventricular tachycardia | Ventricular flutter-fibrillation | Cardiac arrest |
| Control | (Mol. $10^{-6}$/kg) | 30 | 88·5± 4·8 | 204·7±10·6 | 226·0±11·8 | 240·7±11·7 (29/30) | 311·2±12·7 |
| INPEA: 1·5 mg/kg | 6 | 6 | 103·8± 7·2 | 180·1±20·1 | 230·0±19·0 | 253·9±17·3 (6/6) | 299·3±17·1 |
| 3 mg/kg | 12 | 10 | 102·7± 8·6 | 212·9±10·8 | 239·4± 8·9 | 288·1± 9·6 (9/10) | 315·0±11·2 |
| 6 mg/kg | 24 | 10 | 100·4±10·4 | 201·3±14·2 | 242·2± 9·5 | 260·4±11·4 (6/10)† | 350·0±13·0 |
| 12 mg/kg | 48 | 10 | 92·7±14·0 | 249·9± 9·3* | 272·2±10·4* | 308·3 (1/10)† | 349·1±20·5 |
| LB46: 0·0625 mg/kg | 0·25 | 10 | 98·6± 7·8 | 193·5± 9·0 | 223·7± 7·6 | 259·4± 6·1 | 322·3±10·2 |
| 0·125 mg/kg | 0·5 | 10 | 110·5±15·3 | 195·6±10·5 | 247·3±26·6 | 300·6±10·5* (4/10)† | 327·5±11·4 |
| 0·25 mg/kg | 1·01 | 10 | 104·6±13·7 | 216·3±12·8 | 263·5±12·0* | 326·4±13·6* (3/10)† | 352·9±10·1 |
| 0·5 mg/kg | 2·02 | 10 | 97·4± 6·9 | 235·6±12·2 | 278.0±17·9* | None | 355·0±15·1* |
| 1·0 mg/kg | 4·03 | 10 | 113·3± 7·0 | 266·2±20·8* | 277·1±22·2* | None | 354·1±23·0* |

Significant difference from control: * $P<0·01$; † $P<0·001$. The incidence of ventricular fibrillation is given when this was less than 100%, and the mean dose is calculated from the smaller number.

Ventricular intracellular potentials were recorded from strips 1·5–2·0 cm in length, 2–3 mm wide, cut from the free wall of the right ventricle. They were stimulated by platinum plates (0·5 × 2·0 cm) fixed parallel to, but not touching, the tissue, one on each side, at 60/min (duration 2 ms). Atria were stimulated at a frequency about 10% faster than the spontaneous frequency. The solution contained (mM): NaCl, 125; KCl, 5·6; $CaCl_2$, 2·16; $NaHCO_3$, 25; glucose, 11·0; and was gassed with 95% $O_2$, 5% $CO_2$, to give pH 7·4.

The statistical significance of differences was calculated by Student's $t$ test, and the $\chi^2$ test was used for evaluating protection against ventricular fibrillation.

### *Drugs used*

INPEA, 1-*p*-nitrophenyl-2-isopropylamino ethanol hydrochloride, (Selvi); LB46, 4-(2-hydroxy-3-isopropylamino propoxy)-indole base (Sandoz); (−)-propranolol (I.C.I.); (+)-propranolol (I.C.I.); procaine HCl (B.D.H.); atropine sulphate (B.D.H.); strophanthin-G (ouabain; B.D.H.).

## Results

### *Ouabain-induced dysrhythmias*

In anaesthetized guinea-pigs the first sign of dysrhythmia caused by an infusion of ouabain is an irregularity of sinus rhythm associated with a prolongation of the P-R interval on the electrocardiogram. As the infusion proceeds ventricular extrasystoles occur at increasing frequency, until a purely ventricular pacing is estab-

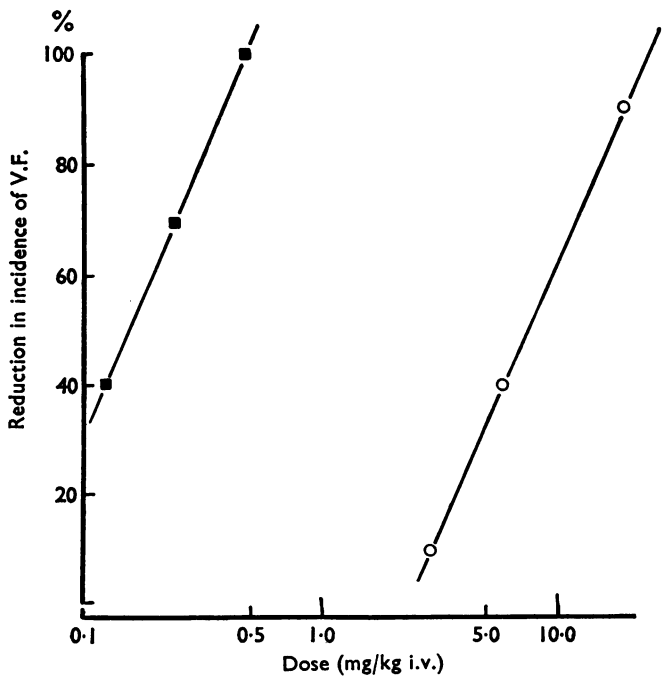


FIG. 1. Protection against ouabain-induced ventricular fibrillation. Ordinate: percentage reduction of incidence of ventricular fibrillation from control incidence. Abscissa: dose, log scale. (■——■), LB46; (○——○), INPEA.

lished, leading to ventricular flutter-fibrillation and cardiac arrest. The mean doses of ouabain required to produce each of these effects in thirty controls, and in animals pretreated with various doses of INPEA and LB46, are presented in Table 1. INPEA had no effect on ouabain toxicity below a dose of 24 $\mu$mol/kg, which reduced the incidence of ventricular fibrillation significantly by 38%, but did not increase the lethal dose of ouabain. Dose-response curves for percentage reduction in ouabain-induced ventricular fibrillation by INPEA and LB46 have been plotted in Fig. 1, and are straight and parallel. They indicate that LB46 was 50 times more effective than INPEA (and 5 times more active than (−)-propranolol from the data of Papp & Vaughan Williams, 1969).

### *β-Adrenoceptor blockade*

Dose-response curves for the effect of isoprenaline on the spontaneous heart rate were obtained in isolated atria before and after exposure to various concentrations of INPEA, LB46, (−)-propranolol and (+)-propranolol. The $pA_2$ values were calculated and are presented in Table 2. The positive inotropic actions of isoprenaline were measured on strips of rabbit right ventricle driven at 1 Hz. The $pA_2$ values (Table 2) of all four compounds were slightly larger for blockade of the inotropic than of the chronotropic responses to isoprenaline, but the present evidence is insufficient to permit any conclusions concerning possible differences in

TABLE 2. *Blockade of the chronotropic and inotropic responses to isoprenaline of isolated cardiac muscle*

| Drug | Chronotropic | Inotropic | Combined |
|---|---|---|---|
| INPEA | 6·00±0·16 | 6·10±0·12 | 6·05±0·09 |
| LB46 | 9·05±0·15 | 9·30±0·06 | 9·17±0·11 |
| (−)-Propranolol | 8·48±0·12 | 8·63±0·12 | 8·55±0·12 |
| (+)-Propranolol | 6·40±0·05 | 6·50±0·09 | 6·45±0·09 |

The figures are calculated $pA_2$ values, ±S.E.M. Each figure is the mean value from three experiments

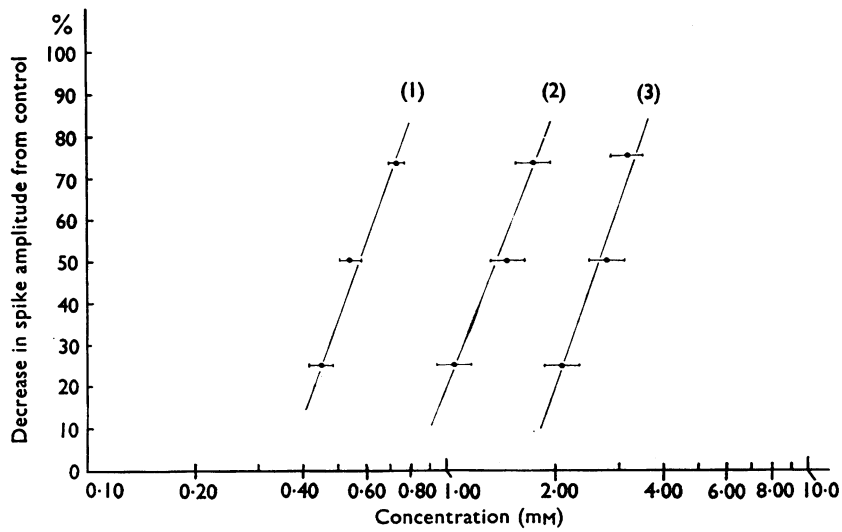


FIG. 2. Local anaesthesia of desheathed frog sciatic nerve. Ordinate: percentage decrease in amplitude of the fastest wave of the action potential. Abscissa: concentration, log scale. (1), Procaine; $n=39$. (2), LB46; $n=8$. (3), INPEA; $n=6$.

the receptors mediating the respective effects. Two features of Table 2 are noteworthy. First, LB46 had more than 1,300 times the activity of INPEA but only 4 times the activity of (−)-propranolol (that is, 8 times the activity of racemic propranolol). Second, (+)-propranolol, sometimes regarded as virtually devoid of β-adrenoceptor blocking activity, was twice as active as INPEA. The ratio of just over 100 for the relative activities of the isomers of propranolol confirms the observations of Barrett & Cullum (1968) in dogs.

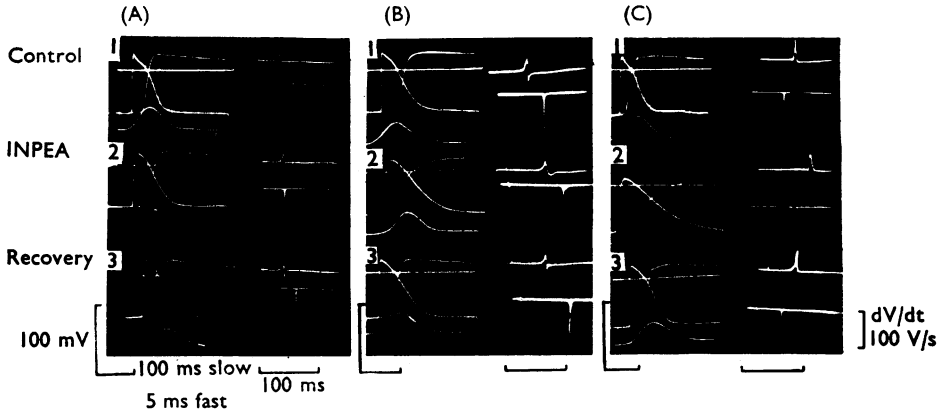


FIG. 3. The effect of INPEA on atrial intracellular potentials. In each frame the records are as follows: LEFT. Horizontal trace, zero potential, with microelectrode outside fibre; middle trace, intracellular potentials at slow and fast sweep speeds; lower trace, contraction. RIGHT. Upper trace, interval between a stimulus from an electrode on the left atrium, and an action potential recorded with a dipolar electrode from the surface of the right atrium; lower trace, differential of the intracellular record. The depth of the spike is proportional to the maximum rate of depolarization (MRD). (A), 12 mg/l. ($4{\cdot}62\times10^{-5}$M); (B), 24 mg/l. ($9{\cdot}24\times10^{-5}$M); (C), 50 mg/l. ($1{\cdot}92\times10^{-4}$M).

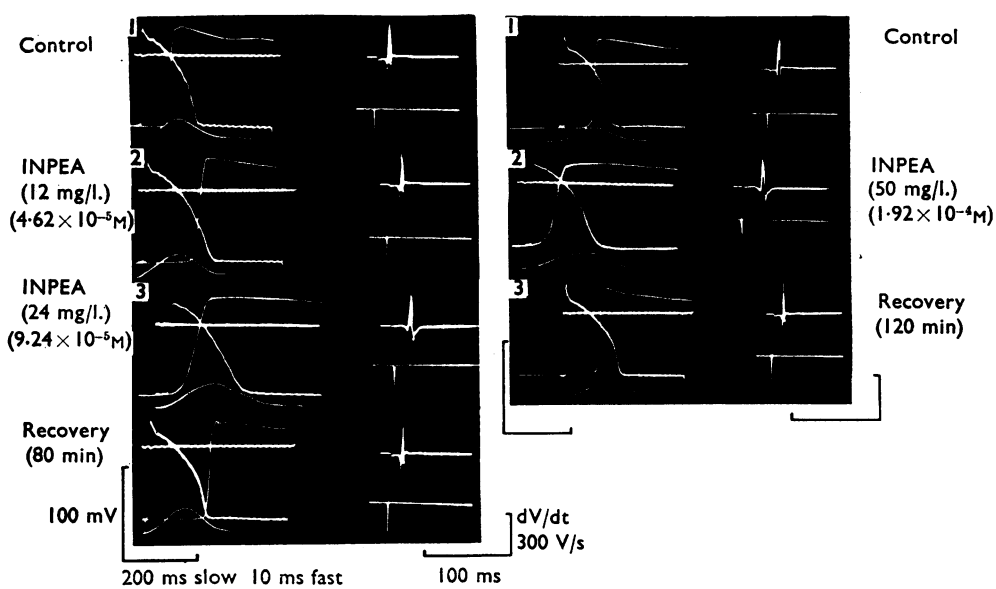


FIG. 4. Effect of INPEA on ventricular intracellular potentials. Description as for Fig. 3.

TABLE 3. *Effects of INPEA on intracellular potentials in isolated rabbit cardiac muscle*

| Concentration of INPEA | | | No. of fibres | Resting potential (mV) | Action potential (mV) | Maximum rate of rise (V/s) | Time to 90% repolarization (ms) |
|---|---|---|---|---|---|---|---|
| **Atrial potentials** | | | | | | | |
| 6 mg/l. | ($10^{-6}$ M) | C | 16 | 60·2±1·0 | 87·2±1·2 | 92·2±2·7 | 106·2±1·3 |
| | | E | 22 | 61·2±0·9 | 88·1±1·0 | 90·0±1·9 | 109·7±1·7 |
| ($n$=2) | 24 | R | 12 | 61·1±1·2 | 87·8±0·9 | 88·7±2·3 | 105·2±2·1 |
| 12 mg/l. | 48 | C | 28 | 64·2±1·2 | 90·2±1·2 | 96·9±2·3 | 102·1±2·1 |
| | | E | 31 | 66·2±1·1 | 89·7±0·9 | 99·1±1·9 | 127·1±1·9† |
| ($n$=3) | | R | 17 | 65·9±0·9 | 89·1±1·6 | 98·2±1·7 | 98·2±1·7 |
| 24 mg/l. | 95·8 | C | 27 | 64·2±0·9 | 88·7±1·6 | 98·7±2·9 | 108·5±1·2 |
| | | E | 32 | 62·7±1·3 | 79·4±1·2* | 57·3±2·1† | 152·7±1·3† |
| ($n$=3) | | R | 19 | 63·9±1·4 | 84·9±1·3 | 88·4±2·3 | 115·8±2·2 |
| 50 mg/l. | 199·5 | C | 23 | 66·2±1·4 | 90·3±1·1 | 90·7±2·4 | 101·7±1·9 |
| | | E | 21 | 64·9±0·8 | 76·4±1·5† | 37·2±1·9† | 197·2±2·3† |
| ($n$=3) | | R | 16 | 64·7±1·4 | 86·8±1·2 | 75·7±3·1 | 112·7±1·7 |
| **Ventricular potentials** | | | | | | | |
| | | C | 24 | 78·7±2·9 | 97·8±3·7 | 321·6±3·9 | 182·8±3·2 |
| 12 mg/l. ($n$=3) | | E | 29 | 81·2±3·1 | 98·7±2·9 | 325·7±5·7 | 217·3±3·6† |
| 24 mg/l. ($n$=3) | | E | 26 | 80·3±1·9 | 88·8±3·9* | 236·7±4·3† | 239·1±3·2† |
| | | R | 18 | 80·8±2·3 | 99·2±2·7 | 326·7±3·7 | 191·8±4·2 |
| | | C | 19 | 83·2±3·6 | 103·2±4·2 | 315·7±4·7 | 178·2±4·2 |
| 50 mg/l. ($n$=3) | | E | 27 | 80·1±2·7 | 87·8±5·1* | 142·7±3·7† | 251·1±4·8† |
| | | R | 16 | 80·7±2·1 | 98·2±3·9 | 302·7±4·3 | 190·2±3·7 |

$n$ indicates the number of experiments from which the results were obtained. The same preparations were used for 12 and 24 mg/l. concentrations of INPEA by the method of cumulative addition, but records were taken only after 1 h of perfusion with each of the two concentrations used. C, control; E, effect after exposure for 60 min to drug; R, after 60 min recovery from drug. Statistical significance of difference from control: *P<0·001; †$P$<0·001.

TABLE 4. *Effects of LB46 on intracellular potentials on isolated cardiac muscle*

| Concentration of LB46 | ($10^{-6}$ M) | | No. of fibres | Resting potential (mV) | Action potential (mV) | Maximum rate of rise (V/s) | Time to 90% repolarization (ms) |
|---|---|---|---|---|---|---|---|
| **Atrail potentials** | | | | | | | |
| 0·3 mg/l. | 1.21 | C | 17 | 62·7±1·0 | 88·7±1·2 | 95·7±2·9 | 106·7±1·3 |
| | | E | 20 | 64·3±0·8 | 90·2±1·4 | 89·5±4·2 | 102·7±2·7 |
| (*n*=2) | | R | 15 | 63·9±0·9 | 90·3±1·1 | 90·3±3·6 | 102·8±1·9 |
| 1·0 mg/l. | 4·03 | C | 27 | 60·8±0·9 | 87·2±1·0 | 89·5±2·7 | 102·7±1·2 |
| | | E | 29 | 62·7±1·0 | 87·7±1·7 | 61·7±2·2† | 101·9±1·6 |
| (*n*=3) | | R | 18 | 61·8±1·1 | 89·1±1·3 | 93·2±1·9 | 98·7±2·4 |
| 3·0 mg/l. | 12·1 | C | 32 | 63·7±1·0 | 91·7±1·2 | 106·2±3·1 | 98·9±1·2 |
| | | E | 27 | 64·1±0·8 | 81·2±1·4* | 58·7±2·2† | 101·6±1·8 |
| (*n*=3) | | R | 20 | 64·7±1·2 | 88·7±1·6 | 98·7±2·6 | 101·9±1·1 |
| 6·0 mg/l. | 24·2 | C | 29 | 66·2±1·3 | 87·8±1·2 | 92·7±2·2 | 107·2±1·3 |
| | | E | 27 | 64·7±1·8 | 76·7±1·8† | 40·1±3·2† | 106·3±1·6 |
| (*n*=3) | | R | 16 | 64·9±1·3 | 86·2±1·1 | 82·7±1·7 | 104·5±2·1 |
| **Ventricular potentials** | | | | | | | |
| | | C | 30 | 82·7±1·6 | 102·7±2·7 | 310·7±4·6 | 192·2±3·2 |
| 1 mg/l. | | E | 26 | 81·8±2·1 | 100·8±2·2 | 272·7±3·9† | 188·7±4·2 |
| 3 mg/l. | | E | 26 | 83·4±1·7 | 92·7±1·9* | 202·7±5·1† | 187·0±3·6 |
| (*n*=3) | | R | 16 | 82·9±2·2 | 103·8±2·3 | 308·0±3·8 | 190·6±2·7 |
| | | C | 25 | 79·7±1·7 | 99·8±2·0 | 296·0±4·1 | 185·2±2·7 |
| 6 mg/l. | | E | 21 | 81·2±1·2 | 90·1±1·6* | 166·2±5·2† | 188·6±3·7 |
| (*n*=3) | | R | 14 | 80·7±1·3 | 101·7±1·7 | 290·8±3·9 | 186·1±2·9 |

C, control; E, effect of exposure for 60 min to drug; R, 60 min after wash out of drug. Statistical significance of difference from control: *P<0·01; †P<0·001.

*Local anaesthesia of frog nerve*

Dose-response curves for INPEA, LB46 and procaine are presented in Fig. 2. INPEA, in spite of the presence of an $-NO_2$ group, had appreciable activity, 0·5 times that of LB46 and 0·2 times that of procaine.

*Intracellular potentials*

The effects of various concentrations of INPEA on atrial and ventricular potentials are shown in Figs. 3 and 4 respectively, and are summarized in Table 3. The maximum rate of depolarization was reduced, in concordance with the drug's activity as a local anaesthetic. The most striking effect of INPEA, however, was a prolongation of the action potential in both atrium and ventricle (class 3 action), similar to that of MJ1999 (Singh & Vaughan Williams, 1970b). LB46 had no class 3 action. The only effect of LB46 of interest on intracellular potentials was the customary reduction of MRD (Table 4).

*Heart rate and electrocardiogram*

The effects of 6, 12, 24 and 48 $\mu$mol/kg of INPEA on the heart rate of anaesthetized guinea-pigs, measured 5 min after intravenous injection, were reductions of $17·7 \pm 2·6$, $23·8 \pm 3·1$, $26·9 \pm 2·9$ and $37·8 \pm 3·4$ respectively. In contrast 0·25 $\mu$mol/kg of LB46 reduced heart rate by $12·2 \pm 1·9\%$, but increasing the dose 50 times caused a further fall in heart rate of no more than $25·5 \pm 2·9\%$, that is 12 $\mu$mol of each drug had approximately equal effects on heart rate, ($n=10$ for all doses of

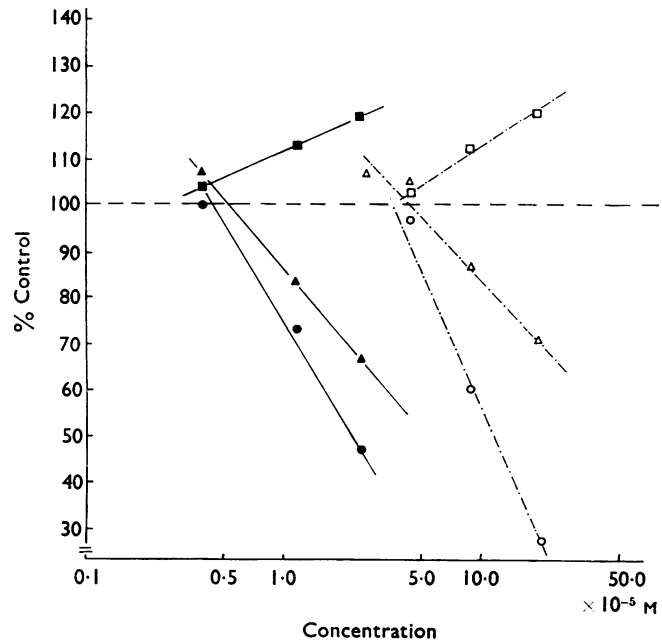


FIG. 5. Effect of INPEA and LB46 on conduction velocity, electrical threshold and contractions in isolated rabbit atria. Ordinate: measurements expressed as percentage of control. Abscissa: concentration, log scale. (■——■), (□—·—□), Electrical threshold for LB46 and INPEA respectively. (●——●), (○—·—○), Conduction velocity for LB46 and INPEA respectively. (▲——▲), (△—·—△), Contraction height for LB46 and INPEA respectively.

both drugs). Neither compound had any effect on the P-R interval of the electrocardiogram, nor on the width of the QRS complex. INPEA, but not LB46, increased the Q-T interval.

*Contractions, conduction velocity and electrical threshold in isolated rabbit atria*

Dose-response curves for percentage changes in the measurements recorded in this section in the presence of INPEA and LB46 are depicted in Fig. 5. The curves for contraction and electrical threshold are reasonably parallel, and indicate that LB46 had about 9 times the activity of INPEA. The dose-related depression of conduction velocity was somewhat steeper with INPEA than with LB46, and showed that a concentration of INPEA about 6 times greater than that of LB46 was required to reduce conduction velocity to 70% of the control. Nevertheless, the concentration of LB46 required for $\beta$-adrenoceptor blockade was much less than for depression of contractions, since a 30% reduction of isoprenaline-induced tachycardia was produced by a concentration 0·02 times that which reduced contractions by 30%.

*Spontaneous and maximum driven frequencies of isolated rabbit atria*

Although prolonged exposure to INPEA and LB46 reduced the spontaneous frequency of the beat in isolated atria, both drugs had a dose-related initial sympathomimetic agonist action. The dose-response relations were not parallel, however (Fig. 6). Both compounds caused a dose-dependent depression of the maximum frequency at which atria would follow a stimulus, but here again, the dose-response curves were not parallel.

All measurements on isolated atria were made at 10, 20, 30, 60 and 120 min after exposure to each concentration of the drugs. The effects were always stable by the end of 60 min, and the dose-response curves below were plotted from 60 min readings.

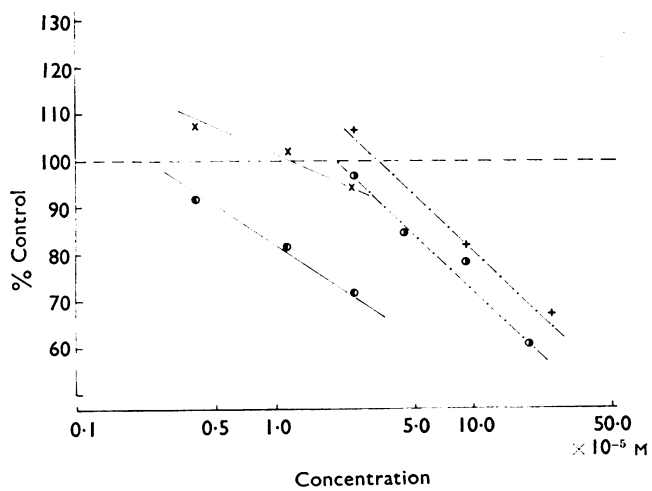


FIG. 6. Effect of INPEA and LB46 on the spontaneous beating frequency of isolated rabbit atria, and on the maximum frequency at which they would follow a stimulus. Ordinate: measurements expressed as percentage of controls. Abscissa: concentration, log scale. (◐——◑), (◑—·—◑), Maximum driven frequency for LB46 and INPEA respectively. (×——×), (+—·—+), Spontaneous atrial frequency for LB46 and INPEA respectively.

## Discussion

Quantitative comparisons of the pharmacological effects of antidysrhythmic drugs over the past few years have permitted their classification into three categories (Vaughan Williams, 1970), possibly four (Singh & Vaughan Williams, 1971). The first class of action consists of a slowing of the maximum rate of depolarization (MRD) in the absence of a change of resting potential or of any significant prolongation of the duration of the action potential (Vaughan Williams, 1958). Most compounds with a class 1 action in cardiac muscle are also local anaesthetics on nerve, but there are wide variations in the relative concentrations required for the two effects. For example, alprenolol depressed MRD by 30% in isolated atria at less than 0·005 times the concentration required to reduce the height of the frog nerve action potential by 25% (Singh & Vaughan Williams, 1970a). Conversely, tetrodotoxin depresses MRD only at a concentration several hundred times greater than that required to block conduction in nerve (Dudel, Peper, Rudel & Trautwein, 1967).

The present experiments have shown that LB46 had 0·4 times the activity of procaine as a local anaesthetic on frog nerve, but was 2 times as potent as INPEA. In reducing MRD in atrial and ventricular muscle, on the other hand, LB46 was more than 20 times as active as INPEA.

The second class of antidysrhythmic action is antisympathetic, either by competitive adrenoceptor blockade or by interference with transmitter release. Although class 2 action is obviously most effective in dysrhythmias associated with sympathetic hyperactivity, it is clear that it contributes to protection against other dysrhythmias also, including ouabain-induced ventricular fibrillation (Dohadwalla, Freedberg & Vaughan Williams, 1969). LB46 was 1,300 times more potent than INPEA in blocking the effect of isoprenaline on cardiac $\beta$-adrenoceptors, and was 50 times more effective in protecting against ouabain-induced ventricular fibrillation. The ratio of the class 1 activities of LB46 and INPEA was only 20. Thus the more active $\beta$-adrenoceptor blocking agent was also the more effective in protecting against ouabain, and although class 1 effects may be involved as well, this observation is consistent with the hypothesis that $\beta$-adrenoceptor blockade at least partly contributes to protection against digitalis toxicity (Vaughan Williams &

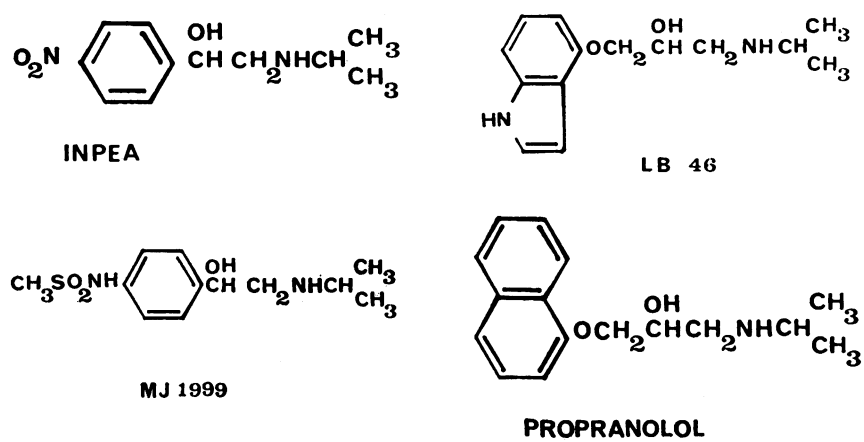


FIG. 7. Structures of some compounds discussed.

Sekiya, 1963), a view supported by much recent evidence (Barrett & Cullum, 1968 ; Raper & Wale, 1968 ; Dohadwalla *et al.*, 1969 ; Blackburn, Byrne, Cullum, Farmer & Levy, 1970), and contrary to the contention of Lucchesi (1965) and Benfey & Varma (1966) that β-adrenoceptor blockade was irrelevant to protection against digitalis-induced dysrhythmias.

The third class of antidysrhythmic action consists of a prolongation of the duration of the cardiac action potential such as is seen in hypothyroidism (Freedberg, Papp & Vaughan Williams, 1970), and in the presence of the antianginal drug amiodarone (Singh & Vaughan Williams, 1970b) and of the β-adrenoceptor blocking drug MJ1999 (Singh & Vaughan Williams, 1970c). MJ1999, like INPEA, has the same side chain as isoprenaline and also has an electron-withdrawing group (methyl sulphonamide) in the para-position of the ring (Fig. 7). It was of interest to find, therefore, that INPEA, but not LB46, had a potent class 3 action in both atrial and ventricular muscle. Measurement of the maximum frequency at which cardiac muscle will follow a stimulus has been a standard test for antidysrhythmic activity for many years (Dawes, 1946), and the dose-response curve paralleled that for class 1 action on the cardiac membrane with several compounds previously studied (Vaughan Williams & Szekeres, 1961 ; Szekeres & Vaughan Williams, 1962). The dose-response curve of INPEA for the decrease in maximum driven frequency was much steeper than that of LB46 and the fact that INPEA, but not LB46, prolonged the action potential may have been responsible for the difference.

Comparison of these experiments with previous work (Dohadwalla, *et al.*, 1969) has indicated that LB46 had about 0·1 times the activity of propranolol as a local anaesthetic on nerve, and the ratio of their class 1 actions in depressing MRD in cardiac muscle was similar. LB46, however, was 8 times more active than racemic propranolol in blocking the action of isoprenaline on isolated muscle, which represents a gain in specificity for class 2 over class 1 actions of about 80 times. LB46 is thus by far the most specific blocking agent for cardiac β-adrenoceptors yet studied. In contrast the β-adrenoceptor blocking action of INPEA was very weak. (+)-Propranolol, which has only 0·01 times the β-adrenoceptor blocking potency of the laevo isomer, is commonly regarded as being virtually devoid of activity, yet it was 2·5 times as potent as INPEA and it seems doubtful, therefore, whether the latter can seriously be classified as a '*β*-adrenoceptor blocking drug' *in vitro*. On the other hand INPEA, like previously studied compounds with electrophilic substituents in the ring (practolol ; Papp & Vaughan Williams, 1969 ; sotalol ; Singh & Vaughan Williams, 1970c) appears to be relatively more active *in vivo* in comparison with lipophilic drugs than would be expected on the basis of its *in vitro* activity. The reason for this has not yet been fully elucidated, but may be related to the attachment of the lipophilic drugs to plasma protein.

B.N.S. is a Nuffield Dominions (New Zealand) Demonstrator, University of Oxford.

## REFERENCES

BARRETT, A. M. & CULLUM, V. A. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43–55.

BENFEY, B. G. & VARMA, D. R. (1966). Antisympathomimetic and antifibrillation effects of pronethalol and propranolol. *Br. J. Pharmac. Chemother.*, **26**, 3–8.

BLACKBURN, C. H., BYRNE, L. J., CULLUM, V. A., FARMER, J. B. & LEVY, G. P. (1970). The pharmacology of 5-(2-t-butylamino-1-hydroxyethyl) salicylamide (AH3474), a β-adrenoreceptor blocking agent. *J. Pharm. Pharmac.*, **21**, 488–497.

DAWES, G. S. (1946). Synthetic substitutes for quinidine. *Br. J. Pharmac. Chemother.*, **1**, 90–112.

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**, 296–297.

FREEDBERG, A. S., PAPP, J. GY. & VAUGHAN WILLIAMS, E. M. (1970). The effect of altered thyroid state on atrial intracellular potentials. *J. Physiol., Lond.*, **207**, 357–370.

GILL, E. W. & VAUGHAN WILLIAMS, E. M. (1964). Local anaesthetic activity of the β-receptor antagonist, pronethalol. *Nature, Lond.*, **201**, 199.

HILL, R. C. & TURNER, P. (1969). Preliminary investigations of a new beta-adrenoceptive receptor blocking drug LB46, in man. *Br. J. Pharmac.*, **36**, 368–372.

LUCCHESI, B. R. (1965). The effect of pronethalol and its dextroisomer upon experimental cardiac arrhythmias. *J. Pharmac. Exp. Ther.*, **148**, 94–99.

MORALES-AGUILERÁ, A. & VAUGHAN WILLIAMS, E. M. (1965). The effects on cardiac muscle of β-receptor antagonists in relation to their activity as local anaesthetics. *Br. J. Pharmac. Chemother.*, **24**, 332–338.

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

RAPER, C. & WALE, J. (1968). Propranolol, MJ1999 and CIBA 39089 Ba in ouabain and adrenaline induced cardiac arrhythmias. *Europ. J. Pharmac.*, **4**, 1–12.

RITCHIE, J. M. & GREENGARD, P. (1965). On the mode of action of local anaesthetics. *Ann. Rev. Pharmac.*, **6**, 405–430.

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

SINGH, B. N. & VAUGHAN WILLIAMS, E. M. (1970a). Local anaesthetic and anti-arrhythmic actions of alprenolol relative to its effect on intracellular potentials and other properties of isolated cardiac muscle. *Br. J. Pharmac.*, **38**, 749–757.

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

SINGH, B. N. & VAUGHAN WILLIAMS, E. M. (1970c). A third class of anti-arrhythmic action. Effects on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ1999 and AH3474. *Br. J. Pharmac.*, **39**, 675–687.

SINGH, B. N. & VAUGHAN WILLIAMS, E. M. (1971). A fourth class of anti-dysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. *Cardiovascular. Res.*, in the Press.

VAUGHAN WILLIAMS, E. M. (1958). The mode of action of quinidine in 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. (1970). Classification of anti-arrhythmic drugs. In: *Symposium on Cardiac Arrhythmias*, ed. Sandøe, E., Flensted-Jensen, E., & Olesen, K. H. A. B. Astra, Sweden: Södertälje.

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

VAUGHAN WILLIAMS, E. M. & SZEKERES, L. (1961). A comparison of tests for antifibrillatory action. *Br. J. Pharmac. Chemother.*, **17**, 424–432.

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