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ANTIFIBRILLATORY EFFICACY OF ENCAINIDE, LORCAINIDE AND ORG 6001 COMPARED WITH LIGNOCAINE IN ISOLATED HEARTS OF RABBITS AND GUINEA-PIGS

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1 In Langendorff-perfused rabbit hearts, electrical stimulation threshold and ventricular fibrillation threshold (VFT) were measured by applying rectangular impulses of 3 ms duration and increasing current at frequencies of 4 and 20 Hz respectively.

2 Perfusion with lignocaine or one of three new antiarrhythmic drugs, encainide, lorcainide and ORG 6001, produced significant, dose-dependent increases in both thresholds.

3 On a dosage basis, encainide was seven times, lorcainide fourteen times and ORG 6001 twice as potent as lignocaine in raising VFT.

4 In Langendorff-perfused guinea-pig hearts, only lorcainide provided complete protection against ouabain-induced ventricular fibrillation, while 6 of 6, 3 of 6 and 2 of 6 hearts fibrillated in the presence of encainide, lignocaine and ORG 6001 respectively, but with infusion durations significantly higher than control.

5 These results indicate the potential antifibrillatory activity of these new antiarrhythmic agents.

Introduction

In the hope of finding more effective and selective antiarrhythmic drugs for the control of life-threatening arrhythmias, three new compounds have recently been added to the list of available drugs. Encainide (4-methoxy-2-(1-methyl-2-piperidyl ethyl) benzanilide hydrochloride), has been found effective in abolishing experimental arrhythmias in a variety of animal models (Byrne, Gomoll & McKinney, 1977). Its electrophysiological properties have been studied on canine Purkinje fibres (Gibson, Somani & Bassett, 1978) and in man (Sami, Mason, Peters & Harrison, 1979). The second drug, lorcainide, is chemically identified as *N*-(4-chlorophenyl)-*N*-(1-methylethyl)-4-piperidenyl benzeneacetamide hydrochloride. Studies in dogs have shown it to be effective in arrhythmias induced by coronary ligation, ouabain-induced ventricular arrhythmias and acetylcholine and aconitine-induced atrial fibrillation (Carmeliet, Janssen, Marsboom, Van Nueten & Xhonneux, 1978). Clinically, it has been found effective in the treatment of patients with ventricular ectopic beats (Kesteloot & Stroobandt, 1977; Klotz, Müller-Seydlitz & Heimburg, 1979). The third drug, a nonhormonal amino steroid, ORG 6001 (3α-amino-5α-androstan-2β-ol-17-one hydrochloride) has a structure rather uncommon for antiarrhythmic drugs. Its antiarrhythmic effect has been compared with that of lignocaine in different experimental models (Vargaftig, Sugrue, Buckett & Van Riezen, 1975). It has also been found to reduce the incidence of ventricular premature beats induced by coronary artery ligation in dogs (Marshall & Parratt, 1975) and pigs (Verdouw, Schamhardt, Remme & De Jong, 1978). Electrophysiological studies of ORG 6001 (Salako, Vaughan Williams & Wittig, 1976), lorcainide (Carmeliet *et al.*, 1978) and encainide (Gibson *et al.*, 1978) have all shown the ability of these drugs to reduce the rate of rise of transmembrane action potential and to prolong the refractory period, actions typical of the local anaesthetic type of antiarrhythmic drugs. Since the effectiveness of these new drugs has, so far, been studied separately by different methods and recent studies (Almotrefi & Baker, 1980; 1981) have shown the reliability of the technique used for the experimental evaluation of the antifibrillatory activity of some other antiarrhythmic drugs, it was decided to compare their antifibrillatory activity with that of lignocaine under the same experimental conditions using two models, electrical stimulation threshold (EST) and ventricular fibrillation threshold (VFT) measurement in isolated heart of the rabbit and ouabain-induced arrhythmias in isolated heart of the guinea-pig.

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Methods

Electrical stimulation threshold and ventricular fibrillation threshold

New Zealand White rabbits of either sex weighing 1 to 1.5 kg were killed by a blow on the head and bled. The isolated hearts were perfused by the Langendorff method with McEwen's solution (1956), gassed with 95% O_2 and 5%CO_2, at a pressure of about 6 KPa (60 cmH_2O) at 37°C. McEwen's solution contained (mmol/1): NaCl 130, KCl 5.6, $CaCl_2$ 2.2, NaH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11 and sucrose 13, and had a pH of 7.3 to 7.4. The methods of recording the amplitude of contraction, the electromyogram, and the stimulation connections have been described in detail previously (Almotrefi & Baker, 1980; 1981). After a stabilization period of at least 40 min of spontaneous beating, EST was measured by applying rectangular impulses of 3 ms duration at a frequency of 4 Hz. The current intensity was increased at a rate of 30 μA per second until the heart followed the stimulus. The minimum current required to drive the heart was taken as the EST. After this threshold was determined, stimulation was stopped and the heart resumed spontaneous beating. VFT was determined by applying impulses of similar duration and increasing current at a frequency of 20 Hz. The minimum current required to induce ventricular fibrillation was taken as the VFT. The moment fibrillation was induced, stimulation was stopped and if normal rhythm had not returned in 60 s, defibrillation was effected by infusing 0.1 ml of 0.54 mol/l potassium chloride into the aortic cannula. In each experiment three determinations of each threshold were carried out at 15 min intervals and the mean was taken as the control value. The heart was then perfused with McEwen's solution containing the test drug and both thresholds were determined after 15, 30 and 60 min exposure to the drug. The heart was then reperfused with the drug-free solution and similar determinations were carried out after 15, 30 and 60 min.

Ouabain-induced arrhythmias

Guinea-pigs of either sex weighing 400 to 500 g were used. The method of perfusion and recording was as described above for rabbit hearts. Ouabain was infused into the aortic cannula at a rate of 5.48 μmol/min. The test drug was added to the perfusion solution 5 min before ouabain infusion was started and perfusion with both continued throughout the experiment. The duration of ouabain infusion required to produce unequal R-R interval, ventricular fibrillation and cardiac arrest in the presence of the test drug was compared with that in ouabain control experiments.

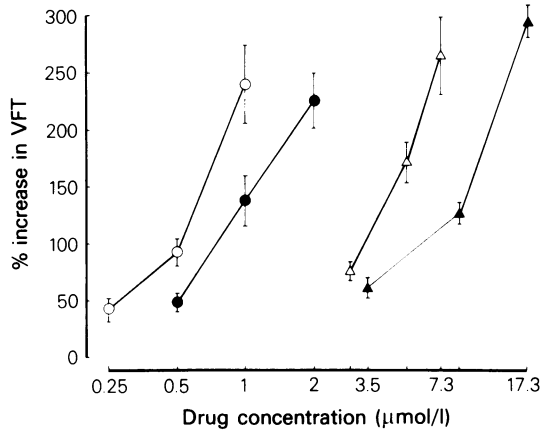

Figure 1 Effects of encainide (●), lorcainide (○), ORG 6001 (△) and lignocaine (▲) on ventricular fibrillation threshold (VFT) measured in isolated heart of rabbit. Abscissa scale: drug concentration on logarithmic scale. Ordinate scale: percentage increase in VFT. Each point is the mean of six experiments; vertical lines show s.e. mean.

Drugs used were encainide hydrochloride (Mead Johnson), lorcainide hydrochloride (Janssen), ORG 6001 (Organon), lignocaine hydrochloride (Astra-Hewlett) and ouabain (BDH). Statistical differences were calculated by Student's *t* test or Student's paired *t* test.

Results

Electrical stimulation threshold and ventricular fibrillation threshold

In 81 rabbits used in the study, EST values ranged between 0.1 and 0.7 mA (0.4 ± 0.02, mean ± s.e.) and VFT values between 0.65 and 14.3 mA (4.62 ± 0.33). Apart from biological variation, reasons for this wide scatter are not obvious and the effectiveness of a drug does not seem to depend upon resting EST or VFT values. In nine untreated hearts, within 1 h of the first determination, EST dropped by 1.9 to 6.3% and VFT by 7.8 to 14.6% of their original values and remained within these limits for another 2 h. Perfusion with various concentrations of all tested drugs produced significant, dose-dependent increases in both thresholds. Dose-response curves of the effect of the three new drugs and that of lignocaine on VFT, after an exposure duration of 1 h, are plotted in Figure 1, which shows a difference between them in potency but the curves are approximately parallel. Similar curves of their effect on EST are plotted in Figure 2. A comparison

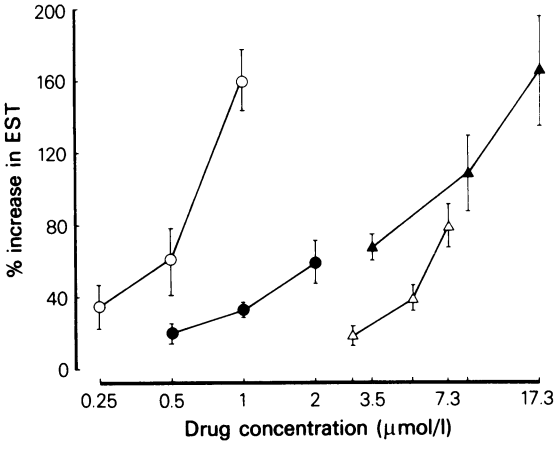

Figure 2 Effects of encainide (●), lorcainide (○), ORG 6001 (△) and lignocaine (▲) on electrical stimulation threshold (EST) measured in isolated heart of rabbit. Abscissa scale: drug concentration on logarithmic scale. Ordinate scale: percentage increase in EST. Each point is the mean of six experiments; vertical lines show s.e. mean.

of Figures 1 and 2 shows that the rise, produced by all drugs in EST is less than that in the corresponding VFT. This difference is most marked with encainide and ORG 6001. The effect of these drugs on both EST and VFT was rapid in onset with the maximum effect being achieved in 15 to 30 min of exposure. However, the effect of all these drugs on VFT was completely removed within 15 min of reperfusion with the drug-free solution, while the effect on EST was not. This large effect of the drugs on VFT was accompanied by small, but significant (mainly with the highest concentrations used) changes in heart rate and amplitude of contraction. These results are summarized in Table 1.

From the dose-response curves shown in Figure 1, the concentration required to raise VFT to 200% above its original value was determined for each drug and accordingly their potency has been compared with that of lignocaine taken as a standard. These concentrations were 11.6, 0.82, 1.62 and 5.62 μmol/l for lignocaine, lorcainide, encainide and ORG 6001 respectively and their relative potencies were lignocaine 1, lorcainide 14.1, encainide 7.2 and ORG 6001 2.1.

Ouabain-induced arrhythmias

Progressive ouabain toxicity, achieved by its continuous infusion into the aortic cannula of Langendorff-perfused guinea-pig hearts, caused reproducible levels of cardiac arrhythmias. In control experiments, ventricular fibrillation occurred in 18 of 18 hearts receiving ouabain alone. Concentrations of the three new antiarrhythmic drugs and lignocaine which produced 60 to 80% rise in VFT (Figure 1) were chosen for study by this method. The results are summarized in Table 2. Only lorcainide provided complete pro-

Table 1 Effects of encainide, lorcainide, ORG 6001 and lignocaine on heart rate and amplitude of contraction in isolated heart of rabbits

	Spontaneous heart rate		*Amplitude of contraction*	
Concentration (μmol/l)	*Control* (beats/min)	*% change*	*Control* (tension, g)	*% change*
Lignocaine				
3.5	179 ± 12	−2.9 ± 2.5	11.38 ± 0.14	−0.7 ± 3.8
8.6	201 ± 14	−3.4 ± 1.1*	10.35 ± 0.43	−6.8 ± 3.5
17.3	202 ± 20	−8.3 ± 2.1**	11.71 ± 0.23	−19.6 ± 2.6***
Encainide				
0.5	196 ± 18	−1.6 ± 1.1	10.5 ± 0.24	+0.9 ± 1.3
1.0	200 ± 9	−3.9 ± 1.6*	8.33 ± 0.37	−6.6 ± 4.3
2.0	224 ± 20	−6.4 ± 1.5**	9.25 ± 0.52	−7.3 ± 3.1*
Lorcainide				
0.25	190 ± 14	−1.4 ± 1.3	10.04 ± 0.25	−3.9 ± 1.3
0.5	188 ± 6	−1 ± 3.1	9.46 ± 0.23	−2.1 ± 1.9
1.0	202 ± 17	−5.8 ± 2.1*	9.63 ± 0.24	−13.2 ± 4.4*
ORG 6001				
2.9	232 ± 15	−5.6 ± 2.9	10.17 ± 0.33	−2.5 ± 1.7
5.1	210 ± 3	−9.7 ± 2.5**	9.5 ± 0.34	−4.3 ± 1.4*
7.3	216 ± 17	−4.4 ± 2.2**	9.38 ± 0.44	−8.9 ± 5.6

Values are means ± s.e. of 6 experiments for each concentration of each drug.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2 Effect of encainide, lorcainide and ORG 6001 compared with that of lignocaine on ouabain-induced arrhythmias in isolated heart of guinea-pigs

Concentration (μmol/l)	*n*	*Mean duration of ouabain infusion (min) required to produce:* *Unequal R-R interval*	*Ventricular fibrillation**	*Cardiac arrest*
Control	18	3.4 ± 0.26	21.3 ± 1.7 (18/18)	71 ± 4.8
Lignocaine 3.5	6	5.5 ± 0.35***	48 ± 13** (3/6)	122 ± 9***
Encainide 0.7	6	4.6 ± 0.65	67 ± 7.8** (6/6)	120 ± 7***
Lorcainide 0.4	6	4.1 ± 0.62	(0/6)	165 ± 14***
ORG 6001 2.9	6	5.2 ± 0.8*	117 ± 20*** (2/6)	167 ± 6***

Values are mean ± s.e.mean.

* The incidence of ventricular fibrillation is given in parentheses.
Statistical significance (*t* test) of the difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0005$.

tection against ouabain-induced ventricular fibrillation, while 6 of 6, 3 of 6 and 2 of 6 hearts fibrillated in the presence of encainide, lignocaine and ORG 6001 respectively. However, the duration of ouabain infusion required to induce fibrillation in these hearts was significantly higher than in control ouabain experiments. Lignocaine and ORG 6001 caused significant increases in the duration of ouabain infusion required to produce unequal R-R intervals, while all four drugs caused significant increases in that required to produce cardiac arrest.

Discussion

From a qualitative point of view, all these three new antiarrhythmic drugs have proved, as did lignocaine, to be effective in raising EST and VFT and in protecting against ouabain dysrhythmogenic cardiotoxicity. These results are expected of compounds with their electrophysiological properties. It is known that the actions of antiarrhythmic drugs in decreasing the rate of rise of transmembrane action potential (with its relation to conduction velocity) and in prolonging the refractory period would tend to reduce the inhomogeneity in excitability and dispersion in recovery, factors known to be of great importance in the initiation of ventricular fibrillation by electrical stimulation (Moore & Spear, 1975). These electrophysiological effects, shared by the other local anaesthetic type of antiarrhythmic drugs, have been found to be possessed by encainide (Gibson *et al.*, 1978), lorcainide (Carmeliet *et al.*, 1978) and ORG 6001 (Salako *et al.*, 1976).

In the present study, lorcainide was most potent in raising both EST and VFT and in protecting against ouabain-induced ventricular fibrillation. In anaesthetized dogs, Carmeliet *et al.*, (1978) compared the effects of lorcainide with those of lignocaine and aprindine in atrial and ventricular arrhythmias induced by different methods. They found lorcainide to have a similar effectiveness to aprindine and that both were more potent than lignocaine. However, in the VFT determination test, they found aprindine, in a similar dose to lorcainide, not effective in raising VFT. In contrast, in a previous study Almotrefi & Baker (1980) using the same methods as described here, found aprindine to be nearly 40 times as potent as lignocaine in raising VFT (lorcainide was found in the present study to be about 14 times as potent as lignocaine). This discrepancy could be due to the differences in technique and species used.

While the effectiveness of the other two drugs, encainide and ORG 6001, has been clearly demonstrated in different experimental arrhythmias (Vargaftig *et al.*, 1975, Byrne *et al.*, 1977), including the coronary artery ligation method (Marshall & Parratt, 1975; Verdouw *et al.*, 1978), their effects on VFT have not previously been investigated. The present study has shown them to raise VFT significantly in concentrations less than those required of lignocaine (Figure 1).

In their rapid onset of action, these three new drugs resembled lignocaine, mexiletine and tocainide, but all differed from aprindine which had a rather slow onset of action (Almotrefi & Baker, 1980). Similarly, the observation that the effect of these new drugs on VFT was completely removed within 15 min of reperfusion with the drug-free solution while their effect on EST was not, has been found previously with phenytoin and mexiletine (Almotrefi & Baker, 1981). The reason for this variation is not clear, but it might indicate that the electrophysiological parameters involved in raising these two thresholds are not exactly the same. Additionally, the greater effect on VFT of all the antiarrhythmic drugs tested in this study, as well as those in previous studies (Almotrefi & Baker, 1980; 1981), in comparison with their effect on the corresponding EST, gives support to the view that VFT determination is a more sensitive and selective measure of antifibrillatory activity (Baum, Eckfeld, Shropshire, Rowles & Varner, 1971).

Just as previously reported by Vaughan Williams & Sekiya (1963) for anaesthetized guinea-pigs, in the present study continuous infusion of ouabain into the aortic cannula of Langendorff-perfused guinea-pig hearts resulted in reproducible levels of cardiac arrhythmias on which the protective action of antiarrhythmic drugs could be evaluated. The results of this method (Table 2) have shown a greater effectiveness of lorcainide, which completely prevented ventricular fibrillation in all hearts. With the other three drugs, where ventricular fibrillation occurred in some trials, the duration of ouabain infusion required to induce fibrillation in these hearts was significantly higher than in controls.

The results of the present study have provided a comparison of the antifibrillatory efficacy of three new antiarrhythmic drugs in electrically-induced and ouabain-induced arrhythmias. Their effectiveness, together with what has been described of their activity after oral administration as well as their minimal haemodynamic effects in experimental and clinical studies (Marshall & Parratt, 1975; Kesteloot & Stroobandt, 1977; Klotz *et al.*, 1979; Sami *et al.*, 1979) makes them a useful addition to the existing antiarrhythmic agents.

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