Br. J. Pharmac. (1976), **57**, 251–262

INVESTIGATIONS TO CHARACTERIZE A NEW ANTI-ARRHYTHMIC DRUG, ORG 6001, INCLUDING A SIMPLE TEST FOR CALCIUM ANTAGONISM

L.A. SALAKO, E.M. VAUGHAN WILLIAMS & J.H. WITTIG
Department of Pharmacology, Oxford University, South Parks Rd., Oxford OX1 3QT

1 The compound Org 6001 (3α-amino-2β-hydroxy-5α-androstan-17-one hydrochloride) was found in recent experiments to exhibit anti-arrhythmic activity. Evidence is presented in this paper concerning its mode of action.

2 Org 6001 was 1.8 times more potent than procaine as a local anaesthetic on desheathed frog nerve.

3 Org 6001 had no effect on the resting potential of isolated cardiac muscle of rabbit, but greatly reduced the maximum rate of depolarization (MRD). The action potential duration (APD) was marginally prolonged in atrial and ventricular muscle.

4 Org 6001 preferentially shortened APD in that part of the Purkinje system in which APD is normally longer than elsewhere, so that APD became more uniform throughout the ventricular conducting system.

5 Org 6001 did not block chronotropic responses to isoprenaline in atrial muscle.

6 Org 6001 had only a small negative inotropic effect in atrial muscle, and did not reduce the positive inotropic effect of raised calcium concentrations.

7 The effect of Org 6001 on MRD was reduced by lowering the external K^+ concentration.

8 It is concluded that Org 6001 is an anti-arrhythmic drug of the first class (local anaesthetic type), and within this group is of a sub-class more closely related to lignocaine than to quinidine.

Introduction

There is still a need in the cardiologist's armamentarium for an anti-arrhythmic drug, rapidly and reliably absorbed when given orally, and with low toxicity and long half-life, for maintenance or prophylactic therapy. Several new compounds have been introduced recently which might fill this role (e.g. mexillitine, Allen, Kofi Ekue, Shanks & Zaidi, 1970; Singh & Vaughan Williams, 1972a) and some established drugs are being re-assessed. The structure of Org 6001 (Figure 1) is quite unlike that of any other anti-arrhythmic drug, and it was naturally of interest to discover whether it might have a novel mode of action also.

There are four main ways in which currently known anti-arrhythmic drugs may act, several compounds possessing more than one of these classes of action (Vaughan Williams, 1970; 1974; 1975). The first consists of a direct membrane 'stabilization', which may be measured in a number of ways (raised electrical threshold, slowed conduction velocity, reduced frequency at which stimuli can be followed), but high concentrations are required to demonstrate these effects, and the most sensitive test is measurement of the maximum rate of depolarization (MRD) with intracellular electrodes. Compounds with this class 1 action on cardiac muscle also have a variable local anaesthetic activity on nerve. The second class of action is anti-sympathetic, by competitive receptor blockade, reduction of transmitter release, or post-synaptic interference with responses to adrenergic stimulation. The third class of action consists of a prolongation of the cardiac action potential, again most reliably recorded with intracellular electrodes, although a large prolongation of ventricular action potentials can usually be detected as a lengthened Q-T interval on the electrocardiogram. Finally, the fourth class of action is an interference with inward calcium currents, but there is, as yet, no simple method for measuring this effect, which is still not as fully authenticated as the other three (Singh & Vaughan Williams, 1972b).

The present investigation has been devoted to determining which, if any, of these actions is exhibited by Org 6001, and a simple test, which gives a positive result with verapamil, has been devised to measure calcium antagonism.

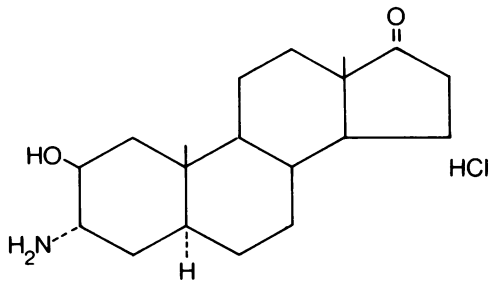

Figure 1 Structure of 3α-amino-2β-hydroxy-5α-androstan-17-one hydrochloride (Org 6001) mol. wt. 341.9, pKA 8.0.

Methods

Atrial recording

Atria removed from rabbits weighing approximately 1 kg of either sex were set up for the recording of mechanical and electrical activity as previously described (Vaughan Williams, 1958; Szekeres & Vaughan Williams, 1962). The atria were suspended in modified Locke solution gassed with 95% O_2 and 5% CO_2 at 32°C. The solution had a composition of (mM) NaCl 125, KCl 5.6, $CaCl_2$ 2.16, $NaHCO_3$ 25, glucose 11, and had a pH of 7.4. During impalements with micro-electrodes the atria were paced at a frequency 10% above the spontaneous frequency by square stimuli, 1 ms duration, strength × 2 threshold. Records were made after 30–60 min exposure to each drug concentration. Contractions were recorded with an RCA 5734 transducer and intracellular potentials with 3M-KCl-filled glass micropipettes.

Ventricular recording

Hearts from stunned 2 kg rabbits of either sex were removed and placed in oxygenated Locke solution at 35°C. Longitudinal cuts, parallel to the septum, were made along the anterior and posterior interventricular grooves. The tricuspid chordae tendiniae were severed at their insertions and the right ventricular free wall was reflected from the septum. The right bundle branch was cut at the level of the membranous septum, and a block of septal muscle (3–5 mm) containing the right bundle was cut free. The bundle becomes a clear strand at the base of the anterior papillary muscle, with two or more branches being inserted into the right ventricular free wall. This portion of the wall was also cut free and the completed dissection was transferred to the recording bath, and comprised: (1) proximal His bundle; (2) right bundle; (3) anterior papillary muscle; (4) false tendons, being the sole connection to (5) the endocardial portion of the free right ventricular wall into which (4) were inserted. The preparation was pinned to a silastic base and superfused with 10 ml/min oxygenated Locke solution at 35°C ± 0.2°C. A bipolar silver stimulating electrode was placed on the proximal remnant of the His bundle. Microelectrode records were taken near the stimulating electrode and at successive intervals of 1–2 mm distal to it, along the His bundle, proximal right bundle, false tendon and ventricular muscle. The region of the conducting system with the longest action potential duration was identified, and one microelectrode was positioned 1 cm proximal to it, and another 2–3 mm distal or in the ventricular muscle itself. The preparation was paced with twice threshold stimuli (2 ms, at 1–1.6 Hz) from the His bundle or proximal right bundle, and every sixth stimulus was followed by an additional ('premature') stimulus after a variable interval shorter than the basal cycle length.

Local anaesthesia

Frog sciatic nerves were stripped of their sheaths and mounted in a three-compartment perspex bath. Cotton wool soaked in frog Ringer solution was placed in the two outer chambers so that the segments of sciatic nerve in these chambers were in an atmosphere of moist air. The central chamber was filled with frog Ringer solution. In one of the outer chambers the nerve was in contact with a pair of platinum stimulating electrodes, in the other, it was in contact with recording electrodes. An indifferent earthing electrode was inserted into the central chamber. Nerves were stimulated with supramaximal pulses (5–10 V) of 1 ms duration and action potentials were displayed on an oscilloscope.

Drugs were added to the central chamber and left in contact with the nerve until a maximal effect on the fastest wave of the action potential was observed. The frog Ringer solution had the following composition: (mM) NaCl 120, KCl 1.88, $CaCl_2$ 1.08, $NaHCO_3$ 2.38 and was kept at a pH of 7.5 by the addition of Tris buffer (0.104 M) 10 ml per litre.

Drugs used were Org 6001 (Organon); isoprenaline sulphate (Burroughs Wellcome); procaine hydrochloride (BDH). Measurements have been presented as means ± s.e., and the significance of differences has been estimated by Student's *t* test, or Student's paired *t* test.

Results

1. Measurement of direct membrane effects (Class 1 action)

Local anaesthesia of frog nerve. Org 6001 at pH 7 had a local anaesthetic potency on frog sciatic nerve

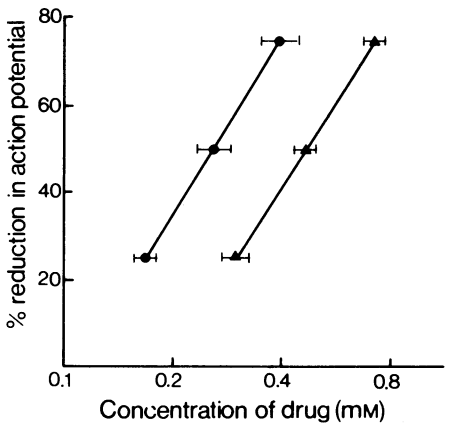

Figure 2 Local anaesthetic activity of Org 6001 (●) on frog sciatic nerve compared with that of procaine (▲). Ordinate scale; % reduction in action potential height. Abscissa scale; concentration of drug (in mM) on logarithmic scale.

1.8 times greater than that of procaine. The mean results of 8 experiments are shown in Figure 2, the ED_{25}, ED_{50} and ED_{75} having been interpolated from each individual dose-response curve to obtain the means. Not only was Org 6001 more potent than procaine, it also had a much slower onset and longer duration of action. Recovery from concentrations of Org 6001 producing greater than a 50% reduction of action potential height was never complete on washout, implying some neurotoxicity (i.e. irreversible inactivation of channels for inward Na-current).

Electrical threshold, conduction velocity and maximum driven frequency. The lowest concentration of Org 6001 studied (2.9 μM), had no effect on the electrical threshold of rabbit isolated atria, but higher concentrations (11.7 and 46.8 μM) caused significant increases. Similarly, conduction velocity was unaffected by the lowest concentration, but was significantly slowed by the higher concentrations. The maximum frequency at which the atria would follow a stimulus was, however, reduced even at the lowest concentration of Org 6001. These results are summarized in Table 1.

Atrial intracellular potentials. Measurements of various parameters of atrial intracellularly recorded potentials are summarized in Table 2, the results having been pooled from seven experiments. Concentrations of Org 6001 up to 46.8 μM had no effect on resting potential, implying a complete absence of any inhibition of ion-pumping by the membrane. A significant fall in the maximum rate of depolarization (MRD) was apparent, however, even at the lowest concentration. The overshoot was significantly reduced by the two higher concentrations, implying direct interference with Na-inward

Table 1 Effects of Org 6001 on rate and force of spontaneous contraction, electrical threshold, conduction velocity and maximum driven frequency in rabbit isolated atria

Concentration of Org 6001 (μM)	*Spontaneous frequency*	*Contractile tension*	*Electrical threshold*	*Conduction velocity*	*Maximum driven frequency*
2.9	−6.3 ± 1.6**	+10.4 ± 4.9	+8.3 ± 4.6	−4.0 ± 2.3	−6.2 ± 2.4*
11.7	−9.9 ± 2.9**	−3.0 ± 4.3	+23.5 ± 7.4	−8.8 ± 2.6**	−10.7 ± 2.2**
46.8	−16.3 ± 2.7**	−9.9 ± 5.9	+30.9 ± 8.8**	−24.3 ± 2.7***	−24.1 ± 3.1***

Values given as % change from control. Mean ± s.e. of 7 experiments.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2 Effect of Org 6001 on rabbit atrial intracellular potentials

Dose of Org 6001 (μM)	*No. of fibres*	*Resting potential (mV)*	*Action potential (mV)*	*MRD (V/s)*	*50% repolarization time (ms)*	*90% repolarization time (ms)*
0	42	73.5 ± 0.54	98.9 ± 1.08	94.7 ± 5.10	51.0 ± 1.0	98.5 ± 1.0
2.9	36	73.0 ± 0.81	97.8 ± 1.08	80.0 ± 4.31*	52.0 ± 1.0	99.0 ± 1.5
11.7	30	74.1 ± 1.08	92.4 ± 1.62**	61.5 ± 5.78***	50.0 ± 1.0	102.0 ± 2.0
46.8	6	74.6 ± 1.08	87.0 ± 2.7**	36.8 ± 5.30***	52.0 ± 1.0	112.5 ± 1.5***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

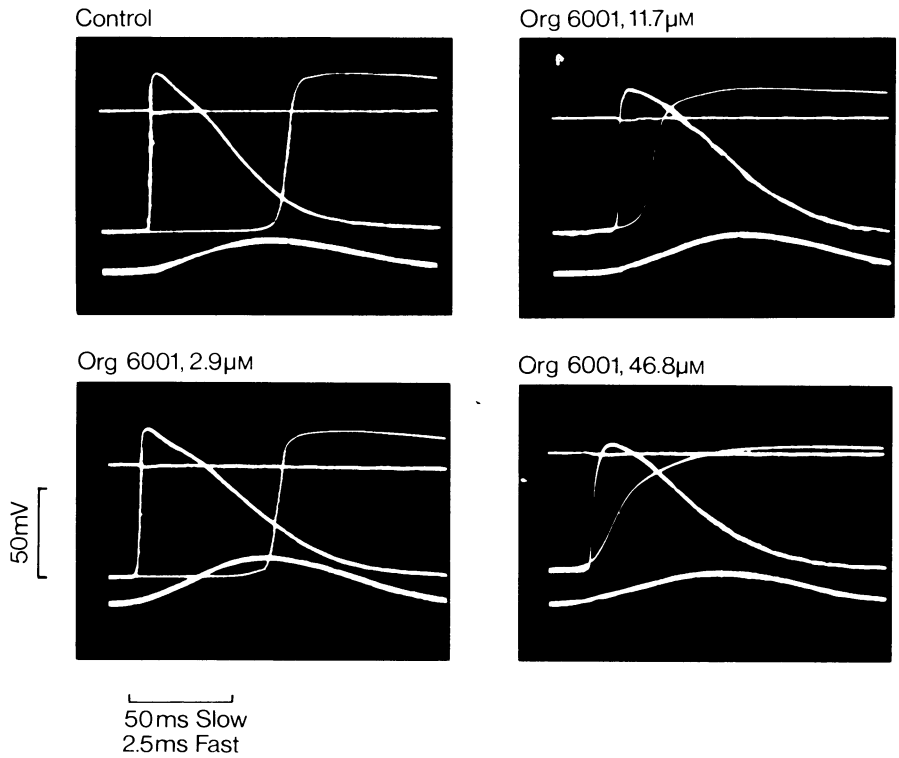

Figure 3 Effect of Org 6001 on rabbit atrial intracellular potentials. In each panel; horizontal trace, zero potential; middle traces, intracellular potentials at slow and fast sweep speeds, superimposed; lowest trace, contraction.

Table 3 Effect of Org 6001 on rabbit ventricular and Purkinje fibre intracellular potentials

	Ventricular muscle			*Purkinje fibre*		
Concentration Organon 6001 μg/ml(μM)	*Action potential height (mV)*	*MRD (V/s)*	*Action potential to 90% re-polarization (ms)*	*Number of fibres*	*Action potential height (mV)*	*MRD (V/s)*
0 (Control) No. of fibres $n = 108$	105.3 ± 1.3	308 ± 4.1	178 ± 2.3	$n = 130$	119.1 ± 3.2	516 ± 11
4 μg/ml (11.7) $n = 56$	103.2 ± 3.1	*232 ± 1.2	*208.3 ± 1.7	$n = 78$	118.6 ± 3.6	*349 ± 5.2
10 μg/ml (29.3) $n = 42$	102.6 ± 2.6	*194 ± 1.1	*210.3 ± 1.3	$n = 83$	116.3 ± 3.1	*157 ± 12.7

* $P < 0.001$ from control values.

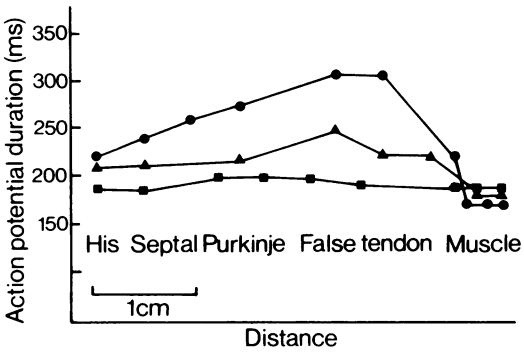

Figure 4 Effect of Org 6001 on the duration of the intracellularly recorded action potential at different parts of the conducting system, and in ventricular muscle. Ordinate scale: action potential duration (ms); abscissa scale: distance from origin of conducting system included within the preparation. (●) Controls; (▲) Org 6001 11.7 μM; (■) Org 6001 29.3 μM.

depolarizing currents, the typical class 1 effect (Figure 3). There was no prolongation of the plateau to 50% repolarization at any concentration, but there was a small but highly significant prolongation of the time to 90% repolarization at the highest concentration. Thus Org 6001 has only a weak class 3 action. Drugs exhibiting this action as a primary feature, e.g. amiodarone, (Singh & Vaughan Williams, 1970) and L 9146 (Vaughan Williams, Salako & Wittig, 1976), typically cause a greater prolongation of the plateau to 50% repolarization than to 90% repolarization.

Ventricular potentials. The His bundle was stimulated at a basal frequency of 1.0 to 1.6 Hz and intracellular recordings were made from Purkinje fibres and ventricular muscle. The resting potential was not altered by Org 6001 in any tissue. Other effects of the drug are summarized in Table 3. In contrast with atria, ventricular action potentials peak amplitudes were not affected by Org 6001 to a significant degree. The effect in ventricular muscle on MRD and action potential duration (APD) was the same as in atria, MRD being reduced and APD lengthened. The effect in Purkinje tissue was similar to that in ventricle with respect to action potential voltage and MRD, but the effect on APD depended on the region from which the record was obtained. The normal pattern of APD in the conducting tissue is that it lengthens progressively from the bundle of His as far as the terminal chordae tendiniae, which have action potentials as much as 100 ms longer than those of the muscle into which they are inserted. Org 6001 11.7 μM shortened the duration greatly in the distal region, much less in the bundle of His. At a concentration of 29.3 μM the APD of the central and terminal sections was reduced so much that it now became as brief as that of the bundle of His itself, and since the ventricular APD was lengthened, the APD was almost uniform throughout the ventricular conduction system. The results from one experiment are depicted graphically in Figure 4 and the mean results from four preparations are presented in Table 4.

Effects on conduction. After every 6th stimulus (S_1) at the basal frequency a 7th was introduced at an interval shorter than the basal interval ('premature' stimulus, S_2). One microelectrode was positioned 1 cm proximal to the region of maximum APD, another 3–5 mm distal to it or in the ventricular muscle itself. The interval between the sixth and the premature stimulus is termed S_1–S_2; the interval between the upstrokes of the 6th and 7th action potentials recorded by the proximal microelectrode is P_1–P_2, and the interval between the upstrokes of the records from the distal electrode is D_1–D_2. As the S_1–S_2 interval was

Table 4 Effects of Organon 6001 on action potential durations in right bundle branch

Drug concentration μg/ml (μM)	*Mean action potential duration (milliseconds)*			
	His	*Septal Purkinje*	*False tendons*	*Free wall ventricular muscle*
0 Control No. of fibres $n=108$	198.5 ± 2.3	246.8 ± 1.8	304.5 ± 4.8	178.0 ± 2.3
4 μg/ml (11.7) $n=36$	204.8 ± 1.2	*210.9 ± 1.3	*225.0 ± 3.6	*208.3 ± 1.7
10 μg/ml (29.3) $n=42$	200.3 ± 1.1	*209.8 ± 0.4	*211.8 ± 0.8	*210.3 ± 1.3

* $P<0.001$ from control values.

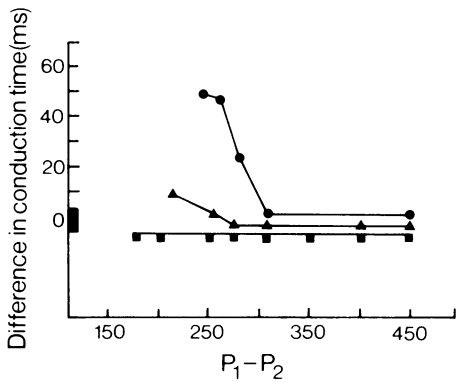

Figure 5 Effect of Org 6001 on conduction in a ventricular preparation. Ordinate scale: Difference in ms between the interval between the time of arrival of normal and premature responses recorded from the distal electrode (D_1–D_2, see text) and the interval between the arrival of normal premature action potentials at the proximal electrode (P_1–P_2). Abscissa scale: The minimal interval in ms between P_1–P_2 at which propagation still occurred of the response to the premature stimulus through to the distal electrode. In the controls D_1–D_2 was much greater than P_1–P_2, because the conduction velocity of D_2 was slowed by travelling in partially refractory tissue. Org 6001 shortened action potential duration in distal Purkinje fibres, so that propagation now occurred in less refractory tissue, and D_1–D_2 was no longer greater than P_1–P_2. (●): Controls; (▲) Org 6001 11.7 μM; (■) Org 6001 29.3 μM.

shortened, propagation of D_2 eventually failed, and the shortest interval of D_1–D_2 at which conduction was successful is the minimum propagation interval through the intervening region (which is sometimes called a 'gate' by e.g. Meyerburg, Steward & Hoffman, 1970; Wittig, Harrison & Wallace, 1973). It must be emphasized that this propagation interval is the difference between the times of arrival of action potentials in tissue distant from the stimulated region, and would include differences of conduction velocity and utilization time of the stimulus. It must, therefore, be distinguished from the 'functional refractory period', which is usually defined as the minimum interval between two stimuli, both of which are successful in eliciting action potentials propagated from the tissue stimulated.

In the control records, because APD was much longer in the conducting segment between the proximal and distal recording electrodes, when the S_1–S_2 interval was shortened P_2 impinged on refractory tissue and conduction failed, so that D_2 was absent. Before conduction failed altogether, P_2 arrived at partially refractory tissue, so that conduction velocity in the intervening segment was slowed, and D_1–D_2 was much longer than P_1–P_2 (Figure 5 (control) and Table 5).

The primary effect of Org 6001 so far demonstrated in both atrial and ventricular potentials was to reduce MRD (class 1 action) and so conduction velocity was slowed (Table 5). However, since APD in the central section was preferentially shortened by the drug, P_2 now reached tissue which had had more time to recover, so that the difference between the minimum

Table 5 Conduction in Purkinje fibres

Org 6001 concentration μg/ml (μM)	*Latency of P_1 (ms)*	*Conduction velocity*		*Intervals (ms)*		
		Proximal segment	*Distal segment*			
		For P_1 (m/s)	*For D_1 (m/s)*	S_1–S_2	P_1–P_2	D_1–D_2
0 Controls	<1	>2	>2	237	237	275
4 μg/ml (11.7)	4	0.25	0.667	198	202	215
10 μg/ml (29.3)	9	0.13	0.4	172	181	185

Latency is the interval between S_1 and P_1, and includes not only conduction time, but utilization time of the stimulus before the action potential takes off from the stimulated region. Conduction velocity for P_1 is the distance between S and P divided by the latency, and is only the 'apparent' conduction velocity because latency included utilization time. In the controls the latency was so short that it could not be measured accurately, so that conduction velocity is 'greater than' 2 m/second. The conduction velocity for D_1 is a true conduction velocity, being the distance P to D divided by the P_1–D_1 interval, and does not include utilization time. The big difference between conduction velocity for P_1 and for D_1 indicates that utilization time was greatly lengthened by the drug.

P_1–P_2 interval (at which D_2 was just conducted) and the minimum D_1–D_2 interval itself was reduced by 11.7 μM Org 6001, and abolished by 29.3 μM. These D_1–D_2 minus P_1–P_2 differences are plotted against P_1–P_2 intervals in Figure 5., but it must be emphasized that the S_1–P_1 interval itself was longer, and the conduction velocity of P_1 and D_1 was slower in the presence of the drug because of its class 1 action (Table 5). A second consequence of the class 1 action was the raising of the electrical threshold of the right bundle from 2.2 V to 3.6 V (±0.2 V) by Org 6001 11.7 μM, and to 4.5 V (±0.3 V) by 29.3 μM. Thirdly, conduction velocity of P_1 and D_1 was greatly reduced (Table 5), much more, in fact, in the Purkinje system than in atrial muscle (Table 1).

Spontaneous frequency and contractions. Drugs with direct membrane effects are often termed 'cardiodepressant', with the implication that reduction of MRD is correlated with a negative inotropic action. Though this may be true in some cases (e.g. with quinidine) it is not necessarily so, either qualitatively or quantitatively, and two drugs, papaverine (Vaughan Williams & Szekeres, 1961) and L 7810 (Bagwell, Polster & Vaughan Williams, 1973) have depressant effects on MRD in association with a positive inotropic action. A similar result was observed at the lowest concentration of Org 6001, which caused a significant reduction in MRD (Table 2) in association with an increase in contractions (Table 1). Even at the highest concentration the small reduction in contraction was not statistically significant, in contrast with the large effects on the membrane.

The spontaneous frequency of the SA node was significantly reduced by all three concentrations of Org 6001, though the effect was not as great as that on MRD.

In the ventricular preparations, spontaneous pacemaker activity was usually present (mean frequency 36 beats/min), and was always completely eliminated by the lowest concentration of Org 6001. Addition of adrenaline (50 μM) raised the spontaneous frequency of the preparations fourfold (to a mean of 136 ± 16 beats/min), but even these highly augmented rhythms were also completely abolished by the lowest concentration of Org 6001.

Effect of external K^+ concentration on MRD. The failure of nerves to recover from the effects of the higher concentrations of Org 6001, and the very large effects on MRD in rabbit atria in relation to its relative potency to procaine (× 1.8) on nerve, suggested that the activity of Org 6001 in blocking depolarizing current might be qualitatively different from the typical 'local anaesthetic' type of drug action. MRD is a function, *inter alia,* of the resting level of the potential from which the action potential 'takes off' (Weidmann, 1955). The effect of drugs with class 1 action (i.e. lignocaine, procaine, procainamide, etc.) is antagonized by low serum potassium, because the K_i/K_o ratio is thereby increased, the resting potential becomes more negative, and MRD is automatically accelerated as a result. If, however, channels for inward Na-current were irreversibly inactivated, lowering K_o, though increasing the resting potential, would no longer be able to restore MRD to normal levels.

To test this hypothesis, the effects of Org 6001 on intracellular potentials were measured in solutions containing 5.6 mM K (the normal plasma K for

Table 6 Effect of Org 6001 on rabbit atrial intracellular potentials at normal and low potassium concentrations

Dose of Org 6001 (μM)	*No. of fibres*	*K^+ (mM)*	*Resting potential (mV)*	*Action potential (mV)*	*MRD (V/s)*	*Repolarization to zero potential (ms)*	*90% Repolarization (ms)*
Control	30	5.6	78.4 ± 0.96	102.7 ± 0.90	109.7 ± 3.93	40.3 ± 1.70	102.7 ± 1.65
	12	2.8	88.7 ± 1.22	110.3 ± 1.42	122.8 ± 5.23	35.8 ± 1.91	107.5 ± 1.83
5.8	30	5.6	78.4 ± 0.89 (0)	100.0 ± 1.09 (−2.6)	83.5 ± 4.59*** (−23.9)	36.2 ± 1.38 (−10.0)	103.4 ± 1.10 (+0.75)
	30	2.8	84.9 ± 1.31 (−4.3)	108.0 ± 1.57 (−2.0)	108.2 ± 3.59* (−11.8)	35.8 ± 1.78 (+0.25)	108.8 ± 1.43 (+1.16)
23.4	30	5.6	75.1 ± 1.38 (−4.1)	93.4 ± 1.42*** (−9.0)	42.9 ± 2.58*** (−60.8)	33.1 ± 2.04* (−17.8)	99.0 ± 1.53 (−3.5)
	30	2.8	83.2 ± 1.79 (−6.1)	103.7 ± 1.02** (−6.0)	69.1 ± 2.57*** (−43.7)	34.0 ± 1.17 (−4.8)	107.7 ± 1.42 (+0.12)

Percent change from corresponding control is indicated in parentheses below the figure for each drug concentration.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

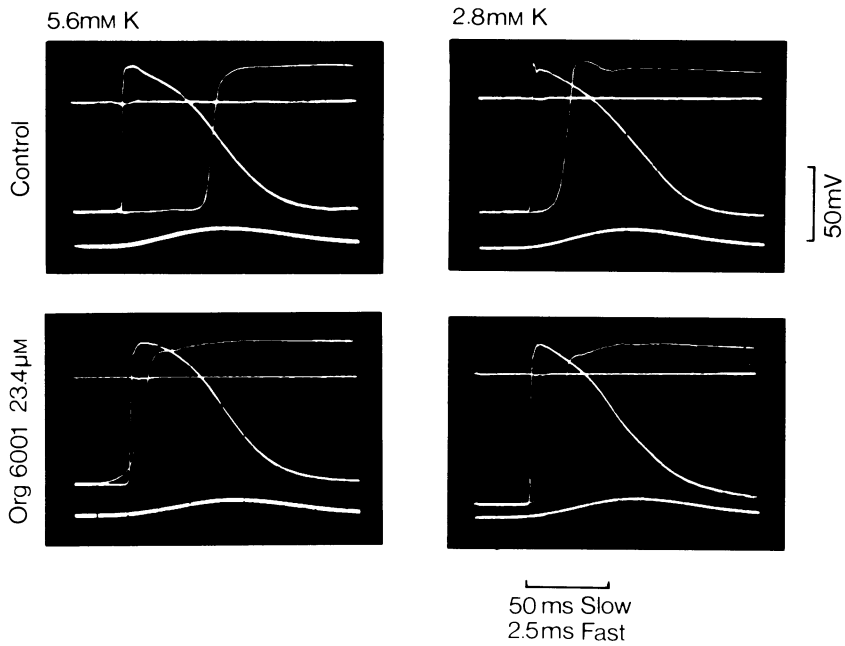

Figure 6 Influence of external potassium concentration on the effect of Org 6001 on rabbit atrial intracellular potentials. Explanation as for Figure 3. Low external potassium counteracted the effect of Org 6001, and shifted the dose-response curve, so that in low K higher concentrations of Org 6001 were required to produce the same effect as in normal K concentration.

rabbits) and 2.8 mM K. The order in which the atria were exposed to the different solutions was as follows: Controls, 5.6 mM K; controls, 2.8 mM K; Org 6001 5.8 μM at 2.8 mM K; Org 6001 5.8 μM at 5.6 mM K; Org 6001 23.4 μM at 5.6 mM K; Org 6001 23.4 μM at 2.8 mM K. Halving the external K raised the resting potential (in the absence of drug) by ten millivolts and MRD by 13 V/second. The effects of Org 6001 at the two levels of external potassium are presented in Table 6. Halving external K simply shifted the whole dose-response curve to the left. Thus at 2.8 mM K MRD in the presence of 23.4 μM Org 6001 was as fast as the control MRD at 5.6 mM K, but was nevertheless significantly slower than the control MRD at 2.8 mM K. The results do not support the view that Org 6001 irreversibly inactivated channels for inward Na current to any substantial extent. Representative examples of these results are depicted in Figure 6.

2. Adrenoceptor blocking effects (Class 2 action)

Dose-response curves for chronotropic and inotropic responses to isoprenaline by rabbit isolated atria were obtained in control solution and in the presence of 11.7 μM Org 6001. The results are plotted in Figure 7. It is apparent that Org 6001 had no blocking action on the responses to isoprenaline of the SA node. There was, however, a small but significant reduction in the inotropic responses to isoprenaline, but this would not be relevant to the arrhythmogenic action of catecholamines. It may be concluded that Org 6001 has no class 2 anti-arrhythmic action at the level of cardiac β-receptors and beyond, though the possibility of a presynaptic or central anti-sympathetic action remains to be examined.

3. Prolongation of action potential (Class 3 action)

The evidence presented in Tables 2 and 6 indicates that, so far as atrial muscle is concerned, Org 6001 had a very minor class 3 activity. In the ventricular conducting system APD is actually shortened, though some lengthening occurs in ventricular muscle, as in atrial muscle.

4. Antagonism to inward calcium currents (Class 4 action)

The suggestion that blockade of inward (depolarizing) calcium current might constitute a fourth class of anti-arrhythmic action was based upon the fact that the

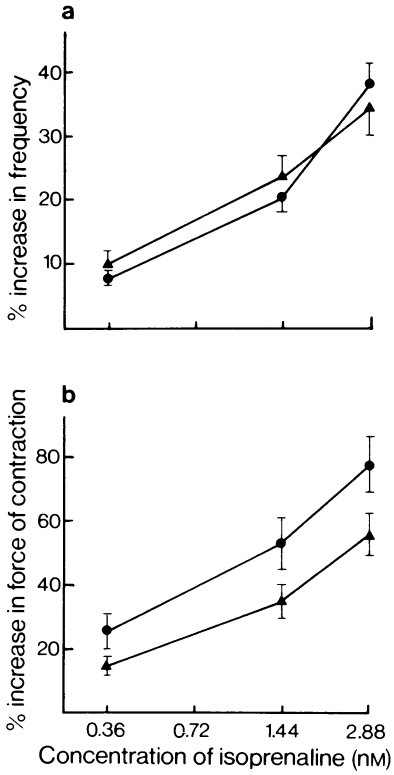

Figure 7 Effect of Org 6001 11.7 μM (4 μg/ml) on chronotropic and inotropic responses to isoprenaline. Ordinate scale; (a) % increases in heart rate and (b) in force of contraction. Abscissa scale; Concentration of isoprenaline (nM) on a logarithmic scale. The effect of Org 6001 on the inotropic responses was statistically significant by Student's paired t test ($P<0.05$). (●): Controls; (▲): Org 6001.

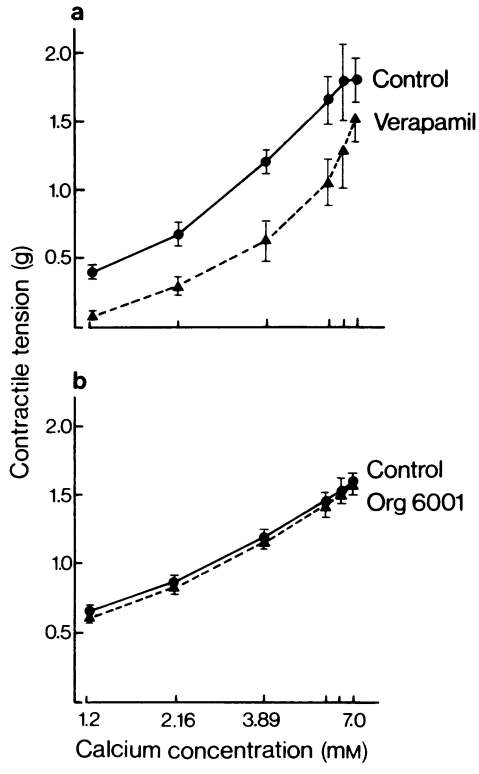

Figure 8 Effects of (a) (0.15 μg/ml) verapamil (a) and (b) Org 6001 (6 μg/ml) on the positive inotropic action of increases in Ca concentration in rabbit isolated atria. Ordinate scale: contractile force in grams. Abscissa scale: concentration (mM) of calcium in Locke solution on logarithmic scale.

drug verapamil, originally introduced as a coronary dilator, was undoubtedly anti-arrhythmic, yet had none of the three actions already described. It was strongly negatively inotropic, flattened the plateau of the action potential (Singh & Vaughan Williams, 1972b), and in high concentration reduced Ca uptake by 'sarcoplasmic reticulum' (Fleckenstein, Döring & Kammermeier, 1968), though this was disputed (Graca & van Zweiten, 1971), and its relevance to surface inward Ca-current is highly doubtful. Thus the classification of blockade of inward Ca-current as a fourth class of anti-arrhythmic action must still retain its question-mark.

The performance of voltage-clamp experiments in cardiac muscle is technically difficult, and the interpretation of the results obtained is controversial (Johnson & Lieberman, 1971). It was, therefore, decided to improvise a simple pharmacological test of the effects of external Ca concentration on contractile force in rabbit isolated atria, and to compare the effect of Org 6001 with that of verapamil. The results are presented in Figure 8. Over the range of Ca concentrations used, the relation between force and log $[Ca]_0$ is nearly linear, and over the lower part of this range verapamil caused a parallel shift to the right. The reason for which the highest Ca concentration had a tendency to 'overcome' the effect of verapamil is not known. It was quite clear, however, that Org 6001 had no effect whatever upon the relation between external Ca and contractile force, and, as thus defined, exhibited no 'calcium-antagonism' of the kind possessed by verapamil.

Discussion

A series of experiments by Marshall & Parratt (1975) indicated that Org 6001 had an anti-arrhythmic potency comparable with that of lignocaine in reducing the number of ectopic ventricular beats after ligation of a coronary artery in anaesthetized dogs. Another series of experiments (Vargäftig, Sugrue, Buckett & van Riezen, 1975) suggested that Org 6001 was one half as potent as lignocaine on desheathed frog nerve. *In vivo* tests in mice (tail-clip test) suggested that Org 6001 was 'much less effective than lignocaine as a local anaesthetic'.

It appeared at first sight, therefore, that although Org 6001 was an effective anti-arrhythmic compound, it could not be classified as a 'local anaesthetic' type. Although anti-arrhythmic compounds with direct effects on the cardiac membrane in reducing MRD usually also have local anaesthetic properties on nerve, the relative activities of various compounds on the two tissues are often very different. In the present paper we have found Org 6001 to be 1.8 times more potent than procaine on desheathed frog nerve. However, the onset of action of Org 6001 is much slower than that of procaine, and nerves exposed to concentrations of Org 6001 greater than the ED_{50} never fully recovered from the effects of the drug. Thus, at any rate at high concentrations, Org 6001 exhibited some neurotoxicity in addition to local anaesthesia. Such 'local anaesthetic' concentrations are of academic interest only, being about a hundred times greater than would be found in the plasma of treated patients.

In rabbit isolated atria Org 6001 had no effect on resting membrane potential at concentrations up to 46.8 μM. At a much lower concentration (2.9 μM) Org 6001 caused a statistically significant reduction in the maximum rate of depolarization (MRD) in association with a positive inotropic action. Even at the highest concentration used, the small depression of contractions by Org 6001 was not statistically significant. It was concluded, therefore, that Org 6001 had high direct membrane activity of class 1 type, with a negligible negative inotropic action. In common with other class 1 drugs, Org 6001 was much less effective in low K solutions than in those containing K in a concentration equal to that of rabbit plasma.

In isolated atria chronotropic responses to isoprenaline were not affected by Org 6001, and so there appeared to be no class 2 (anti-sympathetic) action on the heart. Class 3 action (prolongation of the action potential plateau) was also minor in isolated atria. Finally, by means of a simple new method for testing antagonism to the positive inotropic action of increases in calcium concentration, Org 6001 had no activity of the type exhibited by verapamil (Class 4).

The question arises whether the class 1 activity of Org 6001 is adequate to explain its anti-arrhythmic properties. There may well be types of anti-arrhythmic action other than the four classes described above. It is certain that, within the group of drugs possessing class 1 activity, subdivisions can be made on the basis of various side effects. For example, quinidine and disopyramide have atropine-like properties. Lignocaine and diphenylhydantoin have considerable action on the central nervous system. Lignocaine, and to a lesser extent propranolol also, shorten the action potential at high concentrations, especially in the Purkinje system. Whether these side effects have any relevance to their anti-arrhythmic properties is unproven. Certainly a shortening of the action potential duration would be likely to precipitate arrhythmias rather than to prevent them (Olsson, Cotoi & Varnauskas, 1971; Gavrilescu & Cotoi, 1972). What all these compounds have in common, in addition to their anti-arrhythmic effect, is the ability, in normal K solutions, to reduce MRD in the absence of any change in resting potential. On Occam's principle that hypotheses are not to be unnecessarily multiplied, the onus of proof that an extra hypothesis is necessary rests upon those who claim that the 'class 1' action of local-anaesthetic type drugs is inadequate to explain their anti-arrhythmic action.

In ventricular muscle the effects of Org 6001 on intracellular potentials were similar to those in atrial muscle; no change in resting potential, a large fall in MRD at low dosage, and a small lengthening of action potential duration. This is in contrast to the action of lignocaine, which shortens the APD in ventricular muscle (Wittig *et al.*, 1973).

However, in the ventricular conducting system Org 6001 shortened APD at all points. The effect was small in the bundle of His, but much greater in the central region in which the APD is normally much longer than elsewhere. As a result of these changes (some shortening in His, big effect in the middle, and lengthening in the muscle), the APD was made virtually uniform throughout by a concentration of 29.3 μM Org 6001. The question arises whether this shortening of APD in the conducting system is relevant to the anti-arrhythmic action of the drug. Presumably the very long APD in the distal Purkinje fibres has some functional significance, such as ensuring that ventricular muscle repolarizes first, to block any possibility of retrograde excitation of Purkinje fibres by ventricular muscle. Shortening of Purkinje APD might, therefore, in isolation be pro-arrhythmic, but less so in the presence of a simultaneous strong class 1 effect. Evidence already quoted associates short action potentials with greater probabilities of fibrillation.

On the other hand inhomogeneity is one of the factors predisposing to arrhythmias in 2- or 3-dimensional networks. Non-uniform recovery of excitability in ventricular muscle, which constitutes such a network, was demonstrated by Han & Moe

(1964) and was considered to be likely to enhance a tendency to arrhythmias (Moe, 1962). Thus the elimination of such inhomogeneity in the conducting system has been put forward as a possible reason for the anti-arrhythmic action of lignocaine (Wittig *et al.*, 1973) and propranolol (Harrison, Wittig & Wallace, 1973). However, the arguments for inhomogeneity being a predisposing factor to re-entry would not apply to a linear uni-directional conducting system.

Whether elimination of inhomogeneity in the conducting system (as opposed to the ventricular muscle) is a pro- or anti-arrhythmic effect remains to be proven. Perhaps the other effects of Org 6001 on Purkinje fibres, notably a raised electrical threshold, increased latency, reduced MRD and elimination of spontaneous pacemakers, may be of more significance to its anti-arrhythmic action.

The class 1 action of Org 6001, common to so many anti-arrhythmic drugs, was unequivocal in all tissues studied, and the drug appears to possess none of the other three classes of anti-arrhythmic action to any significant extent, though the small class 3 effect in atrial and ventricular muscle could be a contributing factor. It has virtually no depressant action on contractions in concentrations with high membrane activity. Since it is also rapidly absorbed when taken orally, has a long duration of action, and has very low general toxicity (Vargäftig *et al.*, 1975), Org 6001 obviously merits consideration for clinical trial, especially in maintenance or prophylactic therapy.

We wish to thank Dr M.F. Sugrue, Organon Laboratories, Newhouse, Lanarkshire for a supply of Org 6001, and for unpublished data concerning the compound, and the British Heart Foundation for an equipment grant. L.A.S. is a Wellcome Research Fellow, on leave from the University of Ibadan, Nigeria.

References

ALLEN, J.D., KOFI EKUE, J.M., SHANKS, R.G. & ZAIDI, S.A. (1970). The effect on experimental cardiac arrhythmias of a new anti-convulsant agent, Kö 1173, and its comparison with phenytoin and procainamide. *Br. J. Pharmac.*, **39**, 183–184P.

BAGWELL, E.E., POLSTER, P. & VAUGHAN WILLIAMS, E.M. (1973). The effects on cardiac muscle and nerve of a fluorinated decahydroquinoline derivative, L 7810, rapidly absorbed after oral administration. *Br. J. Pharmac.*, **48**, 183–193.

FLECKENSTEIN, A., DÖRING, H.J. & KAMMERMEIER, H. (1968). Einflus von Beta-receptorblockern und verwandten Substanzen auf Erregung, Kontraktion und Energiestoffwachsel der Myokardfaser. *Klin. Wochenschrift*, **46**, 343–351.

GAVRILESCU, S. & COTOI, S. (1972). Monophasic action potential of right human atrium during atrial flutter and after conversion to sinus rhythm. *British Heart J.*, **34**, 396–402.

GRACA, A.S. & VAN ZWIETEN, P.A. (1971). The influence of iproveratril on calcium movements in isolated heart muscle. *Eur. J. Pharmac.*, **15**, 137–140.

HAN, J. & MOE, G.K. (1964). Non-uniform recovery of excitability in ventricular muscle. *Circulation Res.*, **14**, 44–60.

HARRISON, L.A., WITTIG, J.H. & WALLACE, A.G. (1973). Adrenergic influences on the distal Purkinje system of the canine heart. *Circulation Res.*, **33**, 329–339.

JOHNSON, E.A. & LIEBERMAN, M. (1971). Heart: excitation and contraction. *Ann. Rev. Physiol.*, **33**, 479–532.

MARSHALL, R.J. & PARRATT, J.R. (1975). Anti-arrhythmic, haemodynamic and metabolic effects of 3α-amino-5α-androstan-2β-ol-17-one hydrochloride in greyhounds following acute coronary ligation. *Br. J. Pharmac.*, **55**, 359–368.

MEYERBERG, R.J., STEWARD, J.W. & HOFFMAN, B.F. (1970). Electrophysiological effects of canine peripheral A–V conducting system. *Circulation Res.*, **26**, 361–378.

MOE, G.K. (1962). On the multiple wavelet hypothesis of atrial fibrillation. *Arch. int. Pharmacodyn.*, **140**, 183–188.

OLSSON, B., COTOI, S. & VARNAUSKAS, E. (1971). Monophasic action potential and sinus rhythm stability after conversion of atrial fibrillation. *Acta med. scand.*, **190**, 381–388.

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.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1972a). Investigations of the mode of action of a new antidysrhythmic drug, Kö 1173. *Br. J. Pharmac.*, **44**, 1–9.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1972b). A fourth class of antidysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. *Cardiovasc. Res.*, **6**, 109–119.

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

VARGÄFTIG, B.B., SUGRUE, M.F., BUCKETT, W.R. & VAN RIEZEN, H. (1975). Org 6001 (3α-amino-5α-androstan-2β-ol-17-one hydrochloride), a steroidal anti-arrhythmic agent. *J. Pharm. Pharmac.*, **27**, 697–699.

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

VAUGHAN WILLIAMS, E.M. (1970). Classification of anti-arrhythmic drugs. In *Symposium on Cardiac Arrhythmias*, ed. Sandøe, E., Flensted-Jensen, E. & Olesen, K.H., pp. 449–472. Södertalje, Sweden: AB Astra.

VAUGHAN WILLIAMS, E.M. (1974). Electrophysiological basis for a rational approach to anti-dysrhythmic drug therapy. *Advances in Drug Research*, **9**, 69–102.

VAUGHAN WILLIAMS, E.M. (1975). Classification of antidysrhythmic drugs. *Pharmacology and Therapeutics B*, **1**, 115–138.

VAUGHAN WILLIAMS, E.M., SALAKO, L.A. & WITTIG, J. (1976). The effect on atrial and ventricular potentials, and other pharmacological actions, of L 9146, a non-halogenated benzo(b)thiophene related to amiodarone. *Cardiovasc. Res.* (in press).

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

WEIDMANN, S. (1955). The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J. Physiol., Lond.*, **127**, 213–224.

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

(Received October 29, 1975.
Revised December 10, 1975)