Br. J. Pharmac. (1980), **68**, 25–31

COMPARATIVE ELECTROPHYSIOLOGICAL EFFECTS OF Org 6001, A NEW ORALLY ACTIVE ANTIDYSRHYTHMIC AGENT, AND LIGNOCAINE ON HUMAN VENTRICULAR MUSCLE

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1 The electrophysiological effects of Org 6001, a new orally active antidysrhythmic agent, have been compared with those of lignocaine on the human ventricular action potential *in vitro*.

2 Org 6001 (4 to 16 mg/l) greatly reduced the maximum rate of depolarization (MRD) of the human ventricular action potential but had no effect on resting membrane potential or action potential amplitude.

3 The action potential duration at the 50% repolarization level, but not at the 90% repolarization level, was significantly reduced by Org 6001. The absolute refractory period was unchanged.

4 Lignocaine, at a concentration (4 mg/l) within the therapeutic range, had no significant effect on any measured parameter, either in muscle exposed to a normal (4.0 mM) or high (5.4 mM) extracellular potassium concentration ($[K^+]_o$).

5 Higher concentrations of lignocaine (8 to 16 mg/l) did, however, reduce MRD at both $[K^+]_o$ without changing resting membrane potential or action potential amplitude. The action potential duration was decreased slightly by these higher concentrations of lignocaine whilst the absolute refractory period was lengthened.

6 Org 6001 was found to be more potent than lignocaine in reducing MRD but, unlike lignocaine, the absolute refractory period was not prolonged. These compounds, therefore, differed in their electrophysiological effects on human ventricular muscle although both are characterized as being class 1 antidysrhythmic drugs.

Introduction

Org 6001 (3α-amino-2βhydroxy-5α-androstan-17-one hydrochloride) is a new orally active antidysrhythmic agent. Marshall & Parratt (1975) have shown that Org 6001 is very effective in reducing the number of ventricular dysrhythmias arising from coronary artery ligation in anaesthetized dogs and clinical trials, currently underway, have shown potent antidysrhythmic activity in man (H. Jansen, personal communication). Org 6001 has been categorized as a class 1 antidysrhythmic drug on the basis of its ability to reduce the maximum rate of depolarization of phase zero (MRD) of the rabbit cardiac muscle action potential (Salako, Vaughan Williams & Wittig, 1976). The following studies were undertaken to determine if Org 6001 had similar class 1 effects in human ventricular muscle and to compare its effects with that of the standard antidysrhythmic drug, lignocaine.

There have been a number of studies to determine the electrophysiological basis for the antidysrhythmic effects of lignocaine. Work by Bigger & Mandel (1970) and by Davis & Temte (1969) indicated that therapeutic concentrations of lignocaine increased MRD of canine cardiac tissue. However, Singh & Vaughan Williams (1971) showed that the effects of lignocaine on MRD of rabbit atrial and ventricular muscle depend upon the extracellular potassium concentration ($[K^+]_o$). They found that when $[K^+]_o$ was 5.6 mM, therapeutic concentrations of lignocaine markedly reduced MRD. However, on lowering $[K^+]_o$ to 3 mM this effect was abolished. The mean normal human plasma level of K^+ is reported to be 4.2 mEq/l (Singer, 1964). At this $[K^+]_o$, lignocaine has been shown on canine Purkinje fibres either to have no effect on MRD or to reduce it slightly (Obayashi, Hayakawa & Mandel, 1975; Rosen, Merker & Pippenger, 1976). In view of these conflicting results the effects of lignocaine on human ventricular muscle action potentials were examined at both normal and high $[K^+]_o$ of 4.0 and 5.4 mM respect-

0007-1188/80/010025-07 $01.00

ively. A preliminary account of some of these studies has been presented to the British Pharmacological Society (Kane, 1977).

Methods

The experiments were carried out on small pieces (<1 cm^2) of ventricular muscle obtained from 16 children undergoing corrective open-heart surgery for ventricular septal defects. The patients had not received any antidysrhythmic or cardiotonic medication prior to surgery. The muscle was immersed in cool Tyrode solution and taken to the laboratory within 40 min after excision.

The preparation were pinned to the Sylgard base of the recording chamber and superfused with Tyrode solution at a rate of 6 ml/min. The Tyrode solution had the following composition (mM): NaCl 125, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 0.5, $CaCl_2$ 2.7, glucose 5.5 and KCl either 4.0 or 5.4. The solution, which was equilibrated with 95% O_2 and 5% CO_2, had a pH within the range 7.2 to 7.4. The temperature was maintained at 36 ± 1.0°C.

The preparation was stimulated at a frequency of 1 Hz by rectangular pulses, 1 ms in duration and twice threshold voltage, delivered through a bipolar silver electrode. Through the same electrode a second pulse, 1 ms in duration and three times threshold voltage, could be applied after every sixth driving stimulus for the determination of the absolute refractory period. Transmembrane action potentials were recorded with 3 M KCl-filled microelectrodes, the resistances of which ranged from 5 to 30 MΩ. The electrodes were coupled by a silver-silver chloride wire to an electrometer with a high impedance and capacity neutralization. The maximum rate of depolarization of phase zero of the action potential was determined either by an electronic differentiating circuit (time constant 330 μs), which was linear from 25 to 1000 V/s, or directly from a fast sweep recording of the action potential upstroke. The transmembrane action potentials and the differentiated upstrokes were displayed on a storage oscilloscope and photographed on 35 mm film. The parameters measured were: resting membrane potential, (RMP); action potential height, (AP); maximum rate of depolarization (MRD), the action potential duration at 90% and 50% repolarization levels, (APD_{90} and APD_{50}) and the absolute refractory period (ARP).

Stock solutions of lignocaine hydrochloride B.P. (Astra-Hewlett) and Org 6001 hydrochloride (Organon International) were prepared in distilled water. The stock solution was added to a reservoir of gassed Tyrode solution to obtain final concentrations of 4, 8 and 16 mg/l of each drug. These concentrations are equivalent to 1.72 to 6.87 × 10^{-5} M of lignocaine and 1.17 to 4.68 × 10^{-5} M Org 6001. The tissue which was allowed to equilibrate for 90 min before control action potentials were recorded, was then superfused with the drug solution and records taken 30 to 40 min later. A recovery period of 1 h was allowed before another set on control records was obtained and the muscle exposed to a higher drug concentration.

The statistical significance of differences between means was calculated by Student's t test.

Results

A typical control action potential obtained from human ventricular muscle can be seen in Figure 1. The control parameters obtained from 132 impale-

Table 1 Effect of Org 6001 on human ventricular action potentials

Dose of Org 6001 (mg/l)	*No. of cells*	*Resting potential (mV)*	*Action potential (mV)*	*MRD (V/s)*	*APD_{50} (ms)*	*APD_{90} (ms)*
Control	23	85.5 ± 1.0	114.8 ± 1.5	246 ± 9*	175.1 ± 5.3	267.4 ± 6.4
4	20	81.7 ± 1.2	111.2 ± 1.4	202 ± 11% (−18)	159.4 ± 2.0* (−9)	256.4 ± 2.7 (−4)
Control	33	85.5 ± 0.9	115.4 ± 1.0	227 ± 9	225.3 ± 2.5	306 ± 9.9
8	31	84.0 ± 0.9	111.5 ± 1.2	160 ± 12** (−30)	203.2 ± 6.0** (−10)	287.1 ± 11.0 (−6)
Control	26	82.2 ± 1.3	110.7 ± 1.4	230 ± 12	199.7 ± 7.8	297.5 ± 8.0
16	31	80.5 ± 1.0	106.6 ± 1.2	133 ± 7** (−42)	180.6 ± 6.3* (−10)	293.1 ± 6.3 (−1)

Values are means ± s.e. mean with the percentage change from controls in parentheses. n = number of observations obtained from two or three preparations at each dose level ($[K^+]_o$ = 4.0 mM). * $P < 0.01$; ** $P < 0.001$.

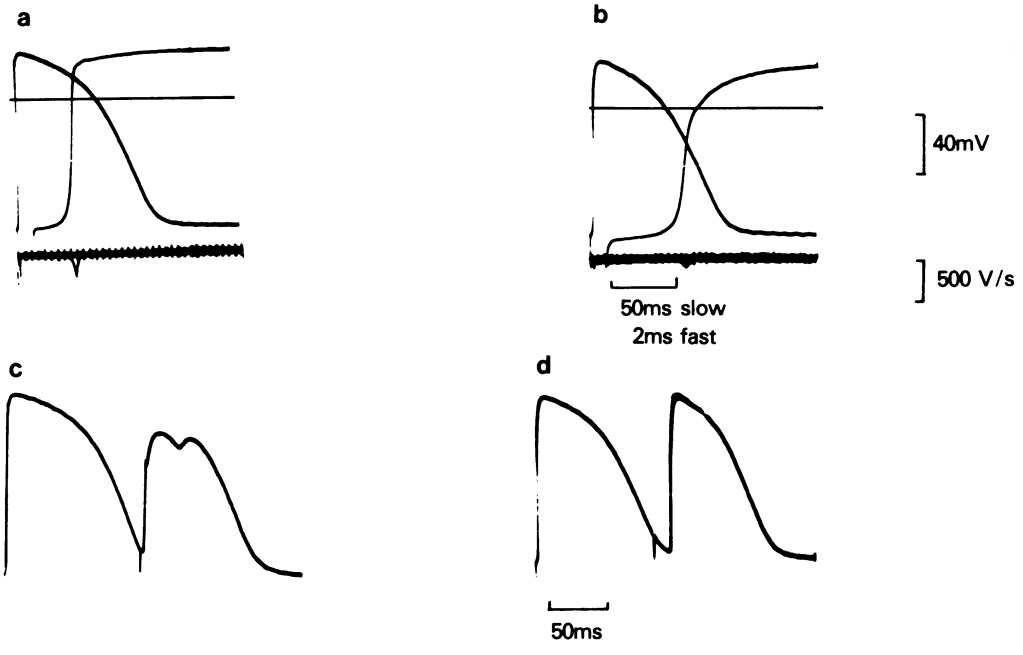

Figure 1 Effect of Org 6001 (8 mg/l) on action potential characteristics of human ventricular muscle exposed to $[K^+]_o$ of 4.0 mM. (a and b): Potentials, from control and drug-treated muscles respectively. In each section, the horizontal lines indicate zero potential; middle traces are intracellular potentials at slow and fast sweep speeds; bottom traces represent the differentiated signal of the action potential upstroke. (c and d): Potentials from control and drug-treated muscles. Each section shows the earliest response, i.e. the end of the ARP, which could be elicited by application of a second pulse.

ments in 12 preparations exposed to $[K^+]_o$ of 4.0 mM were: RMP 81.9 ± 0.5 mV; AP 113.1 ± 0.5 mV; MRD 225 ± 5 V/s; APD_{90} and ADP_{50} 269.9 ± 3.3 and 190.5 ± 2.5 ms respectively. Table 1 shows the effects of Org 6001, in concentrations of 4 to 16 mg/l, on action potential characteristics obtained from 6 preparations. Significant dose-dependent decreases in MRD were produced by these concentrations of the drug. The decreases in MRD were observed without significant changes in RMP or action potential amplitude. APD_{90} was not significantly changed, although APD_{50} was slightly shortened at each dose level. The ARP was not altered by Org 6001 over the concentration range studied. The values of the ARP before and after exposure to 8 mg/l were 304 ± 34 and 293 ± 36 ms respectively ($n = 5$ observations in 4 preparations). Corresponding values before and after a concentration of 16 mg/l were 302 ± 37 and 310 ± 34 ms ($n = 4$ observations in 3 preparations). The records shown in Figure 1 illustrate the effects of a dose of 8 mg/l of Org 6001.

The effects of 4 to 16 mg/l of lignocaine on action potential characteristics in 6 preparations exposed to 4.0 mM $[K^+]_o$ are summarized in Table 2 and an example of an original record obtained at a dose of 8 mg/l is shown in Figure 2. Lignocaine, 4 mg/l, did reduce MRD from 216 ± 6 to 212 ± 6 V/s but neither this effect nor that on any of the other measured parameters was statistically significant. Higher doses of lignocaine (8 and 16 mg/l) did significantly reduce MRD although the effect was not dose-dependent. The effects of lignocaine and Org 6001 on MRD of human ventricular action potentials are contrasted in Figure 3. It can be seen that in the concentration range studied, lignocaine was less potent than Org 6001 in reducing MRD. The reductions in MRD produced by the higher concentrations of lignocaine were not accompanied by a change in RMP or action potential amplitude. APD_{50} was slightly decreased by 8 and 16 mg/l of lignocaine whereas APD_{90} was only significantly reduced by the highest concentration of lignocaine studied. The ARP was significantly increased by the two higher concentrations of lignocaine, but unchanged by a concentration of 4 mg/l. In a concentration of 8 mg/l, lignocaine increased the ARP from 274 ± 12 ms to 322 ± 13 ms ($n = 21$; 4 preparations) and 16 mg/l lignocaine from 304 ± 5 ms to 402 + 19 ms ($n = 18$; 4 prep-

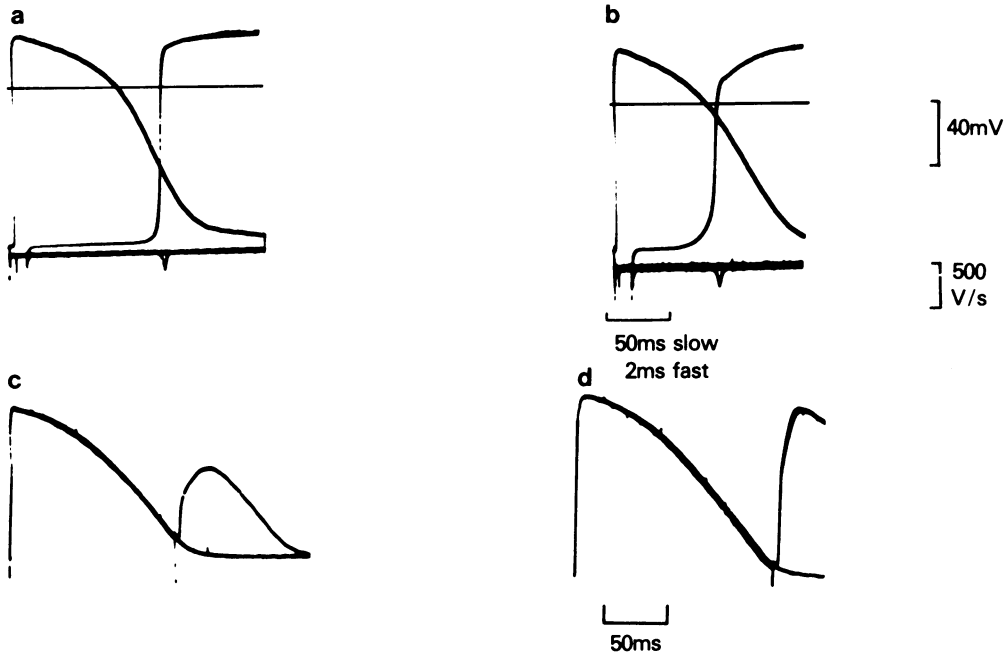

Figure 2 Effect of lignocaine (8 mg/l) on action potential characteristics of human ventricular muscle exposed to $[K^+]_o$ of 4.0 mM. Potentials from control (a and c) and drug-treated (b and d) muscles. Explanation as for Figure 1.

arations). As can be seen from Figure 2, after lignocaine the earliest response which could be produced during an action potential could not be elicited until the membrane had repolarized to a higher level than in the control.

The effects of 4 to 16 mg/l of lignocaine were also studied in 4 preparations in which $[K^+]_o$ was 5.4 mM. In these preparations, control RMP, action potential height and MRD were decreased slightly to respective mean values of 77.6 ± 1.1 mV; 108.4 ± 1.5 mV;

Table 2 Effect of lignocaine on human ventricular action potentials

Dose of lignocaine (mg/l)	*No. of cells*	*Resting potential (mV)*	*Action potential (mV)*	*MRD (V/s)*	*APD_{50} (ms)*	*APD_{90} (ms)*
Control	43	79.1 ± 0.9	111.5 ± 0.8	216 ± 6	183.0 ± 1.4	254.5 ± 2.4
4	36	80.2 ± 0.8	112.0 ± 1.0	212 ± 6	192.3 ± 1.6** (+5)	266.4 ± 2.6* (+5)
Control	39	80.5 ± 0.7	111.4 ± 1.0	212 ± 9	199.1 ± 3.3	274.1 ± 7.6
8	41	80.7 ± 0.8	111.7 ± 0.8	172 ± 6** (−19)	189.9 ± 4.1 (−5)	275.2 ± 3.9
Control	39	85.0 ± 0.9	115.0 ± 2.7	193 ± 8	229.7 ± 3.6	311.7 ± 5.1
16	36	85.1 ± 0.8	115.1 ± 1.0	164 ± 6* (−15)	196.4 ± 2.8 (−14)	277.7 ± 4.9** (−11)

Values are the means ± s.e. mean with the percentage change from controls in parentheses. n = number of observations obtained from at least three preparations at each dose level ($[K^+]_o$ = 4.0 mM) *$P < 0.01$; ** $P < 0.001$.

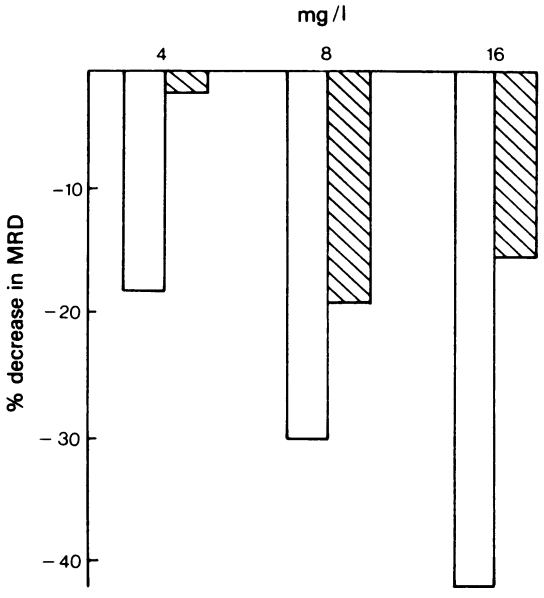

Figure 3 The effect of lignocaine (hatched columns) and Org 6001 (open columns) (4 to 16 mg/l) on maximum rate of depolarization (MRD) of human ventricular muscle.

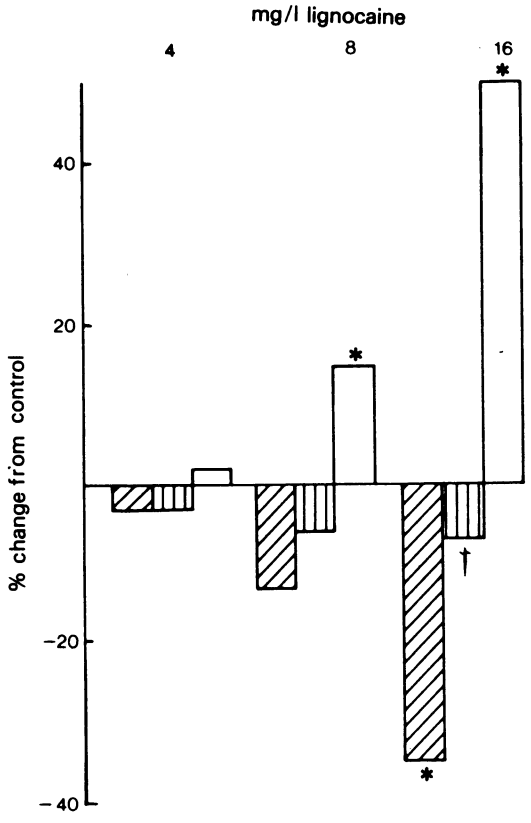

Figure 4 Effect of lignocaine (4 to 16 mg/l) on action potential characteristics of human ventricular muscle exposed to 5.4 mM $[K^+]_o$. Ordinate scale: % change in maximum rate of depolarization (hatched columns), action potential duration at 90% depolarization level (vertically lined columns) and absolute refractory period (open columns). * $P < 0.001$; † $P < 0.02$.

156 ± 11 V/s; whereas APD_{90} and APD_{50} were unchanged with respective mean values of 279.9 ± 8.7 and 210.4 ± 6.2 ms. Figure 4 shows the percentage changes in MRD, APD_{90} and ARP produced by 4 to 16 mg/l of lignocaine in the high $[K^+]_o$. Neither RMP nor action potential height were altered by these concentrations of lignocaine. As in the preparations exposed to 4.0 mM $[K^+]_o$, 4 mg/l of lignocaine had no significant effect on any measured parameter. However, higher doses of lignocaine did produce a dose-dependent decrease in MRD. At these concentrations, the ARP was significantly prolonged whereas APD_{90} was slightly shortened.

Discussion

Very few electrophysiological investigations using intracellular microelectrode techniques have been carried out in human cardiac tissue, partly because of marked variability which has been observed in the action potential charactersitics. In particular, low RMP and action potential amplitudes have been recorded from adult tissue which is often diseased and in which the presence of abundant connective tissue makes impalement with the microelectrode very difficult. Gelband, Bush, Rosen, Myerburg & Hoffman (1972) have shown, however, that action potentials with normal RMP and amplitude can be obtained from human atrial muscle obtained from children. This study was therefore carried out on ventricular muscle obtained from children in whom no evidence of cardiac dysrhythmias or failure was present. The characteristics of the human ventricular muscle action potentials observed in this study were not different from those of other mammalian ventricular tissue and are in agreement with the values observed by Trautwein, Kassebaum, Nelson, & Hecht (1962). The tissue was, therefore, considered to be electrophysiologically normal.

The new antidysrhythmic drug Org 6001, in the concentration range 4 to 16 mg/l, has been shown to produce a marked dose-dependent decrease in MRD of the human ventricular action potential. The result is consistent with the categorization of Org 6001 as a class 1 antidysrhythmic drug (Salako *et al.*, 1976). In the concentration range studied (4 to 16 mg/l), ligno-

caine has been shown to be less potent than Org 6001 in reducing MRD of human ventricular muscle. Lignocaine at 4 mg/l, had no significant effect on MRD either in normal (4.0 mM) or high (5.4 mM) $[K^+]_o$. However, higher doses of lignocaine did decrease MRD at both $[K^+]_o$ concentrations. These results are in agreement with those of Obayashi *et al.* (1975) and Allen, Brennan & Wit (1975) who have shown that when $[K^+]_o$ is 4.0 mM, MRD of normal canine Purkinje fibres is not decreased by concentrations of lignocaine considered to be within the therapeutic range (1 to 5 mg/l). On the other hand, the results obtained are inconsistent with those of Singh & Vaughan Williams (1971) who reported that depression of MRD by lignocaine became more pronounced when the resting membrane potential was decreased by increasing $[K^+]_o$. These inconsistent results may be explained by the fact that the resting membrane potential of human ventricular muscle was not markedly reduced when exposed to the higher $[K^+]_o$ (a similar finding has been reported in human atrial muscle by Gelband *et al.* (1972)) and, as shown by Chen, Gettes & Katzung (1975), 4 mg/l of lignocaine has minimal class 1 activity when the resting membrane potential is approximately -80 mV.

The APD, at both 50 and 90% repolarization levels, was slightly decreased by Org 6001, the effect being significant only on APD_{50}. In rabbit ventricular muscle, Org 6001 was found to prolong the action potential duration slightly (Salako *et al.*, 1976). These divergent results may indicate a species difference in the effect of Org 6001 on APD. The highest concentration of lignocaine studied significantly shortened the human ventricular action potential duration at 50 and 90% repolarization levels, the effect being similar in both $[K^+]_o$. These results are in agreement with those obtained in both canine and rabbit ventricular muscle (Davis & Temte, 1969; Singh & Vaughan Williams, 1971). The ARP was unchanged by Org 6001 (4 to 16 mg/l) whereas it was significantly prolonged, in both $[K^+]_o$, by lignocaine (8 and 16 mg/l). This effect of lignocaine has previously been described on canine ventricular muscle by Davis & Temte (1969). This prolongation of ARP, independent of a lengthening in APD, may be explained by the ability of lignocaine to prolong the reactivation kinetics of the rapid inward sodium current (Chen *et al.*, 1975).

It is not known to what extent the antidysrhythmic properties of lignocaine can be explained by the prolongation of the recovery of the sodium current. As this effect was observed at the same concentrations which reduced MRD in human ventricular muscle, it seems possible the class 1 activity of lignocaine may not fully explain the antidysrhythmic properties of this compound. Org 6001, on the other hand, had a marked class 1 activity without prolonging ARP. This action, which is akin to that of quinidine (Vaughan Williams, 1958), may explain the potent antidysrhythmic properties of Org 6001. The differential effects of Org 6001 and lignocaine on MRD and ARP of human ventricular muscle support the suggestion of Chen *et al.* (1975) that it may prove necessary to subdivide class 1 drugs into those which only alter the steady state variables e.g. quinidine and Org 6001, and those e.g. lignocaine, which also affect the kinetic variables.

I am most grateful to the late Professor P. Caves for so readily making available human ventricular tissue. This work was supported by Organon International Ltd.

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(Received July 28, 1978.
Revised February 1, 1979.)