J. Pharm. Pharmacol. 1992, 44: 996–1000
Received March 23, 1992

Antiarrhythmic, Electrophysiological and Haemodynamic Effects of Prolonged Oral Dosing with Org 7797 in the Anaesthetized Rat

L. DELBRESSINE, N. HARRIS*, K. A. KANE*, A. W. MUIR AND E. WINSLOW

*Organon Laboratories Limited, Newhouse, Lanarkshire ML1 5SH, and *Department of Physiology & Pharmacology, University of Strathclyde, 204 George Street, Glasgow G1 1XW, UK*

Abstract—The antiarrhythmic, electrophysiological and haemodynamic effects of chronic oral administration of Org 7797 ((16α,17β)-17-methylamino-oestra-1,3,5(10)-triene-3,16-diol-(Z)-2-butonedioate) were studied in rats. During dosing (10 mg kg^{-1} twice a day for 10 days) no effects on the electrocardiogram, monitored in conscious animals, were observed despite modest reductions (15–18%) in the maximum rate of depolarization of papillary muscle excised 1 or 6 h after completion of the dosing regime. Following anaesthesia, Org 7797 reduced the severity of arrhythmias induced by coronary artery occlusion and prevented the accompanying decrease in the ventricular fibrillation threshold (VFT) at 1 h after completion of dosing. By 6 h the effect on VFT had waned but protection against ischaemia-induced arrhythmias was retained despite a substantial decrease in Org 7797 plasma levels. Drug treatment did not modify arterial blood pressure, heart rate or stroke volume. We conclude that Org 7797 given chronically via the oral route exerts antiarrhythmic actions which may, at least in part, be due to sodium-channel block. In addition, our results suggest the presence of an active metabolite. The protective effects of Org 7797 were seen in the absence of electrocardiographic or haemodynamic changes suggesting that multiple oral doses of Org 7797 do not compromise normal cardiac function.

Org 7797, (16α,17β)-17-methylamino-oestra-1,3,5(10)-triene-3,16-diol-(z)-2-butenedioate, is a new steroidal class Ic antiarrhythmic agent (Winslow et al 1989a; Campbell et al 1991) currently undergoing clinical evaluation. This compound when given actuely via the intravenous or intra-arterial route prevents ventricular fibrillation in rodent (Winslow et al 1989b), canine (Winslow et al 1991b) and porcine (Janse et al 1990) hearts subjected to acute myocardial ischaemia. Moreover, in canine studies, intravenous Org 7797 in effective antifibrillatory doses does not compromise cardiac function (Winslow et al 1991b).

The aims of the present study were to assess the oral activity of Org 7797 and to evaluate its antiarrhythmic, haemodynamic and electrophysiological actions when given chronically via the oral route.

Materials and Methods

Single dose study: arrhythmias induced by acute coronary artery ligation

Male Wistar rats fed on Labsure CRMX pellets and weighing between 300 and 620 g were anaesthetized with 60 mg kg^{-1} sodium pentobarbitone given intraperitoneally, and were artificially ventilated with room air (10 mL kg^{-1}, 48 strokes min^{-1}). A left thoractomy was performed and the main left descending coronary artery (LAD) prepared for ligation as described by Clark et al (1980). Femoral arterial blood pressure and a limb lead II electrocardiogram (ECG) were recorded on a Mingograph 82 ink-jet recorder. After a 15 min stabilization period, the LAD was ligated. The total number of ventricular premature beats (VPBs) together with the number of animals which developed ventricular fibrillation (VF) during the ensuing 30 min period were noted.

Correspondence: E. Winslow, Organon Laboratories Limited, Newhouse, Lanarkshire ML1 5SH, UK.

Drugs or vehicle (1 mL kg^{-1}, 5% mulgofen in distilled water) were given orally 1 h before coronary artery ligation. Mexiletine, propafenone and disopyramide were included in this part of the study for comparison of oral efficacy.

Chronic oral dosing

Rats weighing between 335 and 549 g were used for this study.

The electrocardiogram and ischaemia-induced arrhythmias. Rats were trained over a 4-day period to stand on copper footplate electrodes for ECG monitoring. At the end of the training period, the animals were orally dosed with 10 mg kg^{-1} Org 7797 given every 12 h for 10 days. Control animals received an equivalent volume (2 mL kg^{-1}) of distilled water. A lead II ECG was recorded at 1 and 6 h after each morning dose. One or 6 h after completion of the dosing regime, coronary artery ligation was performed as described above.

Determination of ventricular fibrillation thresholds (VFT) and haemodynamic measurements. Animals were anaesthetized and artifically ventilated as described above. A left thoracotomy was performed and the chest walls retracted. Two platinum electrodes (6 mm apart) embedded in a narrow rubber strip, were positioned on the left ventricular anterior wall such that the anode was approximately 3 mm below the atrioventricular ring and the cathode on or near the apex. The rubber strip was held in place by means of anchoring threads. Square wave pulses (0·8 ms duration 50 Hz) were passed through a constant current stimulus isolation unit to give an initial current intensity of 100 μA. Current intensity was then gradually increased at a rate of 10 μA s^{-1} until VF developed. All animals reverted spontaneously to normal sinus rhythm on cessation of stimulation. VFT determinations were made 15 min before and 3–4 min

after ligation. Pre-ligation determinations were made 1 or 6 h after the last dose of Org 7797 was given. Control animal determinations were made 1 h after last dose of vehicle.

Arterial blood pressure recorded from the left femoral artery and a lead II ECG were displayed on a Mingograph 82 ink-jet recorder.

Cardiac output was determined using a Gould 3F thermister catheter inserted into the aortic arch via the right carotid artery. A 0·25 mL bolus of 0·9% NaCl (saline) at room temperature (21°C) was injected into the right atrium via the right jugular vein and cardiac output was obtained from a Gould cardiac output computer. Haemodynamic measurements were made before thoracotomy (during spontaneous respiration). Derived parameters were stroke volume, peripheral vascular resistance and external cardiac work.

Recording of transmembrane action potentials

A third group of animals was dosed as described above. One or 6 h after completion of the dosing regime, left papillary muscles were removed and pinned to the base of a recording chamber. The tissues were superfused with physiological salt solution of composition (mM): NaCl 119·6, $NaHCO_3$ 25·0, NaH_2PO_4 1·2, KCl 4·7, $MgCl_2$ 0·57, $CaCl_2$ 2·5 and D-glucose 5·5 gassed with 95% O_2–5% CO_2 and maintained at a temperature of 37 ± 0·5°C. The muscle was stimulated at a frequency of 1 Hz with rectangular pulses of 1 ms duration delivered through a bipolar silver electrode at twice threshold voltage. Cellular transmembrane action potentials were recorded using conventional micro-electrode techniques. The parameters measured were resting membrane potential, action potential height and the times taken to reach 50 and 90% repolarization (APD50 and APD90, respectively). The maximum rate of depolarization (V_{max}) was obtained by electronic differentiation. After a 30 min stabilization period, 8–12 action potentials were recorded from each preparation.

Measurement of Org 7797 plasma levels

Animals were given a single oral dose (20 mg kg^{-1}) of Org 7797 and arterial blood samples taken 1·5 or 4·5 h after administration. The samples were centrifuged and 100 μL of plasma removed for mixing with 0·38% trisodium citrate.

Org 7797 was extracted using SPE C18 cartridges pretreated with one column volume of methanol and one volume of 0·001 M EDTA at pH 3·0. One hundred microlitres of plasma mixed with an internal standard was washed through the column with 1 volume of each of 0·001 M EDTA, 0·1 M NH_4OAc (pH 4·2)/acetonitrile (20:1, v/v) and diethylether. Finally, the cartridge was washed with ethanol. This eluate contained Org 7797 and the internal standard. After evaporation of ethanol, the residue was redissolved in 50 μL methanol/acetonitrile/0·1 M NH_4OAc (pH 4·2) (25:25:50 by volume).

The HPLC system consisted of a Novapak C18-column and an LC-8-DB guard column with 11% acetonitrile in 0·1 M NH_4OAc (pH 4·2) as the mobile phase in conjunction with electrochemial detection (+800 mV). The flow was 1·2 mL min^{-1} and the column was eluted at 40°C. The detection limit was 10 ng. Simultaneously with the plasma samples, a standard calibration curve for Org 7797 and internal standard was processed. The accuracy and precision was judged from plasma samples containing known amounts of drug analysed concomitantly. The Org 7797 concentrations were calculated using the simultaneously processed calibration curve.

Statistics

A Chi-square test was used to detect the significance of differences between the incidences of VF.

A paired *t*-test was used to detect significant differences between pre- and post-occlusion values of VFT.

All other comparisons were made using Student's unpaired *t*-test except where the number of groups being compared with a control group exceeded two, in which case Dunn's test was used (for $P < 0·05$).

Results

Single dose

Early ischaemia-induced arrhythmias. The incidence of VF compared with controls was significantly reduced by 10–20 mg kg^{-1} Org 7797, 50 mg kg^{-1} mexiletine or 100 mg kg^{-1} disopyramide (Table 1). Propafenone (100 mg kg^{-1}) also appeared to reduce the incidence of VF but the result did not attain statistical significance. The total number of VPBs was also significantly reduced by 50 mg kg^{-1} mexiletine, propafenone or disopyramide and by 20 mg kg^{-1} Org 7797.

Plasma levels of Org 7797 following a single oral dose (20 mg kg^{-1}). Plasma levels of Org 7797 were substantially and significantly smaller 4·5 h after dosing than those found 1·5 h after dosing, whereas the antiarrhythmic and antifibrillatory efficacy of the compound was similar at both time intervals (Table 2).

Chronic dosing

The electrocardiogram. The RR, PR and QRS intervals remained unchanged during the dosing regime in both control and Org 7797-treated groups (Fig. 1).

Early ischaemia-induced arrhythmias. The numbers of VPBs in the Org 7797-treated groups were significantly smaller than those recorded from control animals (Table 3). However, although fewer drug-treated animals fibrillated compared with controls, the differences were not significant.

Table 1. Oral activity against early ischaemia-induced arrhythmias following a single dose given 1 h before coronary artery ligation.

Treatment	Dose (mg kg^{-1})	n	Total VPBs (0–30 min)	% VF
Controls		18	1458 ± 206	44
Org 7797	10	7	1470 ± 635	14*
	20	6	675 ± 267*	0*
Mexiletine	20	8	1382 ± 554	50
	50	7	510 ± 217*	0*
Propafenone	50	6	759 ± 152*	33
	100	7	1047 ± 611	14
Disopyramide	50	7	576 ± 244*	29
	100	7	941 ± 481*	0*

n denotes the number of animals in each group. *$P < 0·05$ denotes a significant difference from the appropriate control value.

Table 2. Plasma levels of Org 7797 found 1·5 and 4·5 h after a single oral dose (20 mg kg^{-1}). Coronary artery ligation was performed either 1 or 4 h after dosing.

Treatment		n	Total VPBs (0–30 min)	% VF	Plasma levels (ng mL^{-1})
Controls		20	1322±250	40	—
Org 7797	1–1·5 h	6	438±179*	0*	102±17
	4–4·5 h	5	316±161**	0*	26±2†

*$P<0·05$, **$P<0·01$ denote significant differences from the appropriate control group. †Denotes a significant difference ($P<0·01$) between plasma levels measured at the two time intervals.

Table 3. Effects of chronic oral dosing with Org 7797 (10 mg kg^{-1} twice daily) on early arrhythmias evoked by acute coronary artery ligation either 1 or 6 h after completion of the dosing regime.

Treatment	n	Total VPBs (0–30 min)	% VF
Controls	10	2350±451	50
Org 7797 1 h	10	791±525*	30
6 h	9	439±175*	22

* $P<0·05$ denotes a significant difference from the appropriate control group. n denotes the number of animals in each group. Numbers of ventricular premature beats (VPBs) are given as the mean±s.e.m.

There was no quantitative difference between the anti-arrhythmic efficacy of Org 7797 when coronary artery ligation was performed 1 or 6 h after completion of the dosing regime.

Ventricular fibrillation thresholds. The results are summarized in Fig. 2. In the control animals, coronary artery ligation resulted in a significant ($P<0·05$) decrease in VFT of 42%. Mean pre- and post-ligation values of VFT were significantly higher in the 1 h Org 7797-treated group compared with the controls and VFT did not fall significantly following ligation. However, values of VFT in the 6 h drug-treated group were similar to those recorded in vehicle-treated animals and VFT again fell (by 30%) significantly ($P<0·05$%) after coronary occlusion.

Haemodynamics. Values of arterial blood pressure, heart rate, cardiac output, stroke volume, peripheral vascular resistance and external cardiac work recorded from the control group were not significantly different from those seen in the drug treated groups (Table 4).

Electrophysiology. Table 5 summarizes the action potential characteristics of papillary muscle taken from rats treated with either Org 7797 or distilled water. In both the 1 and 6 h Org 7797 groups, V_{max} was significantly smaller than that recorded in the control group whereas values of resting membrane potential did not differ. Values of both APD50 and APD90 were similar in all groups.

Discussion

The results of the present study show that Org 7797 is orally active against early ischaemia-induced arrhythmias in the rat and is at least 2–5 times more potent than the clinically used sodium channel blocking agents, mexiletine, propafenone and disopyramide, with which it was compared. A single oral dose (10 mg kg^{-1}) of Org 7797 given 1 h before coronary

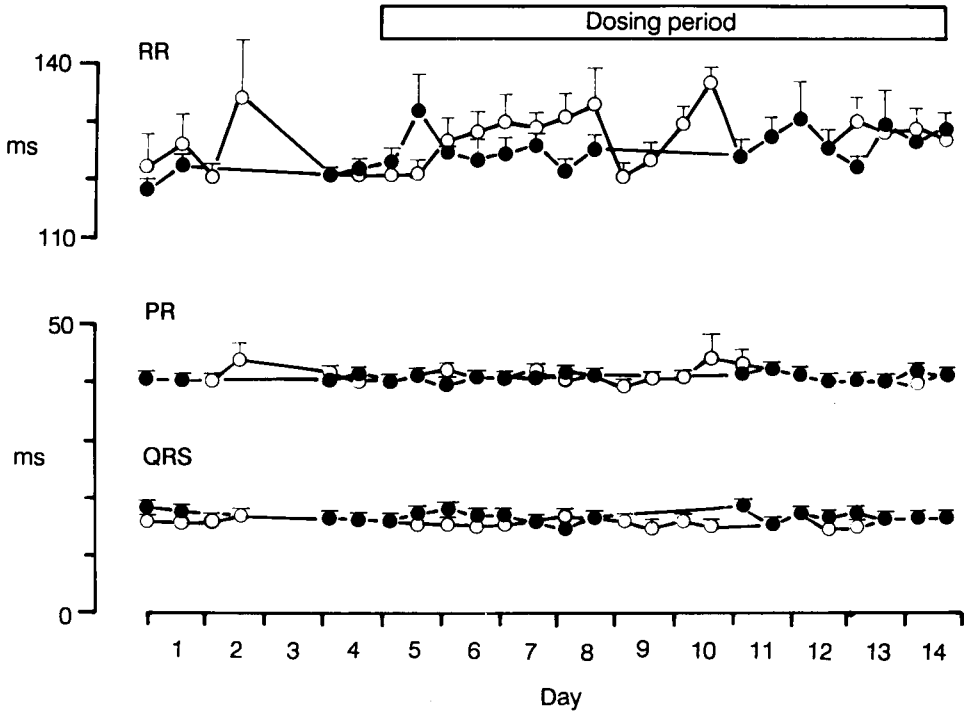

FIG. 1. Effects of chronic oral dosing with Org 7797 (10 mg kg^{-1} twice daily) (○) or distilled water (●) on the RR, PR and QRS intervals in conscious rats. Records were taken twice daily at 1 and 6 h after the morning dose. Each result is the mean±s.e.m. n=10 per group.

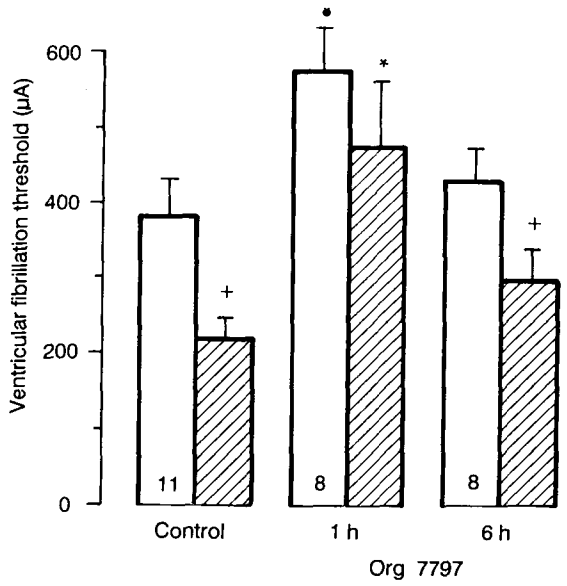

FIG. 2. Ventricular fibrillation thresholds (VFT) recorded 15 min before (□) and 3–4 min after (■) coronary artery ligation in animals chronically dosed with water (controls) or Org 7797 (10 mg kg^{-1} twice daily). Pre-ligation VFT measurements were made either 1 or 6 h after completion of dosing. Each result is the mean ± s.e.m. of results obtained from 8 to 11 animals (shown in the pre-ligation columns). *$P < 0.05$ denotes a significant difference from the appropriate control group and + denotes a significant decrease from the appropriate pre-ligation value.

occlusion was sufficient to almost completely prevent the development of ventricular fibrillation whilst a higher dose (20 mg kg^{-1}) prevented VF and additionally reduced the expected number of VPBs. Four h after single dosing the protection afforded by Org 7797 was just as marked as that seen 1 h after dosing despite a substantial decline (approx. 75%) in Org 7797 plasma levels. These results might suggest an accumulation or trapping of Org 7797 in the myocardium although this seems unlikely since rat liver homogenates metabolize approximately 92% of added Org 7797 within 30–60 min (Delbressine, unpublished). Moreover intravenous Org 7797 is rapidly eliminated from plasma with a half-life in rats of 75 ± 18 min (Planellas et al 1992). It seems more likely that at least one of the six known metabolites of Org 7797 in the rat is active. The most likely metabolite contributing to the pharmacological profile is 2,3,16-triol Org 7797, the major metabolite found in experiments in-vitro. In-vivo experiments in rats also show the conjugated forms of both Org 7797 and this triol derivative together with considerable amounts (21%) of unchanged Org 7797 in the faeces even after intravenous administration (Delbressine, unpublished).

These findings suggest that although the conjugated metabolites would not be expected to contribute to the pharmacological profile, they might be considered as the intrinsic pro-drugs which, via enterohepatic circulation after hydrolysis in the intestine and reuptake, can prolong the pharmacological activity. However, measurement of both cardiac and plasma levels of Org 7797 and its metabolite would be needed to clarify the apparent lack of correlation between Org 7797 plasma levels and antiarrhythmic activity.

Chronic oral dosing with Org 7797 (10 mg kg^{-1} twice a day) also protected animals against early ischaemia-induced arrhythmias for at least 6 h after administration of the last dose. However, in contrast to the effects of a single oral dose of 10 mg kg^{-1}, the expected incidence of VF was only approximately halved whilst the number of VPBs was markedly reduced. This preferential suppression of VPBs is reminiscent of the effects of the major metabolite when given via the intravenous route (Winslow et al 1991a). This metabolite is equipotent with Org 7797 in reducing the number of early ischaemia-induced VPBs in rats but is less than half as potent in preventing VF.

Table 4. Haemodynamic parameters recorded in closed chest spontaneously breathing rats chronically dosed with Org 7797 or distilled water. Recordings were made 1 or 6 h after the last dose of Org 7797 and 1 h after the last dose of distilled water.

		Org 7797	
	Controls	1 h	6 h
Mean systemic blood pressure (mmHg)	151 ± 7	145 ± 11	150 ± 8
Heart rate (beats min^{-1})	415 ± 18	429 ± 15	439 ± 20
Cardiac output (mL min^{-1})	80 ± 4	90 ± 5	90 ± 6
Stroke volume (mL $beat^{-1}$)	0·19 ± 0·013	0·20 ± 0·011	0·21 ± 0·017
Peripheral vascular resistance (dynes s $cm^{-5} \times 10^3$)	161 ± 13	138 ± 15	143 ± 14
External cardiac work (kg m min^{-1})	0·172 ± 0·011	0·168 ± 0·016	0·179 ± 0·015

Table 5. Action potential characteristics of papillary muscle from rats chronically treated with Org 7797 or distilled water. Five preparations were used in each group.

Treatment	Number of cells	Resting membrane potential (mV)	Action potential height (mV)	V_{max} ($V\ s^{-1}$)	APD50 (ms)	APD90 (ms)
Control	50	74·2 ± 0·8	93·0 ± 1·1	120 ± 5	16·4 ± 0·7	41·3 ± 1·7
Org 7797 1 h	49	76·8 ± 0·4	90·8 ± 0·8	98 ± 4*	15·4 ± 0·7	41·4 ± 1·4
6 h	48	75·7 ± 0·7	91·0 ± 0·6	102 ± 4*	15·4 ± 0·8	42·9 ± 2·0

*$P < 0.01$ denotes a significant difference from the appropriate control value.

Our results might therefore suggest an accumulation of the active metabolite during chronic dosing or a more rapid biotransformation compared with acute dosing. Taken together, results from available pharmacokinetic studies might indicate that the dose regime used in the present study resulted in plasma levels of Org 7797 or its metabolite below the threshold necessary to prevent completely ischaemia-induced fibrillation. Nevertheless chronic dosing with Org 7797 raised VFT and attenuated the post-ligation decrease in VFT. This effect had, however, waned by 6 h which may reflect different potencies of Org 7797 and its major metabolite. Previous studies in isolated papillary muscle showed that the major metabolite was less than half as potent as Org 7797 with respect to its class I action (Winslow et al 1991a). Different plasma levels of the two compounds might, therefore, account for the differential effects on VFT as seen at different times after dosing. Fibrillation thresholds are increased by sodium-channel blocking agents (Marshall & Winslow 1982; Camm 1984; Almotrefi 1985) and higher levels of Org 7797 (as adjudged from single dosing with 20 mg kg^{-1}) would be expected at 1, as compared with 6 h after dosing. However, in the in-vitro studies on excised papillary muscle, we observed a modest, but statistically significant, reduction in V_{max} with no change in resting membrane potential (indicative of a class I action) and this change in V_{max} was similar in muscles excised at 1 and 6 h after the last dose of drug was given.

As in porcine isolated ventricular muscle exposed to Org 7797 (Winslow et al 1989a), action potential duration was unchanged after chronic dosing with Org 7797. Thus the evidence obtained from this study regarding the mechanism underlying the antiarrhythmic effect would support that of a class I action but we have no clear explanation for the lack of effect on VFT at 6 h compared with 1 h after administration of the last dose of the drug. However, it should also be noted that the class I effect observed in excised muscle was not associated with a prolongation of the QRS interval, an action expected from a class Ic drug (Harrison 1985) and previously demonstrated following intravenous administration of Org 7797 to anaesthetized dogs (Campbell et al 1991). This suggests that the degree of sodium-channel block induced by chronic administration of Org 7797 was not sufficient to slow ventricular conduction in-vivo at least during normal sinus rhythm. In this context, it is interesting to note that both Janse et al (1990) and Kirchhof et al (1991) concluded from phase mapping activation studies in porcine and canine hearts, that the likely mechanism underlying the antifibrillatory action of Org 7797 is an ability to attenuate physiological shortening of the refractory period induced by high frequency stimulation, an action which outweighs its effect to slow conduction. The resulting prolongation of wavelength reduces the likelihood of fibrillation (Rensma et al 1988). It is not yet known if the major metabolite of Org 7797 shares this action. With hindsight, it would obviously have been of interest to measure the frequency dependence of refractory periods in the excised muscle so that a clearer understanding of the mechanism of action of the drug might have been obtained. However, it does seem unlikely that the modest drug-induced reduction in V_{max} seen in the present study can solely explain the observed antiarrhythmic effects.

We found no evidence of detrimental haemodynamic actions following chronic oral administration of Org 7797. Values of arterial blood pressure, cardiac output and stroke volume in drug-treated animals were similar to those found in controls. In addition, Org 7797 was devoid of effects on the PR or RR intervals indicating normal conduction, at least at physiological frequencies, in both atrial and nodal tissue.

In conclusion, Org 7797 given chronically via the oral route to rats exerts antiarrhythmic actions in the absence of detrimental electrocardiographic or haemodynamic changes. Although we found some evidence of sodium-channel block, it seems unlikely that this is the only mechanism through which prolonged oral dosing with Org 7797 exerts its protective effects in rats. In addition, levels of the 2-hydroxy metabolite may also be important.

References

Almotrefi, A. A. (1985) Effect of aprindine, verapamil and nifedipine on ventricular fibrillation threshold in isolated rabbit hearts. Arch. Int. Pharmacodyn. 274: 89–96

Camm, A. J. (1984) Cardiac electrophysiology of four new antiarrhythmic drugs—encainide, flecainide, lorcainide and tocainide. Eur. Heart J. 5: 75–79

Campbell, J. K., Marshall, R. J., Winslow, E. (1991) Comparison of the electrophysiological effects of Org 7797, disopyramide, mexiletine and propafenone in anaesthetised dogs with myocardial infarcts. Br. J. Pharmacol. 194: 433–439

Clark, C., Foreman, M. I., Kane, K. A., McDonald, F. M., Parratt, J. R. (1980) Coronary artery ligation of anaesthetised rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J. Pharmacol. Meth. 3: 357–368

Harrison, D. C. (1985) Antiarrhythmic drug classification: new science and practical applications. Am. J. Cardiol. 56: 185–187

Janse, M. J., Wilms-Schopman, F., Opthof, T. (1990) Mechanism of antifibrillatory action of Org 7797 in regionally ischemic pig heart. J. Cardiovasc. Pharmacol. 15: 633–643

Kirchhof, C., Wijffels, M., Brugada, J., Planellas, J., Allessie, M. (1991) Mode of action of a new class Ic drug (Org 7797) against atrial fibrillation in conscious dogs. J. Cardiovasc. Pharmacol. 17: 116–124

Marshall, R. J., Winslow, E. (1982) The effects of sodium channel inhibitors on early arrhythmias associated with acute myocardial ischaemia. In: Parratt, J. R. (ed.) Early Arrhythmias Resulting from Myocardial Ischaemia. Mechanisms and Prevention by Drugs. Macmillan, London, pp 251–294

Planellas, J., Winslow, E., Yih, T., Timmer, C. (1992) Org 7797, a new and unusual antiarrhythmic agent. Cardiovasc. Drug Rev. In press

Rensma, P. L., Allessie, M. A., Lammers, W. J. E. P., Bonke, F. I. M., Schalij, M. J. (1988) Length of excitation wave and susceptibility to reentrant atrial arrhythmias in normal conscious dogs. Circ. Res. 62: 395–410

Winslow, E., Martorana, M., Bell, P. (1989a) Electrophysiologic effects of Org 7797, a new steroidal antiarrhythmic agent. Comparison with class Ia, Ib and Ic drugs. J. Cardiovasc. Pharmacol. 14: 205–212

Winslow, E., Campbell, J. K., Marshall, R. J., Martorana, M., Muir, A. W. (1989b) Antiarrhythmic and electrophysiological effects of the aminosteroid, Org 7797, a new class Ic drug. Br. J. Pharmacol. 96: 185P

Winslow, E., Barron, E., Campbell, J. K. (1991a) Antiarrhythmic and class III electrophysiological actions of the 2,3,16-triol metabolite of Org 7797, a class Ic antiarrhythmic agent. Ibid. 102: 339P

Winslow, E., Campbell, J. K., Barron, E., Marshall, R. J., Muir, A. W. (1991b) Effects of Org 7797 on early, late and inducible arrhythmias following coronary artery occlusion in rats and dogs. Ibid. 104: 853–858