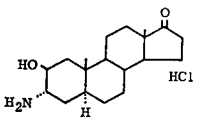
ORG 6001 (3α-amino-5α-androstan-2β-ol-17-one hydrochloride), a steroidal anti-arrhythmic agent

Potent local anaesthetic activity is possessed by 3α-amino-2β-hydroxy and 2β-amino-3α-hydroxy-5α-androstanes (Buckett, Hewett & others, 1967; Buckett, Marwick & Vargaftig, 1975). Since compounds such as lignocaine are both local anaesthetics and anti-arrhythmics, several members were screened for anti-arrhythmic activity. Activity was observed and further compounds were synthesized. One such compound was Org 6001 (3α-amino-5α-androstan-2β-ol-17-one-hydrochloride; I) a non-hormonal amino steroid whose pharmacological profile is now presented.



I

Org 6001 was examined for anti-arrhythmic activity in several pharmacological test models. Antagonism of aconitine-induced arrhythmias was studied in fasted male Wistar rats (190–210 g) by the method of Vargaftig & Coignet (1969). Ability to antagonize ouabain-induced ventricular tachycardia was studied in anaesthetized dogs (8–10 kg) as described elsewhere (Buckett & others, 1975). Post-infarct arrhythmias were induced in dogs (8–10 kg) by the method of Harris (1950) except that a four stage ligation procedure was used for the left anterior descending branch of the left coronary artery in order to reduce the mortality experienced following the conventional two stage method. Anti-arrhythmic activity was studied 20 to 40 h after ligation. In all experiments doses refer to the salts.

The i.v. injection of Org 6001 two min before aconitine infusion was extremely effective in antagonizing arrhythmias induced by aconitine in rats. Antagonism was dose-dependent and results were expressed as ED50 values which were defined as the dose of drug required to increase the arrhythmia-inducing dose of aconitine by 50%. The i.v. ED50 values for Org 6001 and lignocaine hydrochloride were 1·9 and 14·0 mg kg^{-1} respectively, whereas the i.v. LD50 (95% confidence limits) values for Org 6001 and lignocaine in rats were 147 (118–184) and 28·5 (18·7–43·0) mg kg^{-1} respectively. The ED50 value for Org 6001 administered orally 1 h before aconitine infusion was 10·3 mg kg^{-1}. Orally administered lignocaine at doses up to 60 mg kg^{-1} had no protective effect. The ability of Org 6001 to antagonize aconitine-induced arrhythmias in rats was long lasting. Statistically significant anti-aconitine activity was present not only 24 h after the i.v. injection of 5 mg kg^{-1} but also 18 h after 20 mg kg^{-1} administered orally. The i.v. infusion of Org 6001 (0·5 to 4·0 mg kg^{-1} min^{-1}) to dogs for 10 min immediately after commencement of ouabain-induced ventricular tachycardia resulted in a dose-dependent almost complete restoration of sinus rhythm although Org 6001 was slightly less potent than lignocaine in this respect. The ability of a 10 min i.v. infusion of Org 6001 (1 and 2 mg $^{-1}$ kg^{-1} min^{-1}) to correct post-infarct arrhythmias was essentially comparable to that of similar doses of lignocaine in conscious dogs (Fig. 1). Unlike Org 6001, lignocaine could not be infused for 10 min at a dose of 4 mg kg^{-1} min^{-1} as it induced convulsions and marked QRS widening. Orally administered Org 6001 (50 mg kg^{-1}) to coronary ligated dogs was effective in restoring sinus rhythm 1, 3 and 6 h after administration. On the other hand, the oral administration of lignocaine (50 mg kg^{-1}) exerted very little corrective effect at these times.

Dog blood pressure (−13%), heart rate (−18%) and cardiac contractility (−38%)

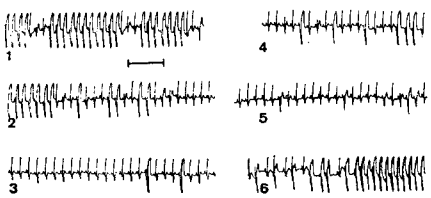


FIG. 1. The corrective effect of Org 6001 on ventricular arrhythmias in a dog 24 h after coronary artery ligation. In 1, the electrocardiogram (lead II) before drug administration; in 2, three min after commencement of a 2 mg kg^{-1} min^{-1} infusion of Org 6001; in 3, ten min after cessation of a 10 min infusion of 2 mg kg^{-1} min^{-1}; in 4, thirty min after cessation; in 5, fifty min after cessation; and in 6, 20 h after cessation. Time scale = 2 s.

were partially depressed 5 min after the i.v. injection of a large dose of Org 6001 (30 mg kg^{-1}). However, these effects of Org 6001 were less than those evoked by the same dose of lignocaine at the same time of testing. The corresponding values for lignocaine were blood pressure (−25%), heart rate (−24%) and cardiac contractility (−50%). Furthermore, in Org 6001-treated dogs carotid flow was not depressed whereas lignocaine reduced flow in the carotid vascular bed. Effects on atrio-ventricular conduction were studied in pentobarbitone sodium-anaesthetized dogs as described by Duchene-Marullaz (1970). The infusion of Org 6001 (2 mg kg^{-1} min^{-1}) and lignocaine (2 mg kg^{-1} min^{-1}) for 10 min elicited reductions of 9·0 and 19·1% respectively in ventricular maximum following rate 5 min after cessation of infusion. The ability of isoprenaline both to antagonize 5-hydroxytryptamine-induced broncho-constriction in the anaesthetized guinea-pig and to increase cat heart rate and decrease blood pressure respectively was unaltered by i.v. doses of Org 6001 up to 20 mg kg^{-1}. Hence Org 6001 would appear to be devoid of blocking effect on β_1 and β_2 adreno-ceptors. In the mouse tail clip test of Bianchi (1956), lignocaine had an ED50 of 8·2 mg ml^{-1} whereas Org 6001, at concentrations up to 15 mg ml^{-1}, had no detectable local anaesthetic activity. However, Org 6001 was approximately 0·5 times as potent as lignocaine when tested for conduction anaesthesia in the *in vitro* desheathed frog sciatic nerve.

Org 6001 is an effective anti-arrhythmic in three experimental models and shows potential advantages over lignocaine, in particular oral activity. Other advantages include reduced adverse haemodynamic effects and lack of central nervous system stimulation. Furthermore, the fact that it is neither a potent local anaesthetic in the mouse *in vivo* nor an β-adrenoceptor blocker makes Org 6001 an extremely interesting compound. Lignocaine is comparatively ineffective in the acute stage of myocardial infarction (Epstein, Beiser & others, 1973) and the observation by Parratt & Marshall (personal communication) that Org 6001, at doses having no adverse haemodynamic effects, completely prevented ventricular arrhythmias which follow the occlusion of the major branch of the left coronary artery of the dog points to the possible clinical potential of Org 6001.

Organon R.D. S.a.r.l.
Eragny-sur-Epte
France

B. B. VARGAFTIG*

* Present Address: Merrell International Research Centre, 67000-Strasbourg, France.

S.D.G. Organon,
Organon Laboratories Limited,
Newhouse,
Lanarkshire ML1 5SH, U.K.

M. F. SUGRUE†
W. R. BUCKETT**

Scientific Development Group,
Organon,
Oss, The Netherlands

H. VAN RIEZEN

April 15, 1975

** Present Address: Novo A/S, 2880-Bagsvaerd, Copenhagen, Denmark.
† To whom correspondence and reprint requests should be addressed.

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Experimental atherosclerosis in the rat: biochemical evaluation

The rat has been generally considered to be resistant towards the development of atheromatous arterial lesions, induced by a cholesterol-rich diet (Fillios, Andrus & others, 1956). However, in resistant species, such as baboon or rat, factors such as mechanical injury (Gutstein, Lazzarini-Robertson & La-Taillade, 1963), vitamin D_2 administration (Bajwa, Morrison & Ershoff, 1971) or immunization with foreign proteins (Howard, Patelski & others 1971) are often used in addition to the cholesterol diet. Altman (1973) found that rats treated for five consecutive days with an olive oil solution containing vitamin D_2 and cholesterol, exhibited extensive atherosclerotic lesions in the aorta, e.g. calcification as well as lipidic plaque formation.

To characterize these histological observations biochemically, male and female Sprague-Dawley rats, 310 $\pm$ 10 g, were randomized, divided into 4 different groups of 10 rats each and treated orally (dose kg^{-1} day^{-1}) for 5 days as follows: I, olive oil, 1·5 ml; II, vitamin D_2, 8 mg in olive oil, 1·5 ml; III, cholesterol, 40 mg in olive oil, 1·5 ml; IV, vitamin D_2, 8 mg + cholesterol, 40 mg in olive oil, 1·5 ml. At the end of the treatment the animals were fasted for 24 h and killed by a blow on the head.

Plasma and aorta samples were collected for estimation of lipids. The tissue was homogenized in physiological saline and then lyophilized. Total cholesterol, triglycerides and phospholipids were estimated respectively by slightly modified methods of Bloor (1916), Van Handel, Zilversmit & Bowman (1957), and Svanborg & Svennezholm (1961), after lipid extraction by the method of Carlson (1963). Vitamin D had no effect on cholesterol concentration.

The effect of treatments is shown in Table 1. In plasma the treatment with vitamin D_2, alone and with cholesterol, increased the fat concentrations, while cholesterol alone did not change the plasma lipid pattern. No significant interaction between D_2 and cholesterol was observed.

In aorta the combination of cholesterol with vitamin D_2 significantly increased the lipid concentrations but the individual treatments had no effect, thus the administration of cholesterol with vitamin D_2 affected significant changes in the triglyceride and