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ANTIARRHYTHMIC, HAEMODYNAMIC AND METABOLIC EFFECTS OF 3α-AMINO-5α-ANDROSTAN-2β-OL-17-ONE HYDROCHLORIDE IN GREYHOUNDS FOLLOWING ACUTE CORONARY ARTERY LIGATION

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1 The antiarrhythmic, haemodynamic and metabolic effects of a new amino steroid, ORG6001, have been investigated in experimental acute myocardial infarction in anaesthetized greyhounds.

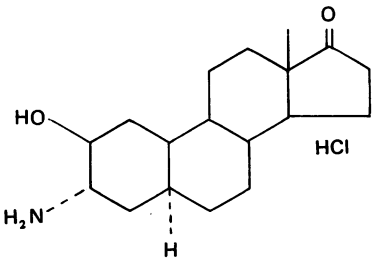
2 ORG6001 administered either intravenously (1-10 mg/kg) or orally (50 mg/kg) significantly reduced the incidence of ventricular ectopic beats in the first 30 min after ligation of the left anterior descending coronary artery.

3 In dogs pretreated with ORG6001, metabolic changes indicative of myocardial ischaemia (lactate production and potassium efflux) were less marked than those occurring in control animals.

4 Antiarrhythmic doses of ORG6001 caused only minimal transient haemodynamic effects.

5 These results suggest that ORG6001 may possess distinct advantages over presently-used antiarrhythmic drugs in the prevention and treatment of the early arrhythmias which occur after myocardial infarction.

Introduction



Formula of ORG6001
(3α-amino-5α-androstan-2β-ol-17-one HCl)

It has recently been reported that ORG6001 (3α-amino-5α-androstan-2β-ol-17-one hydrochloride) protects animals from cardiac arrhythmias induced either by aconitine, by ouabain or by multiple stage coronary artery ligation one day previously (Vargaftig, Sugrue, Buckett & Van Riezen, 1975). In addition, these workers have stated that ORG6001 may possess advantages over lignocaine since it has less adverse haemodynamic effects and is active orally. Acute one-stage ligation of the left anterior coronary artery in anaesthetized greyhounds results in reproducible and marked ventricular ectopic activity for 30 min after the occlusion and, in addition, causes metabolic changes in the venous blood draining the ischaemic region which do not occur in blood draining the remaining, essentially normal, areas of myocardium (Marshall, Parratt & Ledingham, 1974; Marshall & Parratt, 1974). The purpose of the present work was to investigate in this experimental model the haemodynamic and antiarrhythmic effects of ORG6001, administered both intravenously and orally, and to assess whether the drug was capable of modifying the metabolic consequences of acute coronary artery ligation.

Methods

Experiments were carried out using twenty-nine greyhounds of either sex weighing between 26 and 31 kg. Anaesthesia was induced by intravenous administration of sodium thiopentone

(20 mg/kg). After endotracheal intubation, respiration was applied from a positive-pressure ventilation pump (25 strokes/min), with 100% oxygen containing 0.5-1.0% trichlorethylene. The tidal volume of the pump was adjusted to maintain an arterial P_{CO_2} of 35-40 mmHg (1 mmHg = 1.33 mbar). Reflex movements were prevented by the intermittent intramuscular administration of suxamethonium chloride (100 mg).

Catheters were placed in the descending aorta (via the right femoral artery), the right atrium (via the right saphenous vein) and the coronary sinus (via the left external jugular vein). A catheter-tip transducer (Millar Instruments, Inc., Houston, Texas) was inserted into the lumen of the left ventricle (via the left carotid artery in the neck) for the measurement of left ventricular pressure and also dP/dt (using an Elema-Schonander differentiating circuit). The frequency response of this transducer system is flat to 200 Hz. Records of left ventricular pressure at high gain allowed accurate assessments to be made of left ventricular end-diastolic pressure (LVEDP). The correct location of all catheters was confirmed using a Siemen's image intensifier. Cardiac output was measured by dye-dilution, indocyanine green (2.5 mg) being injected as a bolus into the right atrium and blood withdrawn at a constant rate through a densitometer (Waters Co., Rochester, Minnesota). This blood was immediately replaced. All cardiac output determinations were made in duplicate.

The heart was exposed through a left thoracotomy and the pericardium overlying the anterolateral aspect of the heart was incised. Blood flow in the circumflex branch of the left coronary artery was measured with a Nycotron 372 electromagnetic flow meter using a calibrated probe of 1.5-2.5 mm diameter. The anterior descending branch of the left coronary artery, at a point distal to the septal artery branch, was prepared for ligation with minimum dissection. A major branch of the main vein adjacent to the artery (the anterior coronary vein) was catheterised using the Seldinger technique with a 10 cm Longdwel teflon catheter (size 20G). This local coronary vein catheter was not tied in position and was manipulated until its tip lay well into the region of the myocardium which was to be made ischaemic. It has been shown that such a coronary vein catheter, after coronary artery ligation, drains blood predominantly from the ischaemic region (Fisher, Heimbach, Ledingham, Marshall & Parratt, 1973; Marshall *et al.*, 1974).

Blood samples were taken, without exposure to air, at regular intervals and were analysed for oxygen and carbon dioxide tensions, oxygen content and pH, as outlined by Ledingham, McBride, Parratt & Vance (1970), except that the value used for haemoglobin oxygen-binding capacity was 1.39. Blood samples from the aorta, coronary sinus and coronary vein were taken immediately before and 30 min after coronary artery ligation and were analysed for lactate (Hohorst enzymatic method using a Boehringer test combination) and for plasma potassium using standard flame photometry. Potassium determinations were discarded if there was any obvious haemolysis.

After a stabilisation period, the ligature was tied in one stage and the number of ventricular ectopic beats counted during each 5 min period for 30 min; no blood samples were taken during this time since manipulation of the coronary venous catheter sometimes itself induced arrhythmias. No arrhythmias occurred in any of the animals after 30 minutes.

In order to investigate the possible anti-arrhythmic activity of ORG6001, the following protocol was used:

GROUP I—Intravenous ORG6001 (10 mg/kg), 15 min prior to ligation ($n = 6$)
GROUP II—Intravenous ORG6001 (10 mg/kg), 1.5 h prior to ligation ($n = 6$)
GROUP III—Intravenous ORG6001 (1.0 mg/kg), 15 min prior to ligation ($n = 5$)
GROUP IV—Oral ORG6001 (50 mg/kg) 4 h prior to ligation ($n = 7$)
GROUP V—Oral placebo (lactose), 4 h prior to ligation ($n = 5$)

Groups IV and V were studied double-blind and the key to the code was not revealed until all the results had been collated. One dog (which was revealed later to be in Group V) fibrillated during left ventricular catheterisation and was thus discounted from the trial. The results obtained in these groups of dogs were compared with those previously obtained in control and lignocaine-treated animals (Marshall & Parratt, 1974). At the end of each experiment, a bolus of diffusible dye was injected at a pressure of 50 mmHg into the peripheral stump of the ligated coronary artery. The dog was immediately fibrillated with potassium chloride and the dyed muscle quickly excised and weighed. The mass of this dyed ischaemic area of muscle was expressed as a percentage of the free ventricular wall.

Systemic arterial pressure (pulsatile and meaned by electronic integration), mean right atrial pressure, left ventricular pressure and dP/dt, LVEDP, left circumflex coronary blood flow and the electrocardiogram (standard limb lead II) were recorded on an Elema-Schonander ink-jet writing recorder (Mingograph 81).

Myocardial oxygen availability and consumption were calculated as outlined by Marshall &

Parratt (1973) and cardiac work, peripheral vascular resistance, whole body oxygen consumption and oxygen extraction as described by Ledingham, Parratt, Smith & Vance (1971). Statistical analysis of the results was carried out using Students' *t* test for paired or unpaired data.

Results

The haemodynamic effects of ORG6001

After the rapid intravenous injection of ORG6001 (1 or 10 mg/kg) into the right atrium, there were immediate, transient, and apparently dose-dependent, decreases in diastolic blood pressure and in left ventricular d*P*/d*t max* and increases in heart rate and in coronary blood flow. These immediate haemodynamic effects of ORG6001 are summarised in Table 1 and illustrated in Figure 1. However, 10 min after the intravenous injection,

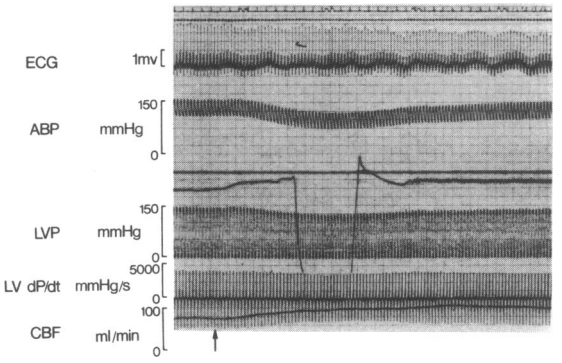


Figure 1 The immediate effects of an intravenous injection of ORG6001, 10 mg/kg (at arrow) on (from top to bottom), the electrocardiogram lead II (ECG), aortic blood pressure (ABP), left ventricular pressure (LVP), left ventricular d*P*/d*t* (LV d*P*/d*t*) and left circumflex coronary blood flow (CBF). The time marker at the top of the trace represents 1 s intervals.

Table 1 The immediate (0-60s) haemodynamic effects of ORG6001 administered intravenously to anaesthetized greyhounds.

	ORG6001	
	1.0 mg/kg (n = 6)	*10 mg/kg (n = 9)*
Decrease in diastolic blood pressure (mmHg)	−10 ± 1 (8%)	−27 ± 7* (22%)
Increase in heart rate (beats/min)	+17 ± 8 (9%)	+11 ± 6 (6%)
Decrease in left ventricular d*P*/d*t max.* (mmHg/s)	−117 ± 36 (5%)	−218 ± 57 (9%)
Increase in coronary blood flow (ml/min)	+22 ± 5* (43%)	+31 ± 8* (39%)

Values are mean ± s.e.
* Change significant at $P < 0.05$ level.

Table 2 Haemodynamic changes induced by ORG6001, 10 min after intravenous administration in anaesthetized greyhounds.

	ORG6001			
	1.0 mg/kg		*10 mg/kg*	
	Before	*After*	*Before*	*After*
Mean blood pressure (mmHg)	145 ± 2	143 ± 1	143 ± 3	143 ± 3
Heart rate (beats/min)	185 ± 13	180 ± 12	183 ± 1	187 ± 7
Cardiac output (l/min)	3.4 ± 0.5	3.2 ± 0.4	4.3 ± 0.3	4.4 ± 0.4
External cardiac work (kg. m min^{-1})	3.8 ± 0.6	3.7 ± 0.7	4.6 ± 0.4	4.5 ± 0.5
Left ventricular dP/dt max (mmHg/s)	3216 ± 421	3050 ± 376	2810 ± 141	2760 ± 141
LVEDP (mmHg)	7 ± 1	7 ± 1	8 ± 1	9 ± 1
Coronary blood flow (ml/min)	49 ± 2	52 ± 3	73 ± 7	83 ± 10
Myocardial oxygen consumption (ml/min)	8.4 ± 0.5	9.7 ± 0.9	9.8 ± 1.2	11.1 ± 1.6

Values are mean of 9 observations at each dose level ± s.e.

there were no significant changes in blood pressure, heart rate, cardiac output, left ventricular d*P*/d*t max.*, LVEDP, coronary blood flow, myocardial oxygen consumption and external cardiac work (Table 2). ORG6001, at either dose, did not affect coronary or peripheral vascular resistance or whole body oxygen consumption (Table 3).

Effects of ORG6001 on ventricular arrhythmias occurring in the immediate 30 min post-ligation period.

The results of this present investigation are illustrated in Figure 2 and are compared with previous results (Marshall & Parratt, 1974) obtained in control dogs and in dogs pretreated with lignocaine in Table 4. Ventricular arrhythmias are common in this preparation and include sustained runs of ventricular tachycardia (defined as more than 6 consecutive ventricular beats) particularly during the 16-20 min post-ligation period (Table 4 and Figure 2). Two out of the 4 dogs which received oral placebo fibrillated during this time. In contrast, significantly fewer arrhythmias were seen during the entire 30 min post-ligation period in dogs pretreated with ORG6001, administered intravenously in a dose of 10 mg/kg (15 min or 1.5 h before ligation) or orally, in a dose of 50 mg/kg, 4 h previously (Figure 2 and Table 4); ventricular tachycardia was not observed in any dog pretreated with ORG 6001. Results obtained in the dogs given ORG (1.0 mg/kg i.v.) 15 min before ligation were more equivocal. Although these animals had fewer arrhythmias than the controls, the difference was only significant in the first 20 min period. Overall, only 2 out of the 17 dogs pretreated with intravenous ORG6001 fibrillated in the first 30 min and none given the drug orally 4 h before ligation succumbed during this period. Further indication of the duration of ORG6001 is apparent from the protection afforded by 10 mg/kg intravenously administered 1.5 h before ligation. None of the 6 animals administered this dose died and the number of arrhythmias was significantly less than in the control, untreated, animals over the 30 min post-ligation period.

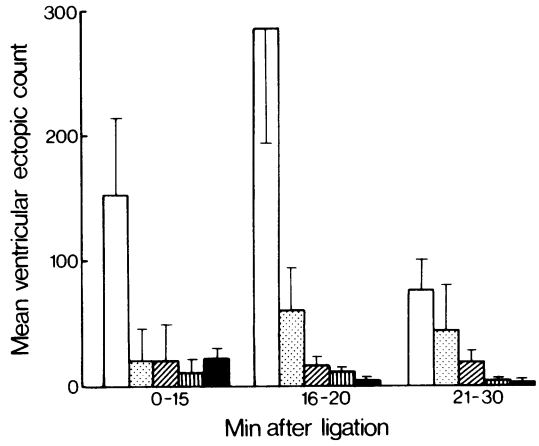


Figure 2 Mean ventricular ectopic counts in the immediate 30 min period after coronary artery ligation in control dogs and in dogs pre-treated with intravenously or orally administered ORG6001. The left hand set of histograms represents counts in the first 15 min, the middle set represents the crucial 16-20 min period and the right hand histograms represent the final 10 min period. Pre-treatment with ORG6001 suppressed the number of arrhythmias seen in any of these periods during the first 30 min after ligation. Columns (in each block of histograms) are, from left to right, control; ORG6001, 1 mg/kg 15 min i.v.; ORG6001, 10 mg/kg 1.5 h i.v.; ORG6001, 10 mg/kg 15 min i.v.; ORG6001, 50 mg/kg 4 h orally.

Haemodynamic and metabolic consequences of ligation

The haemodynamic consequences of coronary artery ligation in this experimental model have been described in full elsewhere (Fisher *et al.*,

Table 3 Changes in vascular resistance and whole body oxygen handling before, and 10 min after, the intravenous administration of ORG6001 to anaesthetized greyhounds

	ORG6001			
	1.0 mg/kg (n = 6)		10 mg/kg (n = 9)	
	Before	After	Before	After
Peripheral vascular resistance (units)	44 ± 5	45 ± 6	36 ± 3	35 ± 3
Coronary vascular resistance (units)	24 ± 3	22 ± 2	20 ± 2	18 ± 2
Right atrial oxygen extraction (%)	11 ± 1	15 ± 3	12 ± 1	13 ± 2
Whole body oxygen consumption (ml/min)	107 ± 23	155 ± 52	148 ± 17	130 ± 13

Values are mean ± s.e.

Table 4 Analysis of the number of ventricular ectopic beats at various times after the acute ligation of the anterior descending branch of the left coronary artery in control (untreated) dogs and in dogs pretreated with lignocaine, ORG6001 or placebo

Group	*0-5 min*	*6-10 min*	*11-15 min*	*16-20 min*	*21-25 min*	*26-30 min*	*TOTAL*	*Deaths within 30 min*
Control	120 ± 42	150 ± 60	94 ± 67	286 ± 92	96 ± 38	52 ± 21	904 ± 260 (193-2250)	4/12
Lignocaine (2 mg/kg i.v.) 10 min pre-ligation	129 ± 61	201 ± 78	77 ± 73	283 ± 124	86 ± 32	3 ± 1*	813 ± 378 (104-1605)	2/16
ORG6001 (10 mg/kg i.v.) 15 min pre-ligation	19 ± 9*	14 ± 8*	2 ± 2*	11 ± 2*	5 ± 2*	2 ± 2*	43 ± 13* (9-84)	1/6
ORG6001 (10 mg/kg i.v.) 1.5 h pre-ligation	5 ± 3*	47 ± 45*	14 ± 8*	16 ± 7*	17 ± 9*	19 ± 12	116 ± 43* (33-324)	0/6
ORG6001 (1.0 mg/kg i.v.) 15 min pre-ligation	27 ± 18	8 ± 12*	30 ± 18	59 ± 34	32 ± 24	56 ± 52	210 ± 132* (9-698)	1/5
ORG6001 (50 mg/kg) ORAL 4 h pre-ligation	28 ± 18	33 ± 21*	9 ± 5*	3 ± 2*	1 ± 1*	4 ± 3*	78 ± 29* (3-185)	0/7
Placebo ORAL 4 h pre-ligation	42 ± 28	98 ± 27	143 ± 80	264 ± 96	110 ± 40	46 ± 12	—	2/4

Values are mean ± s.e., range in brackets.
* $P < 0.025$, Significantly different from control.

1973; Marshall *et al.*, 1974). The haemodynamic effects of ligation in this series of dogs pre-treated with ORG6001 (Table 5) were essentially similar and included marked decreases in cardiac output and external cardiac work. These changes are secondary to a decreased cardiac contractility, as manifested by transient decreases in left ventricular dP/dt *max* with more sustained increases in LVEDP (Table 5).

When a major branch of the left coronary artery is ligated, metabolic changes occur in the venous blood draining the ischaemic region which do not occur in blood draining the remaining, essentially normal areas. These changes in the control dogs have been described in detail by Marshall *et al.*, (1974) and include reductions in coronary venous PO_2, pxygen content, pH and lactate extraction and an increase in PCO_2. The administration of intravenous or oral ORG6001 before coronary artery ligation modified these metabolic consequences of myocardial ischaemia (Table 6). There were only slight, and insignificant, reductions in coronary vein PO_2 and oxygen content and the increase in PCO_2 and decrease in pH appeared to be less marked than in the control series previously described (Marshall *et al.*, 1974). The effects of ORG6001 on lactate handling by the ischaemic myocardium were especially striking and for the high intravenous dose (10 mg/kg 15 min before ligation) are summarized in Figure 3. None of the five dogs administered this dose of ORG6001, were producing lactate 30 min after ligation. This is to be compared with 5 out of the 8 dogs that produced lactate at this time in the control series. Myocardial lactate extraction in the ischaemic myocardium decreased slightly in the ORG6001 treated dogs (from a mean of +32% to a mean of +13%; $P > 0.05$) but changed significantly from extraction (+41%) to production (−8%) in the control, untreated dogs ($P < 0.025$). There were insignificant changes in lactate extraction by the normal myocardium following coronary artery ligation in either control or ORG6001 treated animals (Figure 3). Thus ORG6001 (10 mg/kg, i.v.) appeared to reduce the shift from aerobic (lactate extraction) to anaerobic metabolism (lactate production) in the acutely ischaemic zone of myocardium. Similar results were obtained in the dogs pretreated orally with ORG6001 (50 mg/kg). Before ligation both areas of myocardium extracted lactate to the same extent (coronary sinus 39 ± 6%; coronary vein 40 ± 5%). Thirty min after ligation lactate extraction in the normal region remained unchanged (34 ± 6%) and although in 2 out of 7 dogs, lactate production was evident in the ischaemic zone, the mean extraction value (19 ± 10%) was not significantly different from the pre-ligation control.

The lactate results in the dogs given ORG6001 (10 mg/kg) 1.5 h before ligation showed that 2 out of 5 dogs were producing lactate after 30 min; the mean change in extraction was from 32 ± 6% pre-ligation to 1 ± 9%, 30 min post-ligation ($P < 0.025$). The corresponding lactate extractions in the normal myocardium (assessed by coronary sinus sampling) were 33 ± 5 and 37 ± 6% respectively. The smaller dose of ORG6001 (1 mg/kg) given 15 min before ligation also had no effect on lactate production in the ischaemic myocardium. Two of the 5 dogs were producing lactate after 30 min ischaemia and the mean change (from 40 ± 4% to 9 ± 11%) was significant ($P < 0.025$) and similar to the change observed in the control series.

The effects of ORG6001 on potassium efflux from the ischaemic myocardium

An examination of the effects of ORG6001 on potassium efflux from the ischaemic myocardium was undertaken using simultaneous analysis of blood from the coronary vein (draining the ischaemic region) and from the coronary sinus (draining the essentially normal regions of the myocardium). As far as we are aware, there have been no previous studies on the effects of an antiarrhythmic drug on potassium efflux into a local vein draining an ischaemic region.

In the control series (no drug treatment) clear evidence was obtained of K^+ efflux into the local coronary vein. Before coronary artery ligation the

Table 5 The haemodynamic effects of acute coronary artery ligation in control dogs and in dogs pretreated with ORG6001

		Cardiac output (l/min)		*Left ventricular dP/dt max (mmHg/s)*		*LVEDP (mmHg)*	
	n	*Pre-ligation*	*30 min post ligation*	*Pre-ligation*	*30 min post ligation*	*Pre-ligation*	*30 min post ligation*
CONTROL	8	3.2 ± 0.4	2.2 ± 0.2*	2910 ± 280	2600 ± 190	6 ± 1	11 ± 2*
ORG6001 (10 mg/kg i.v.) 15 min pre-ligation	5	4.2 ± 0.2	3.5 ± 0.3*	3100 ± 193	2700 ± 114*	8 ± 1	10 ± 2
ORG6001 (10 mg/kg i.v.) 1.5 h pre-ligation	6	2.9 ± 0.4	2.4 ± 0.3*	2833 ± 487	2683 ± 490	7 ± 2	10 ± 3*
ORG6001 (1.0 mg/kg i.v.) 15 min pre-ligation	4	3.5 ± 0.6	2.6 ± 0.1*	3025 ± 388	2700 ± 363	7 ± 2	9 ± 3
ORG6001 (50 mg/kg) ORAL 4 h pre-ligation	7	4.1 ± 0.4	3.5 ± 0.3*	3014 ± 380	2886 ± 359	11 ± 2	14 ± 2*

Values are mean ± s.e.
* $P < 0.05$ Paired 't' test.

Table 6 Changes in coronary vein oxygen extraction, PCO_2 and pH before and 30 min after, coronary artery ligation in control dogs and in dogs treated with ORG6001.

	Oxygen extraction %		*PCO_2 (mmHg)*		*pH (Units)*	
	Pre-ligation	*Post ligation*	*Pre-ligation*	*Post ligation*	*Pre-ligation*	*Post ligation*
CONTROL	54 ± 3	69 ± 3*	45 ± 2	60 ± 3*	7.347 ± 0.014	7.265 ± 0.023*
ORG6001 (10 mg/kg i.v.) 15 min pre-ligation	49 ± 3	61 ± 4*	58 ± 2	71 ± 4*	7.310 ± 0.011	7.274 ± 0.009*
ORG6001 (10 mg/kg i.v.) 1.5 h pre-ligation	48 ± 3	52 ± 2	57 ± 6	62 ± 8	7.312 ± 0.048	7.282 ± 0.062
ORG6001 (1.0 mg/kg i.v.) 15 min pre-ligation	51 ± 4	52 ± 4	52 ± 2	57 ± 3	7.318 ± 0.031	7.278 ± 0.030
ORG6001 (50 mg/kg) ORAL 4 h pre-ligation	55 ± 2	63 ± 3*	59 ± 4	65 ± 4	7.326 ± 0.014	7.309 ± 0.010

Values are mean ± s.e.
* $P < 0.05$.

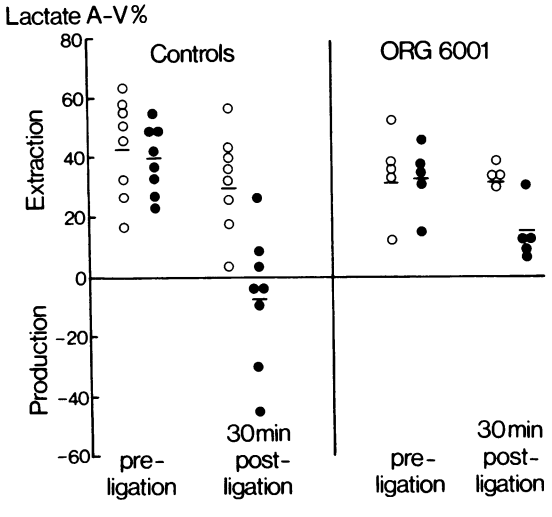


Figure 3 The effects of coronary artery ligation on the extraction of lactate by both normal (○, coronary sinus) and acutely ischaemic (●, coronary vein) regions of the myocardium in control dogs and in dogs given ORG6001 (10 mg/kg i.v.) 15 min before ligation. Mean values are represented by the bars. Lactate production in the ischaemic zone only occurred in the control dogs. * $P < 0.025$.

arterial, coronary sinus and local coronary vein potassium values were similar (3.7 ± 0.1, 3.5 ± 0.2 and 3.5 ± 0.1 mEq/l respectively). Samples from these sites 30 min after coronary artery ligation gave values of 3.6 ± 0.2, 3.5 ± 0.2 and 4.3 ± 0.3 mEq/l respectively; $n = 12$). There was thus a substantial and significant ($P < 0.05$) efflux of potassium into the local coronary vein (i.e. from 3.5 ± 0.1 to 4.3 ± 0.3 mEq/l). It should be noted that potassium efflux into the coronary sinus was not observed in these control experiments and this result again clearly demonstrates the value of local venous sampling in metabolic studies on myocardial ischaemia.

No significant potassium efflux into the local coronary vein was observed after the intravenous administration of ORG6001 (1 mg/kg or 10 mg/kg 15 min before ligation); the coronary vein values were 3.5 ± 0.2 (pre-ligation) and 3.6 ± 0.2 mEq/l (30 min post ligation) after the lower dose of ORG6001 and 3.1 ± 0.4 and 3.5 ± 0.5 mEq/l respectively at the 10 mg/kg dose level. Similar results were obtained in the group of dogs given oral ORG6001 (50 mg/kg). Immediately prior to ligation, the arterial, local coronary vein and coronary sinus potassium levels were 3.5 ± 0.1, 3.6 ± 0.1 and 3.4 ± 0.3 mEq/l respectively. These levels remained unchanged 30 min after ligation,

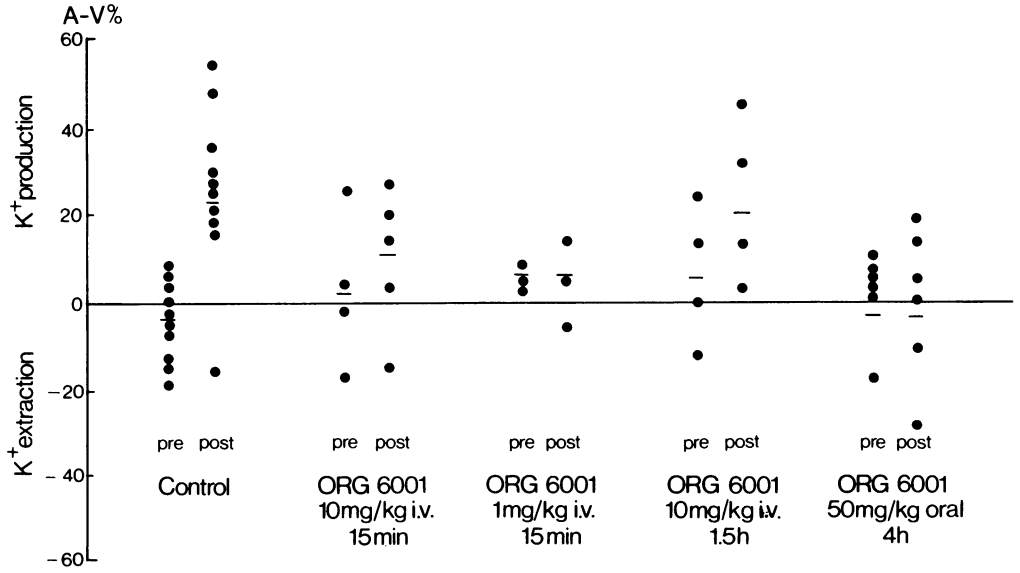


Figure 4 The effects of coronary artery ligation on potassium efflux into a local coronary vein in control dogs and in dogs pretreated with intravenously or orally administered ORG6001. Mean values are represented by the bars. Intravenous or oral ORG6001 prevented the marked efflux of potassium which was evident in control dogs, 30 min after ligation. * $P < 0.025$.

being 3.5 ± 0.1, 3.7 ± 0.1 and 3.4 ± 0.3 mEq/l respectively.

The potassium results are summarized in a rather different way in Figure 4 and it is clear that potassium efflux is evident 30 min after coronary artery ligation in the untreated dogs but not in those pretreated with intravenous or oral ORG6001.

Discussion

The most important reasons for the decrease in mortality of patients suffering from ischaemic heart disease have been the early detection and treatment of cardiac arrhythmias in the immediate period after myocardial infarction. Sixty-one per cent of deaths from myocardial infarction among patients younger than 65 occur within 1 h of onset (Gordon & Kannel, 1971), ventricular fibrillation being the terminal event in more than 90% of these early deaths (Pantridge & Adgey 1969). At present the most commonly used drugs for the treatment of these early ventricular arrhythmias are quinidine, procaineamide and lignocaine but there are drawbacks inherent in their use. Prominent side effects such as depression of myocardial contractility, hypotension and conduction abnormalities have especially been described with the use of quinidine and procaineamide (Jewitt, 1971; Mason, De Maria, Amsterdam, Zelias & Massumi, 1973). Lignocaine, the most effective of these three drugs, is thought not to produce marked haemodynamic effects (Austen & Moran, 1965) but suffers from the disadvantages that it is short acting (Harrison & Alderman, 1971), that it is not well absorbed orally, and most important, that it is less effective in the control of ventricular dysrhythmias that occur within 2 h of the onset of infarction, than those occurring later (Pantridge, 1971; Adgey, Allen, Geddes, James, Webb, Zaidi & Pantridge, 1971). There is thus a major need for an antiarrhythmic drug which produces minimal haemodynamic effects, which is effective against arrhythmias occurring in the early stages of infarction and which is reliably absorbed after oral administration. Such an orally active drug could then be given prophylactically to patients in whom the risk of a recurring myocardial infarct is great.

In these studies on experimental myocardial infarction, ORG6001 administered intravenously or orally, significantly reduced the number of ventricular ectopic beats in the first 30 min after acute coronary artery ligation. In addition, in contrast to the control animals, few of the treated animals succumbed to ventricular fibrillation during this period. The incidence of these early arrhythmias after acute coronary ligation depends critically on many factors which include the site of the ligature and size of the infarct produced (Thomas, Shulman & Opie, 1970), temperature, the anaesthetic used and the haemodynamic state of the animals. In these experiments, all ligations were made on the main intraventricular (anterior descending) coronary artery at a position 10-25 mm from the tip of the left atrial appendage and distal to the main septal branch. There was no difference in the size of ischaemic zone produced in the control dogs (21 ± 2% by weight of the free left ventricular wall) and in those treated with ORG6001 (23 ± 4%). In addition, control and treated animals did not differ immediately prior to ligation as regards oesophageal temperature (36.8 ± 0.6 and 37.7 ± 0.5°C respectively) haemodynamics (Table 5) or blood gases (Table 6).

Some indication of the duration of action of ORG6001 was given by the fact that, when administered intravenously 1.5 h before ligation, the drug was still capable of suppressing these early arrhythmias. Even more striking was the marked reduction in arrhythmias in dogs given ORG6001 (50 mg/kg) by mouth 4 h before ligation. Although this high oral dose of ORG6001 did not cause any obvious side-effects, it would be of interest to investigate the threshold oral dose of the drug which still exerted antiarrhythmic activity. Of relevance is the observation that two dogs given 25 mg/kg of ORG6001 by mouth, 4 h prior to ligation had respectively only 62 and 77 ventricular ectopic beats in the first 30 min after ligation. Reduction in the oral dose to 10 mg/kg in an additional two dogs did not markedly protect (218 and 320 ventricular ectopic beats respectively). In these studies, no attempt was made to elucidate the mode of action of ORG6001 but it is of note that the drug (2 mg/kg i.v.) is capable of reducing ventricular maximum following rate in anaesthetized dogs (Vargaftig *et al.*, 1975) and increases, in a dose-dependent manner, the effective refractory period of electrically driven guinea-pig atrial muscle in concentrations between 0.3 and 3.0×10^{-4} M (Marshall, unpublished observations). Previous work *in vivo*, also suggests that the antiarrhythmic properties of ORG6001 cannot be ascribed to either β-adrenoceptor blocking or local anaesthetic activity (Vargaftig *et al.*, 1975).

Although ORG6001, administered intravenously or orally, protected against these early ventricular arrhythmias, the drug did not affect the depression of myocardial contractility and cardiac output induced by coronary artery ligation. However the metabolic changes which occurred in the ischaemic myocardium were less marked in dogs treated with intravenous or oral ORG6001. One of the most marked metabolic changes which occurs in ischaemic cardiac tissue

and which can be detected by selective coronary venous sampling is the production of lactate (Opie, Owen, Thomas & Samson, 1973; Marshall *et al.*, 1974). The normal heart is capable of extracting lactate (Figure 3). The discharge of lactate or decreased lactate uptake evident in the ischaemic myocardium is the result of increased glycolysis and a relative decrease in the rate of pyruvate entry into the Krebs cycle. The stimulus for the increased glycolysis in ischaemia is probably both increased glucose uptake and glycogen breakdown. It is now widely accepted that in most cases, lactate production represents a shift from aerobic to anaerobic metabolism (Opie *et al.*, 1973) and can be used as an index of impaired myocardial tissue oxygenation (Gorlin, 1972). Intravenous or oral ORG6001 largely prevented the occurrence of lactate production in the ischaemic zone of myocardium. Whether this is due to a direct beneficial effect of ORG6001 on blood flow or oxygenation in the ischaemic myocardium or whether it is secondary to the reduction in arrhythmias which themselves tend to decrease blood flow (Samet, 1973; Marshall *et al.*, 1974) is at present unknown.

Pretreatment with ORG6001 also prevented the efflux of potassium ions into coronary venous blood. The relevance of the potassium ion to the occurrence of lethal ventricular arhythmias soon after the production of acute myocardial ischaemia has been the subject of a number of experimental studies. Some authors have deduced a causal relationship between cumulative potassium loss and the incidence of ventricular arrhythmias in the early stages of experimental myocardial infarction (Harris, Bisteni, Russell, Brigham & Firestone, 1954; Regan, Harman, Lehan, Burke & Oldewurtel, 1967) while others have failed to show any relationship (Wexler & Patt, 1960; Thomas *et al.*, 1970).

Although in this study only one estimation of potassium was made after ligation (at 30 min), we attempted to correlate the total number of ventricular ectopic beats with the egress of potassium in individual animals. We found no correlation ($r = 0.223$) between potassium egress and ventricular ectopic activity and therefore cannot assign a direct arrhythmogenic role to potassium ions. On the other hand, there was a good correlation between potassium egress and lactate production ($r = 0.763$). This may be explained by the fact that the further decrease in blood flow within the ischaemic region caused by arrhythmias not only results in an increased shift to anaerobic metabolism but, either by increasing the permeability of the cell membrane or by inhibiting $Na^+ - K^+$ ATPase, also induces an increased egress of potassium ions from the ischaemic region.

In doses which significantly suppressed early ventricular arrhythmias, ORG6001 caused only slight and transient haemodynamic changes. The immediate effects comprised modest falls in diastolic blood pressure, left ventricular pressure and left ventricular dP/dt *max* accompanied by tachycardia (presumably reflex in nature) and increased coronary blood flow. Ten minutes after injection, all parameters had returned to control values. Thus there was no evidence that ORG6001, in doses up to 10 mg/kg i.v., caused lasting myocardial depression, an effect readily seen with quinidine and procaineamide at lower doses in our experimental model (Marshall & Parratt, unpublished) and in man (Jewitt, 1971). In addition, animals pretreated with a high oral dose (50 mg/kg) of ORG6001, 4 h previously, were, just prior to coronary artery ligation, haemodynamically similar to control dogs.

We conclude that the non-hormonal amino steroid, ORG6001, is effective when administered both intravenously and orally, in suppressing the early arrhythmias induced by acute one-stage coronary artery ligation in anaesthetised dogs. ORG6001 would seem to hold important advantages over presently used antiarrhythmic drugs in that it has a long duration of activity and does not cause myocardial depression in effective antiarrhythmic doses.

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