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Electrical and mechanical effects of new aminosteroids on guinea-pig isolated ventricular muscle

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1 LND 623 and LND 796 are two aminosteroid derivatives which exert similar positive inotropic effects to digitalis. Their electrophysiological, toxic and inotropic effects were investigated in both normal and partially K^+-depolarized ventricular muscle.

2 In guinea-pig myocardial fibres, LND 623 and LND 796 required tenfold higher concentrations than digoxin to induce the same signs of toxicity; e.g. triggered activities generated from delayed afterdepolarizations, leading to the marked depression of action potential characteristics and inexcitability. These abnormal rhythms and delayed afterdepolarizations were abolished by 1 mM caffeine. The toxic effects were reversed by washout, particularly in the case of LND 796.

3 In normal-K^+ solution, LND 623 and LND 796 exhibited concentration-dependent positive inotropic effects on guinea-pig papillary muscle and increased concomitantly resting membrane potential and action potential amplitude. The range of active concentrations (0.1 to 1 μM) of LND 623 was larger than that of digoxin (0.3 to 1 μM). Like digoxin, LND 796 exerted negative inotropic effects at the lowest concentrations (0.01 to 0.03 μM) and positive inotropic effects at high concentrations (1 and 3 μM).

4 In partially K^+-depolarized papillary muscle, in the presence of 2 μM histamine, LND 623 (3 and 10 μM) and LND 796 (10 and 30 μM) enhanced the two components P_1 and P_2 of the contraction and increased slow action potential amplitude, resting potential and maximal rate of depolarization. Low concentrations (0.03 to 0.3 μM) of LND 796 induced negative inotropic effects. β-Adrenoceptor blockade with atenolol (1 μM) did not modify the activity of LND 623 but significantly enhanced the negative inotropic effect on P_2 induced by 1 and 3 μM LND 796 and reduced the positive inotropic effect on P_1 and P_2 of the highest concentration (30 μM) studied.

5 In the presence of either caffeine (1 mM) or Ca^{2+}-free, Sr^{2+}-rich (3.6 mM) solution, LND 623 and LND 796 produced a positive inotropic effect which was stronger with LND 623.

6 It is suggested that two mechanisms are involved in the inotropic effects of these aminosteroids: (i) an enhanced Ca^{2+} entry via the slow calcium channels partially brought about by a local release of endogenous catecholamines in the case of LND 796, (ii) an inhibitory effect on Na^+–K^+ ATPase which, at the highest concentrations, lead to similar signs of cellular toxicity to those described for digitalis drugs. Because of their enlarged positive inotropic range, both aminosteroids may be of interest in the treatment of congestive heart failure.

Introduction

The compounds LND 623 and LND 796 are two aminosteroid derivatives with positive inotropic effects that are similar in several respects to those of digitalis (Jarreau *et al.*, 1983). They differ chemically from digitalis by the absence of the 14-β hydroxyl group and by the side chain attached to the 17-β position on the steroid nucleus; LND 623 is a 3β-rhamnosyloxy 14β-amino 5B(H) pregnan 20β-ol (Figure 1) and LND 796 a close derivative. Although both of these characteristics have been postulated as major determinants of the positive inotropic effects of cardiac glycosides (Thomas *et al.*, 1980), LND 623 and LND 796 exert strong positive inotropic effects that have only been briefly reported (Bidouard *et al.*, 1983; Jarreau *et al.*, 1984; Biour *et al.*, 1986; Adamantidis *et al.*, 1987) and inhibit Na^+-K^+ ATPase activity (Swynghedauw *et al.*, 1983). In addition LND 623 has been found to have greater potency

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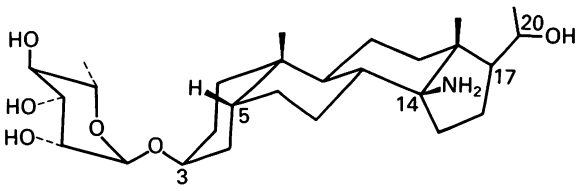

Figure 1 Chemical structure of LND 623.

and lower toxicity than digitalis (Jarreau *et al.*, 1983; Halpryn *et al.*, 1987); thus a greater therapeutic range than with cardiac glycosides may be expected.

It was therefore interesting to examine the electrophysiological and mechanical effects of LND 623 and LND 796 on cardiac ventricular preparations and to compare them with digitalis. Because other mechanisms not directly related to the Na^+-K^+ pump blockade may be involved in their inotropic actions, further investigations were performed in guinea-pig papillary muscle partially depolarized by elevating extracellular K^+ concentration. Such conditions lead to a biphasic contraction which has been demonstrated as suitable for distinguishing between Ca^{2+} arising from the sarcoplasmic reticulum (first component of contraction P_1) and Ca^{2+} entering the cells along with the slow inward calcium current (second component of contraction P_2) (Honoré *et al.*, 1986; 1987a,b). Atenolol, caffeine and strontium were used to clarify the mechanisms underlying the inotropic actions and the toxicity of LND 623 and LND 796. The effects of aminosteroids were compared with those of digoxin obtained under the same conditions (Adamantidis *et al.*, 1984; Adamantidis & Honoré, 1987; Honoré *et al.*, 1987b).

Methods

Tissue preparation and data recording

Guinea-pigs of either sex (300–400 g) were killed by cervical dislocation and exsanguinated. The heart was quickly removed and placed in normal Krebs-Henseleit solution at room temperature (20 ± 1°C). Either papillary muscles (3.0 to 4.0 mm in length, 0.5 to 0.7 mm in diameter) in electromechanical studies or longitudinal strips (4 to 5 mm in length, 1.5 to 2 mm in width, 1 mm in thickness) in toxicity studies were dissected from the right ventricle. Preparations were placed in a 3 ml plastic chamber and continuously superfused at a constant flow (3 ml min^{-1}) with Krebs-Henseleit solution at 36 ± 0.5°C. The composition of the bathing solution was as follows (mM): Na^+ 135, K^+ 4, Ca^{2+} 1.8, Mg^{2+} 1, Cl^- 117.8, HCO_3^- 25, $H_3PO_4^-$ 1.8 and glucose 11. The pH of this solution when gassed with 95% O_2 and 5% CO_2 was 7.35 ± 0.05. The ventricular strips were pinned (the endocardial surface upward) to the silicone bottom of the experimental chamber. The basal end of papillary muscles was pinned to the floor of the chamber and the tendinous end attached by a hooked stainless steel needle and a thin silk thread to a force-displacement transducer (Kulite BG 10). The muscles were carefully stretched at the start of experiments until the peak of the length-tension curve was reached. Preparations were allowed to recover for at least 60 min before the experiment was begun.

Transmembrane potentials were recorded with conventional glass microelectrodes (10–20 MΩ) filled with 3M KCl, connected to an electrometer amplifier (W.P.I. Instruments, Inc., model 750). Electrical and mechanical activities were displayed on a Tektronix model 5111 dual beam oscilloscope and photographed. In addition, action potentials were collected and analysed using a high resolution data acquisition system (Micromed Aquitec SK 64) and the mechanical activity was monitored on a rectilinear chart recorder (Gould Allcoscript EN 216).

Experimental protocols

Electrophysiological study of toxicity in ventricular strips The preparations were stimulated at 0.625 Hz with a bipolar teflon-coated steel wire electrode positioned on the surface of the preparation. Rectangular pulses, 1 ms in duration and 2 times the threshold intensity were delivered by a stimulator (Savita G2). After an equilibration period of 90 min, the drug was added to the bathing solution and superfused until inexcitability occurred. Then the drug was washed out for 60 min. Whenever spontaneous activity developed, electrical stimulation was stopped. In some experiments, the effects of caffeine on spontaneous arrhythmias and delayed afterdepolarizations (DAD) were investigated. Caffeine was added as a bolus (0.1 ml of 0.1 M solution) in the experimental chamber, so allowing rapid recovery from its effects.

Electromechanical study of papillary muscles in normal K^+ Papillary muscles were continuously stimulated at a frequency of 1 Hz. After 90 min equilibration, the drug was added to the bathing solution at increasing concentrations which were superfused for 20 min. Drug superfusion was stopped once arrhythmias occurred and the chamber washed out for 30 min.

Electromechanical study in K^+-depolarized papillary muscles Papillary muscles were stimulated at a frequency of 0.5 Hz. After 90 min equilibration in normal Krebs-Henseleit solution, preparations were partially depolarized by elevating extracellular K^+

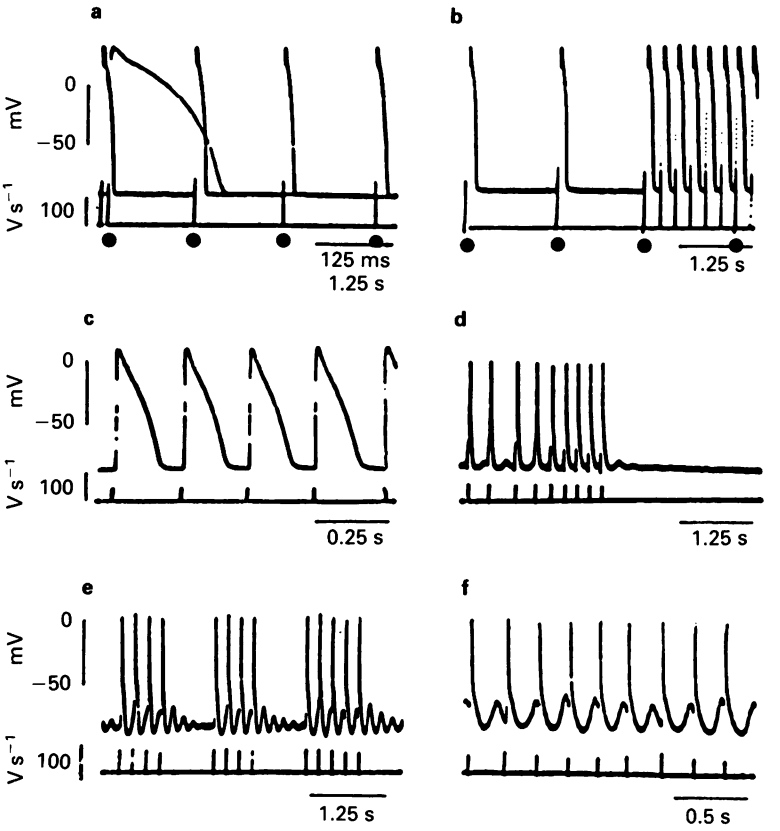

Figure 2 Arrhythmogenic effects of LND 623 (12.8 μM) observed in guinea-pig ventricular fibre. In each panel, upper trace is action potential (AP), lower trace is V_{max}. Each driven AP is labelled with a dot. (a) Control AP before LND 623 superfusion. (b) Spontaneous rhythmical activity occurs after 12 min of LND 623 superfusion. (c) No apparent alteration in membrane diastolic potential is seen. (d) Irregularities in the rhythm and brief pauses unmask delayed afterdepolarizations (DAD). (e) Later (204 min), DADs amplitude are increased and pauses allow damped oscillations to develop. (f) Large DADs are recorded at a more rapid speed.

concentration from 4 to 19 mM. Histamine (2 μM) was added to the K^+-rich solution to restore cellular excitability. Under these experimental conditions slow action potentials and biphasic contraction occurred (Adamantidis & Honoré, 1987). After 60 min stabilisation in K^+-rich solution in the presence of histamine, increasing concentrations of drugs were added to the medium and superfused for 20 min. In order to evaluate the influence of a possible release of endogenous catecholamines, the experiments were repeated in the presence of the cardioselective β-adrenoceptor blocking agent atenolol (1 μM) which was chosen because it is devoid of β-mimetic intrinsic activity and membrane-stabilizing effects (Barrett *et al.*, 1973). The effects of LND 623 and LND 796 on the second component which is related to the slow inward Ca^{2+} current (Honoré *et al.*, 1987a) were more accurately studied with caffeine (1 mM) and substitution of 1.8 mM Ca^{2+} ions by 3.6 mM Sr^{2+} ions in a separate series of experiments, both manoeuvres leading through different mechanisms to the suppression of the early component (King & Bose, 1983; Honoré *et al.*, 1986; 1987a). Atenolol (1 μM) was always present in these series of experiments to avoid any influence of catecholamines. Each concentration of aminosteroid was superfused for 15 min. Washout of the drug was estimated to be complete after 15 min of drug-free superfusion in the modified medium.

Measurements and data analysis

Action potentials were analysed for the following parameters: resting membrane potential (RMP) in mV, action potential amplitude (APA) in mV, maximal rate of rise of depolarization (V_{max}) in $V s^{-1}$,

Table 1 Concentration-dependence of LND 623 and LND 796 in producing the delays required to induce electrophysiological signs of toxicity: comparison with digoxin

	Concentration (μM)	Delay	
		First arrhythmias (min)	*Inexcitability* (min)
LND 623	6.4	26 ± 16	200
	12.8	8 ± 3	100 to 200
LND 796	6.4	19 ± 11	200
	12.8	8 ± 2	100 to 200
Digoxin	1.28	15 ± 1	180 ± 30

action potential duration at 30, 50, and 90% repolarization (APD_{30}, APD_{50} and APD_{90} respectively) in ms.

The amplitude of the monophasic contraction was measured at the peak of the twitch. For the biphasic contraction recorded in K^+-rich solution, the amplitudes of the first component (P_1) and of the second component (P_2) were measured. In some cases, the two components were not well-separated and one peak was not sufficiently defined. In such cases, measurements of that component were made at the time the rising or relaxation phase showed a marked change in its time course (e.g. a shoulder). However because of the overlap of P_1 and P_2, measurements of the components can only be viewed as an approximation of the actual contribution of each component to the observed twitch. Thus a possible overestimation of the quantitative accuracy of the results was avoided.

The results are expressed as the mean ± s.e. mean. In each protocol, control drug-free experiments were run in parallel. Therefore an artifact due to a continuous natural decline of contractile force in the tissue was avoided. Drug effects were evaluated by statistical analysis of comparison of the means with Student's t test. $P < 0.05$ was regarded as significant. LND 623 and LND 796, (kindly provided from Laboratoire Nativelle) were made up daily as a concentrated stock solution (1 mM) of the powder dissolved in HCl solution (0.01 N). Aliquots were then added to the perfusing medium to give the final concentration. The pH of each solution was always readjusted to 7.35 ± 0.05 before superfusion. Atenolol was a gift of ICI Pharma Laboratory and digoxin a gift of Laboratoire Nativelle. Other agents used in this study, including histamine dihydrochloride and caffeine were obtained commercially.

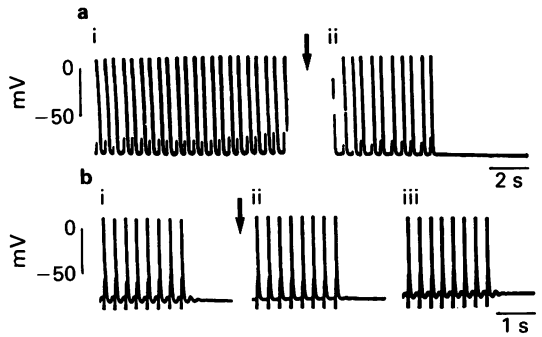

Figure 3 Abolition by caffeine of arrhythmogenic effects induced by LND 796 (12.8 μM) in guinea-pig ventricular fibre. The arrows indicate exposure to caffeine, which was added to the bath as a bolus (0.1 ml of 0.1 M solution). (a) (i) After 18 min of LND 796 superfusion, phase of stable arrhythmias is obtained; (ii) within 20 s after caffeine bolus, arrhythmias abruptly cease. (b) Each action potential (AP) is electrically elicited: (i) after 72 min of LND 796 superfusion, delayed afterdepolarizations (DAD) followed each AP; (ii) 3 min after caffeine bolus, DADs are completely abolished; (iii) 12 min after caffeine bolus DADs reappear.

Results

Electrophysiological study of the toxicity of LND 623 and LND 796

Prolonged exposure of ventricular strips to LND 623 ($n = 8$) or LND 796 ($n = 7$) induced electrophysiological signs of toxicity quite similar to those obtained under the same conditions with digoxin (Adamantidis *et al.*, 1984). Typical examples of prominent features recorded with aminosteroids are given in Figure 2. A first phase of rhythmical activity occurred (Figure 2b) with a delay depending on the concentration used (Table 1) but without apparent alteration in membrane diastolic potential (Figure 2c). Later irregularities in the rhythm of spontaneous activity (Figure 2d) unmasked delayed afterdepolarizations (DADs) which increased in amplitude along with the progressive intoxication. In a second phase of arrhythmias, brief pauses allowed

damped oscillations to develop (Figure 2e) and spontaneous action potentials were clearly initiated from the DADs (Figure 2f). Action potential amplitude and duration progressively decreased. Ultimately spontaneous rhythmical activity ceased. DADs needed stimulation to appear, then preparations became inexcitable within 100 to 200 min. Table 1 shows the delays required for the onset of the first arrhythmias and for the occurrence of inexcitability observed with LND 623 and LND 796. Occurrence and development of arrhythmias induced by 12.8 μM LND 623 and LND 796 were quite similar to those observed with 1.28 μM digoxin (Adamantidis *et al.*, 1984).

As illustrated in Figure 3, caffeine abolished within 30 s the sustained rhythmical activity (Figure 3a) and the delayed afterdepolarizations (Figure 3b) induced by LND 796. Caffeine washout allowed the toxic signs to reappear (Figure 3b). Similar effects of caffeine were obtained in 4 other experiments with LND 796, and 5 experiments with LND 623.

Interestingly, washout of LND 796 always led to complete recovery from arrhythmogenic effects within 43 $\pm$ 2 min. As shown in Figure 4, washout of unresponsive fibres stimulated at a frequency of 0.625 Hz (Figure 4a) led to the transient reappearance of arrhythmias (Figure 4b). After arrhythmias stopped (Figure 4c), electrical stimulation of the preparations elicited markedly depressed action potentials (Figure 4d) which recovered normal characteristics within the following 20 min (Figure 4e, f). After washout of either LND 623 or digoxin, no

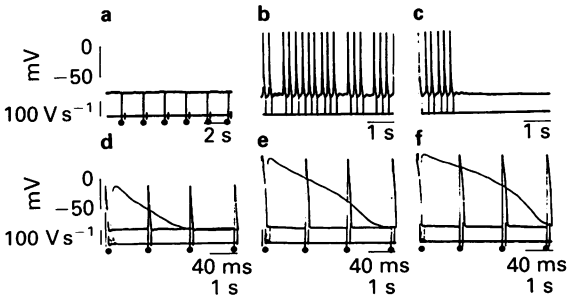

Figure 4 Electrical recovery from toxic effects induced by LND 796 (12.8 μM) in a ventricular fibre. Upper trace is action potential (AP), lower trace is V_{max}. Each driven AP is labelled with a dot. (a) After 105 min of LND 796 superfusion, the fibre was inexcitable. (b) After 13 min of washout, arrhythmias reappear transiently. (c) Rhythmical activity spontaneously ceases after 14 min of washout. (d) After 22 min of washout, electrical driving elicits markedly depressed APs. (e) and (f) After 32 and 45 min of washout respectively, AP characteristics progressively and completely recover from the toxic effects of LND 796.

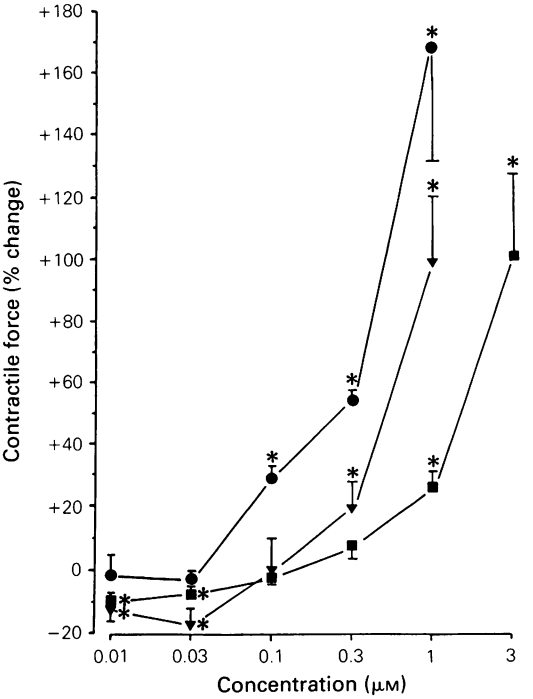

Figure 5 Cumulative dose-effect curves for LND 623 (●), LND 796 (■) and digoxin (▼) on the contractile force of guinea-pig papillary muscle in normal K^+ medium. Results show means with s.e.mean (bars) from 5 separate experiments for each drug. Significant difference from control drug-free experiments, $*P < 0.05$ using Student's *t* test.

such rapid recovery was observed and arrhythmias were still present after 60 min in drug-free solution.

Electromechanical effects in papillary muscles in normal K^+ solution

The effects of each aminosteroid were tested in 5 experiments and digoxin was studied in parallel ($n = 5$). As shown in Figure 5, 0.1 μM of LND 623 exerted a significant positive inotropic effect but LND 796 and digoxin induced first a significant negative inotropic effect at the 2 lowest concentrations (0.01 and 0.03 μM), then a significant positive inotropic effect from 1 μM and 0.3 μM respectively. At a concentration of 3 μM, arrhythmias occurred with both digoxin and LND 623. A higher concentration (10 μM) of LND 796 was required to induce abnormal rhythms. In addition Figure 5 shows that LND 623 exerted a more potent positive inotropic effect than LND 796 and digoxin.

As indicated in Table 2, the low concentrations of LND 623 and LND 796 significantly increased the

Table 2 Concentration-dependent effects of LND 623 and LND 796 on action potential characteristics in ventricular fibres exposed to normal K^+ solutions

Concentrations (μM)		*RMP* (mV)	*APA* (mV)	V_{max} (Vs^{-1})
LND 623	Control	-86.5 ± 0.5	123.3 ± 1	179 ± 9
	0.01	-88.1 ± 0.2*	125.1 ± 0.4*	184 ± 9
	0.03	-88.7 ± 0.4*	127.2 ± 0.6*	184 ± 6
	0.1	-89.4 ± 0.3*	127.7 ± 1	173 ± 8
	0.3	-88.0 ± 0.4*	124.4 ± 1.4	170 ± 7
	1	-85.2 ± 1.0	115.4 ± 2.2*	134 ± 3*
LND 796	Control	-85.6 ± 0.4	122.8 ± 0.7	164 ± 8
	0.01	-87.5 ± 0.6*	125.9 ± 0.9*	175 ± 6
	0.03	-89.3 ± 0.6*	129.0 ± 0.9*	180 ± 8
	0.1	-89.1 ± 0.4*	128.5 ± 0.6*	181 ± 14
	0.3	-89.5 ± 0.4*	128.9 ± 1.0*	188 ± 8
	1	-88.9 ± 0.5*	128.4 ± 0.9*	188 ± 8
	3	-88.7 ± 0.7*	123.9 ± 1.8	185 ± 10

* $P < 0.05$.
RMP: resting membrane potential; APA: action potential amplitude; V_{max}: maximal rate of rise of phase 0 depolarization.

resting membrane potential to more negative values and the action potential amplitude without concomitant modifications in V_{max}. These effects were more obvious with LND 796 than with LND 623. Action potential duration was not significantly modified. As expected, arrhythmogenic concentrations led to a loss in resting membrane potential and a decrease in action potential amplitude, duration and V_{max} (not indicated in Table 2).

Electromechanical effects in K^+-depolarized papillary muscles

On the biphasic contraction recorded in K^+-depolarized (19 mM) papillary muscle in the presence of 2 μM histamine, LND 623 exerted a positive inotropic effect on both components P_1 and P_2 (Figure 6). This effect was significant, as compared to control drug-free experiments, from a concentration of 3 μM upwards (Figure 7). Lower concentrations were without significant inotropic effect. At the upper concentration of 10 μM, the contractile force was greatly increased. This effect was significantly more important on P_1 than on P_2 and led P_1 and P_2 to overlap concomitantly with the appearance of aftercontractions (Figure 6). Low concentrations (0.01 to 3 μM) of LND 796 exerted a significant dose-dependent negative inotropic effect on both components (Figure 8) which was significantly reversed at a concentration of 10 μM. At the upper concentration of 30 μM, both a large increase in contractile force and aftercontractions were observed.

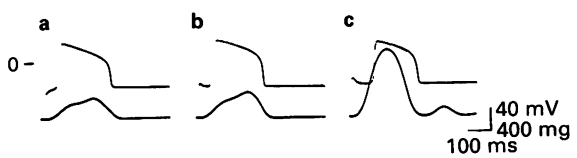

Figure 6 Electromechanical effects of LND 623 on the biphasic contraction obtained in K^+-depolarized papillary muscle in the presence of 2 μM histamine. Upper trace is action potential, lower trace is biphasic contraction. (a) Control action potential and biphasic contraction. (b) LND 623 3 μM produces similar increase in P_1 and P_2 amplitude. (c) LND 623 10 μM markedly enhances P_1 and P_2 amplitude and P_1 and P_2 overlap concomitantly with the occurrence of aftercontraction and depression in action potential characteristics.

In the presence of atenolol at a concentration of 1 μM (which completely blocked the inotropic effects of 0.1 μM isoprenaline), the inotropic effects of LND 623 were not significantly modified (Figure 7). In contrast, with LND 796, the negative inotropic effects of low concentrations were significantly more pronounced and the positive inotropic effects of high concentrations attenuated in the presence of atenolol (Figure 8).

As shown in Table 3, low concentrations of both aminosteroids significantly increased action potential amplitude, overshoot and V_{max} and shifted resting membrane potential to more negative values. Action potential duration was not significantly lengthened. In the presence of atenolol, these modifications in AP parameters were not significantly altered.

Figure 9 illustrates the inotropic effects of LND 623 and LND 796 in the presence of caffeine in comparison with control experiments. Caffeine has been

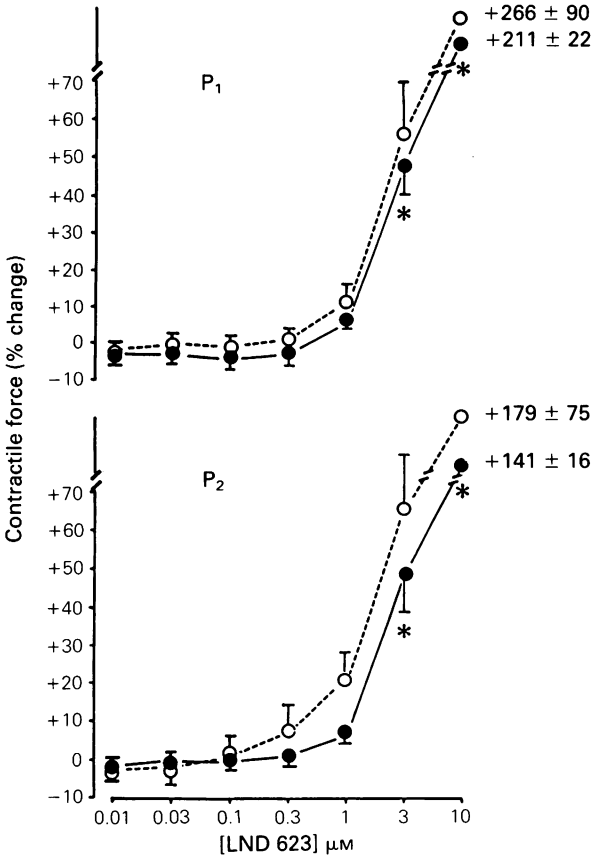

Figure 7 Cumulative dose-effect curves for LND 623 on the two components P_1 and P_2 of the biphasic contraction recorded in K^+-depolarized guinea-pig papillary muscle in the presence of 2 μM histamine. The LND 623-induced effects are studied in the absence (●) and in the presence (○) of β-adrenoceptor blockade with atenolol (1 μM). Results show means with s.e.mean (bars) from 5 separate experiments for each protocol. Significant difference from control drug-free experiments. * $P < 0.05$ using Student's t test.

shown to abolish P_1 and increase P_2 (Honoré *et al.*, 1986; 1987a,b). As shown in Figure 10b, LND 623 caused a significant positive inotropic effect which increased from 1 to 10 μM. A much slighter positive inotropic effect was observed with 3, 10 and 30 μM LND 796. However, this effect was significant as compared to the control experiments which exhibited a progressive decline of the contractile force. Washout of both drugs led to complete recovery within 15 min.

When Ca^{2+} ions are totally substituted by Sr^{2+} ions, the contraction becomes monophasic and corresponds to the second component of the biphasic contraction (King & Bose, 1983; Honoré *et al.*, 1987a). Figure 10a shows the inotropic effects of increasing concentrations of LND 623 and LND 796 in Ca^{2+}-free Sr^{2+} (3.6 mM) solution. At 0.3 and 1 μM, LND 623 significantly enhanced the contractile force. A progressive increase in the resting tension (contracture) reversed this positive inotropic effect at a concentration of 3 μM, leading to severe negative inotropic effects at 10 μM. LND 796 at concentrations of 1, 3 and 10 μM increased significantly the contractile force. Only a slight contracture was observed at the highest concentration studied (30 μM).

Discussion

The present study was undertaken in order to establish more clearly the inotropic and toxic effects of the new aminosteroids LND 623 and LND 796 in guinea-pig ventricular myocardium. In agreement with brief previous reports (Bidouard *et al.*, 1983; Jarreau *et al.*, 1983; Swynghedauw *et al.*, 1983), the results show that both drugs induce a more powerful positive inotropic effect and have a lower toxicity than digitalis. They required tenfold higher concentrations than digoxin (Adamantidis *et al.*, 1984) to induce the same electrophysiological signs of intoxication i.e. occurrence of spontaneous rhythms generated from delayed afterdepolarizations concomitantly with a reduction in resting membrane potential, amplitude and duration of action potentials. The delayed afterdepolarizations that initiate triggered activities have been described in cardiac tissues exposed to a variety of experimental conditions including toxic levels of digitalis (Ferrier & Moe, 1973; Ferrier *et al.*, 1973; Rosen *et al.*, 1973a,b; Ferrier, 1977; Reder & Rosen, 1982), low external K^+ (Kass *et al.*, 1978; Eisner & Lederer, 1979), K^+-free Ca^{2+}-rich solutions (Hiraoka *et al.*, 1981; Hiraoka & Kawano, 1984), ischaemia (Friedman *et al.*, 1973; Singer *et al.*, 1981; El-Sherif *et al.*, 1983), hypoxia and acidosis (Coraboeuf *et al.*, 1980; Adamantidis *et al.*, 1986). They are believed to result from a cyclic release of Ca^{2+} from the sarcoplasmic reticulum (Fabiato, 1983) as a consequence of intracellular Ca^{2+} overload (Kass & Tsien, 1982; Kort & Lakatta, 1984). Either the reduction or the blockade of Na^+-K^+ ATPase activity leads to such Ca^{2+} overload by elevating intracellular Na^+ and reducing Ca^{2+} efflux via the Na^+-Ca^{2+} exchange (Bers & Ellis, 1982; Sheu & Fozzard, 1982).

Caffeine has been shown to abolish specifically digitalis-induced arrhythmias and delayed afterdepolarizations in Purkinje fibres and ventricular muscles (Di Gennaro *et al.*, 1983; 1984; Adamantidis *et al.*, 1984) and in single isolated ventricular cells

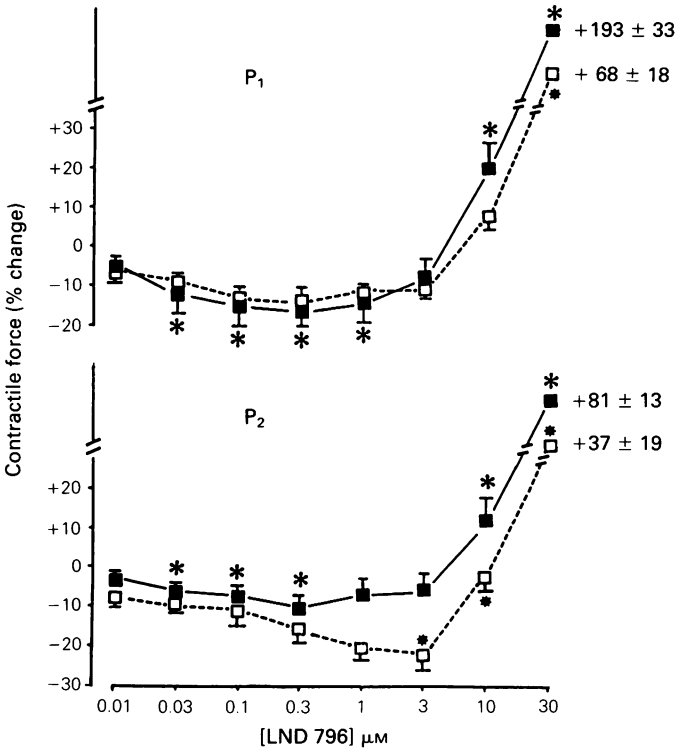

Figure 8 Cumulative dose-effects curves for LND 796 on the two components P_1 and P_2 of the biphasic contraction recorded in K^+-depolarized guinea-pig papillary muscle in the presence of 2 μM histamine. LND 796-induced contractile effects are studied in the absence (■) and in the presence (□) of β-adrenoceptor blockade with atenolol (1 μM). Results show means with s.e.mean (bars) from 5 separate experiments. Significant difference from control drug-free experiments, $*P < 0.05$ using Student's t test. Significant difference from the presence or the absence of atenolol, $*P < 0.05$ using Student's t test.

(Matsuda *et al.*, 1982). These effects of caffeine have been attributed to the elimination of oscillatory potentials by the inhibition of Ca^{2+} movements into the sarcoplasmic reticulum (Fabiato, 1983; Di Gennaro *et al.*, 1984). The present results show that caffeine reversibly eliminated the delayed afterdepolarizations induced by LND 623 and LND 796 and suppressed ventricular arrhythmias. These data allow us to propose that both aminosteroids induce abnormal rhythms through basic mechanisms common with those of digitalis and related to the strong inhibition of Na^+-K^+ ATPase (Swynghedauw *et al.*, 1983).

In normal K^+ medium, both LND 623 and LND 796 were found to exert, in a dose-dependent manner, a positive inotropic effect from concentrations of 0.1 μM and 1 μM respectively. However, only LND 796 induced a significant negative inotropic effect at lower concentrations. The monophasic inotropic effect of LND 623 and the biphasic effect of LND 796 were observed in normal K^+ medium as well as in K^+-rich solution. The elevation of extracellular K^+ concentration only shifted the dose-effect curves to the right in a similar way to that observed with digoxin under the same conditions (Adamantidis & Honoré, 1987).

It has been suggested that the biphasic contraction obtained in K^+-depolarized guinea-pig papillary muscle is related to the Ca^{2+} arising from sarcoplasmic reticulum (first component of contraction P_1) and the Ca^{2+} entering the cell through the slow Ca^{2+} channels (second component of contraction P_2) (Honoré *et al.*, 1986; 1987a,b). P_1 amplitude was increased to a significantly larger extent than P_2 by the highest concentrations of LND 623 and LND 796 used (10 μM and 30 μM respectively). Moreover the strong positive inotropic effect on P_1 was always accompanied by the appearance of aftercontractions.

Table 3 Concentration-dependent effects of LND 623 and LND 796 on action potential characteristics in ventricular fibres exposed to K^+-rich (19 mM) solutions in the presence of histamine (2 μM)

Concentrations (μM)		*RMP* (mV)	*APA* (mV)	V_{max} (Vs^{-1})
LND 623	Control	-50.0 ± 0.3	85.8 ± 0.9	19.5 ± 2.8
	0.01	-50.8 ± 0.3	87.8 ± 0.8	19.8 ± 3.3
	0.03	-51.8 ± 0.4*	89.3 ± 1.6*	21.4 ± 2.1
	0.1	-53.0 ± 0.3*	93.0 ± 1.3*	27.0 ± 2.3*
	0.3	-53.8 ± 0.3*	94.0 ± 1.6*	26.8 ± 2.5*
	1	-54.0 ± 0.4*	94.3 ± 2.1*	25.8 ± 1.9
	3	-53.3 ± 0.1*	92.3 ± 2.0*	24.3 ± 2.9
	10	-45.0 ± 0.4*	76.8 ± 1.4*	16.0 ± 3.2
LND 796	Control	-49.0 ± 0.7	86.0 ± 1.8	18.3 ± 3.7
	0.01	-50.5 ± 0.9	90.8 ± 1.7	20.3 ± 4.4
	0.03	-50.8 ± 1.3	91.8 ± 2.0*	20.8 ± 4.9
	0.1	-51.6 ± 0.8*	93.1 ± 1.0*	22.6 ± 4.4
	0.3	-53.8 ± 0.7*	94.0 ± 1.1*	23.5 ± 5.7
	1	-54.0 ± 0.4*	95.4 ± 1.7*	19.3 ± 3.5
	3	-54.3 ± 0.5*	96.5 ± 2.0*	18.3 ± 3.6
	10	-51.8 ± 1.2	93.2 ± 2.6*	17.8 ± 4.0
	30	-48.0 ± 1.2	78.2 ± 6.9*	12.3 ± 5.6

* $P < 0.05$.
RMP: resting membrane potential; APA: action potential amplitude; V_{max}: maximal rate of rise of phase 0 depolarization.

Similar features have been reported with digoxin (Adamantidis & Honoré, 1987) and related to the Ca^{2+} overload, produced by Na^+-K^+ ATPase inhibition. These data support the above proposition that the effects of toxic levels of both aminosteroids and digitalis involve similar underlying mechanisms.

The negative inotropic effect induced by low concentrations of LND 796 was associated with a significant increase in action potential amplitude, overshoot and V_{max} and a shift of resting membrane potential to more negative values. This effect may be ascribed to a stimulating effect of LND 796 on Na^+-K^+ ATPase comparable to that reported with digitalis (Hart *et al.*, 1983; Adamantidis & Honoré, 1987). The results obtained in the presence of β_1-receptor blockade, which eliminates a possible influence of endogenous catecholamines, strengthen this assumption: the negative inotropic effect was increased only on P_2 which has been found to be more sensitive to catecholamines than P_1 (Honoré *et al.*, 1987a). As previously suggested for digoxin (Adamantidis & Honoré, 1987), β_1-receptor blockade would avoid the development of a positive inotropic effect induced by released endogenous catecholamines which may counterbalance the negative inotropic effect attributable to Na^+-K^+ ATPase stimulation. Such an action of released catecholamines is further supported by the fact that the positive inotropic effect induced by high concentrations of LND 796 was decreased in the presence of atenolol. In contrast, LND 623 did not induce any negative inotropic effect and did not seem to activate adrenergic mechanisms in its positive inotropic effect since atenolol did not modify its effects on contractile force.

The range of positive inotropic concentrations of LND 623 was found to be greater than with LND 796 and digoxin (Adamantidis & Honoré, 1987). A similar observation was made by Jarreau *et al.* (1983; 1984) and required further investigation in order to establish whether mechanisms other than Na^+-K^+ ATPase inhibition were involved in the positive inotropic effect of LND 623. For this reason the effects of LND 623 and LND 796 were investigated in the presence of caffeine. Caffeine is known to increase release from and to block reuptake of Ca^{2+} into sarcoplasmic reticulum which results in the biphasic contraction becoming monophasic through the disappearance of the first component (Honoré *et al.*, 1986; 1987a). In these conditions LND 623 and, to a lesser degree LND 796, exerted a significant positive inotropic effect. Digoxin has been reported to induce a negative (rather than a positive) inotropic effect and contracture (Adamantidis & Honoré, 1987). The positive inotropic effect observed on P_2 amplitude with aminosteroids, especially with LND 623, may be explained by an increased Ca^{2+} entry via the slow Ca^{2+} channels.

This proposition is reinforced by the results obtained in the experiments performed in Ca^{2+}-free,

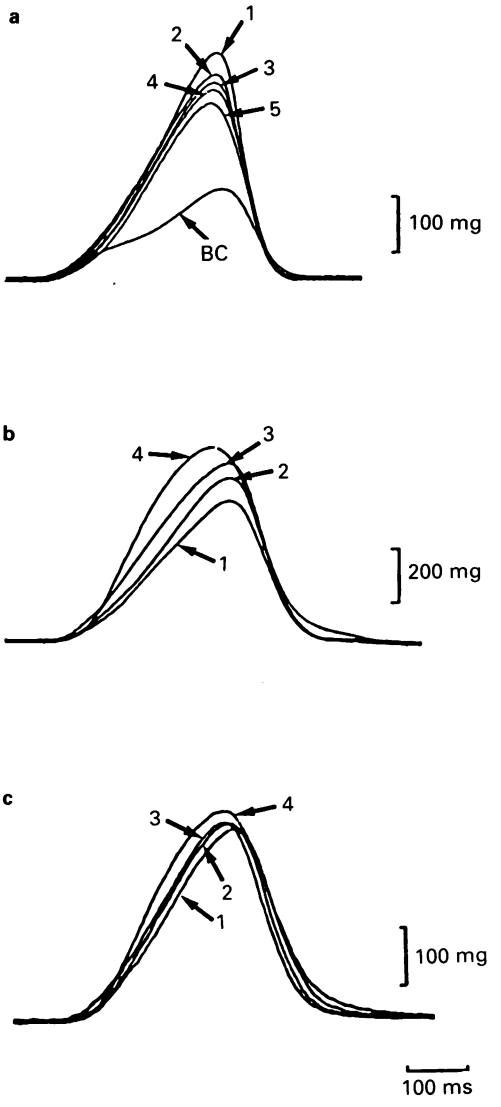

Figure 9 Contractile effects of LND 623 and LND 796 on the biphasic contraction in the presence of caffeine (1 mM) and atenolol (1 μM). In (a) are shown the effects of caffeine on the biphasic contraction (BC) and the progressive decline with time observed after 15 min (1), 30 min (2), 45 min (3), 60 min (4) and 75 min (5). Panel (b) shows the effects of cumulative concentrations of LND 623 on K^+-depolarized papillary muscle exposed to caffeine (1 mM) for 15 min (1) then after 15 min of 1 μM LND 623 (2), 15 min of 3 μM LND 623 (3) and 15 min of 10 μM LND 623 (4). Similarly panel (c) shows the effects of cumulative concentrations of LND 796 on K^+-depolarized papillary muscle exposed to caffeine (1 mM) for 15 min (1) then after 15 min of 3 μM LND 796 (2), 15 min of 10 μM LND 796 (3) and 15 min of 30 μM LND 796 (4).

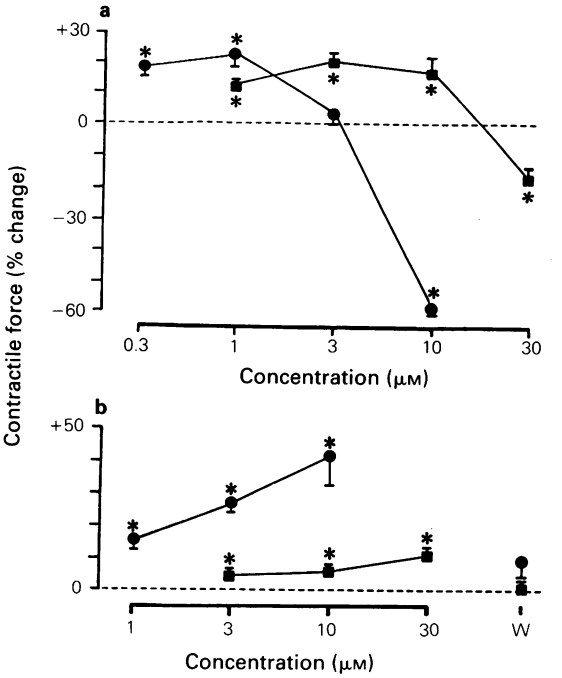

Figure 10 Contractile effects of LND 623 (●) and LND 796 (■) on the second component of the biphasic contraction (in the presence of 1 μM atenolol). (a) The second component of the biphasic contraction obtained in Ca^{2+}-free, Sr^{2+} (3.6 mM) medium; (b) the second component of the biphasic contraction obtained in the presence of 1 mM caffeine. Results show means with s.e.mean (bars) from 5 separate experiments for each protocol. Significant difference from control drug-free experiments, * $P < 0.05$ using Student's t test. W = after 15 min of washout of the drug.

Sr^{2+}-rich solutions. In these conditions only the P_2 component was recorded (Honoré *et al.*, 1987a). Both aminosteroids were found to induce a significant positive inotropic effect which, at high concentrations, was reversed to a negative inotropic effect with the development of contracture. These effects may be explained by an increased entry of Sr^{2+} through the slow Ca^{2+} channels (King & Bose, 1983) followed by an intracellular accumulation of Sr^{2+} ions. Since it has been demonstrated that Sr^{2+} ions can replace Ca^{2+} ions to produce a Na^+-Sr^{2+} exchange (Van Kerkhove & Carmeliet, 1971), an increased Sr^{2+} influx via the Na^+-Sr^{2+} exchange may also occur because of the increased intracellular Na^+ concentration resulting from the Na^+-K^+ ATPase inhibition, so leading to intracellular Sr^{2+} accumulation.

Thus, although the contribution of Na^+-Ca^{2+} exchange in the positive inotropic effect of amino-

steroids can be neither ruled out nor directly implicated from the results of our experiments, it seems likely that aminosteroids act through inotropic mechanisms other than Na^+-K^+ ATPase inhibition. It is suggested that LND 623 could enhance Ca^{2+} entry via the slow Ca^{2+} channels without involvement of adrenergic mechanisms while endogenous release of catecholamines may play a role in the inotropic effects of LND 796. In addition, low concentrations of LND 796 could exert a stimulating effect on Na^+-K^+ ATPase activity comparable to that reported with digoxin (Adamantidis & Honoré, 1987). Interestingly, a rapid and complete recovery from aminosteroid-induced effects was observed on washout, particularly with LND 796. These observations may be explained by a weaker stability of the association of the drug with its receptor and may play a role in the lower toxicity of aminosteroids as compared with digitalis.

An enlarged range of positive inotropic concentrations associated with a lower toxicity may have clinical implications in the treatment of congestive heart failure but further investigations are needed to study more accurately the haemodynamic (Biour *et al.*, 1986) and other cardiovascular effects of these aminosteroids.

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