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LOCAL ANAESTHETIC AND ANTIARRHYTHMIC PROPERTIES OF AN AMINOSTEROID: 3α-DIMETHYL-AMINO-5α-ANDROSTAN-2β-OL-17-ONE (ORG. NA13)

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1 The aminosteroid Org. NA13 (3α-dimethylamino-5α-androstan-2β-ol-17-one) was shown to be a more potent local anaesthetic than lignocaine in rats and guinea-pigs.

2 Experimental arrhythmias induced in mice by chloroform, in rats by aconitine and in dogs by coronary artery ligation were corrected by Org. NA13 at doses from 10 to 50 mg/kg intravenously.

3 In contrast to lignocaine, other local anaesthetics and β-adrenoceptor blocking drugs, Org. NA13 did not show any activity against the arrhythmias induced by ouabain in dogs.

4 The acute toxicity in whole animals and myocardial toxicity in the rabbit isolated atrium appeared to be less than that observed with lignocaine.

Introduction

Recent advances in the pharmacology of steroids have led to the clinical introduction of therapeutic agents possessing pharmacodynamic properties other than those of an endocrinological or related nature. In particular the class of steroids possessing extranuclear amino groups, known as amino-steroids, has yielded many different types of activity (Buckett, 1972). For example, potent neuromuscular blocking agents such as pancuronium bromide (Pavulon) (Buckett, Marjoribanks, Marwick & Morton, 1968), analgesics (Craig, 1968), central nervous system depressants (Hewett, Savage, Lewis & Sugrue, 1964) and local anaesthetics (Buckett, Hewett, Marwick & Savage, 1967; Hewett & Savage, 1968) have all been reported.

This paper describes the properties of Org. NA13, 3α-dimethylamino-5α-androstan-2β-ol-17-one (Figure 1), a potent local anaesthetic which was selected for further pharmacological study from the series described by Buckett *et al.* (1967). The evidence for its local anaesthetic and anti-arrhythmic activity is presented in comparison with the standard drug, lignocaine hydrochloride.

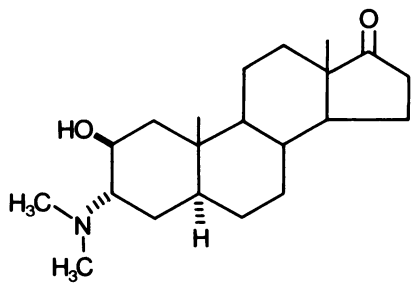

Figure 1 The chemical structure of 3α-dimethyl-amino-5α-androstan-2β-ol-17-one (Org. NA13).

Methods

Local anaesthesia

Intracutaneous local anaesthetic potency was determined by the method of Bülbring & Wajda (1945) using groups of six female albino guinea-pigs (600-700 g body wt.). For each experiment three concentrations of test and standard drug were injected intracutaneously into six injection sites. The flinch response to a needle prick on each site at 6 min intervals up to 36 min was recorded and converted to a percentage for

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each concentration, allowing a potency ratio to be determined.

Surface anaesthetic potency was determined in guinea-pigs (600 g body wt.) according to the method of Chance & Lobstein (1944). Drugs were dissolved in McIlwain buffer (pH 6.0) and solutions instilled into the open eye for 15 seconds. The corneal reflex was tested by touching the eye surface with a glass rod at 1 min intervals for 10 minutes. Potency ratios were determined from dose-response lines obtained from three concentrations of each drug.

Conduction anaesthesia was estimated by rat sciatic nerve block according to the method of Åstrom & Persson (1961) using groups of six male albino Wistar rats (150-200 g body wt.).

Anti-arrhythmic actions

Prevention of chloroform-induced arrhythmias was examined in male albino mice (20-24 g) by the method of Lawson (1968).

Delay in onset of aconitine-induced ventricular arrhythmias was examined in male albino Wistar rats (350-400 g body wt.) anaesthetized with pentobarbitone sodium (60 mg/kg i.p.). Animals were pretreated with NA13, lignocaine or 0.9% w/v NaCl solution (saline) intravenously 2 min before infusion of aconitine 10 μg/ml by the intravenous route according to the method described by Vargaftig & Coignet (1969).

Changes in auricular refractory period were measured essentially by the method of Dawes (1946) using isolated atria of rabbits. Atria were suspended by stainless steel stimulating electrodes in a 50 ml isolated organ bath containing Ringer-Locke solution at 30°C equilibrated with 95% O_2 and 5% CO_2. Contractions were recorded via a force displacement transducer (Statham FT03) on a polygraph (Grass 7) from which atrial rate and amplitude of contraction could be measured directly. The spontaneously beating preparation was allowed a stabilization period of 1 h before stimulation by trains of impulses of increasing frequency for 20 s in each minute using a Grass S4 stimulator. The first frequency at which atria did not respond 1 : 1 to stimulation was taken as the reciprocal of the functional refractory period. For determining drug effects, the test anti-arrhythmic drug was added in solution to the bath for 10 min prior to stimulation, during which time direct effects on the spontaneously beating atria could also be observed.

Antagonism of ouabain intoxication in pentobarbitone anaesthetized dogs was studied by infusion of antiarrhythmic drugs at 1 mg kg^{-1} min^{-1} intravenously for 10 min after injections of ouabain. The intoxication was produced by intravenous ouabain at 0.04 mg/kg followed 30 min later by 0.02 mg/kg and then at 10 min intervals by 0.01 mg/kg until ventricular tachycardia was observed on the electrocardiogram which was then continuously monitored throughout the infusion of anti-arrhythmic drug and for 30 min thereafter.

Correction of post-infarction arrhythmias was demonstrated in six male dogs (8-10.3 kg body wt.) prepared essentially according to the method of Harris (1950) except that a four stage ligation procedure for the anterior descending branch of the left coronary artery was used to reduce the mortality experienced after the two-stage method. Five to 10 h post-operatively the initial sinus rhythm was replaced by ventricular tachycardia. The anti-arrhythmic drugs were infused over a period of 10 min to unanaesthetized dogs 20-40 h after surgical preparation and electrocardiogram recorded from three derivations (Alvar Reega VIII).

Toxicity

Acute toxicity of anti-arrhythmic drugs in mice was determined after intravenous, subcutaneous, intraperitoneal and oral administration to albino male mice (18-22 g) with LD_{50} values and 95% limits being calculated by standard methods.

Mucosal irritation was examined by applying drug solutions to the right eyes of groups of three New Zealand white rabbits at a concentration of 1% in normal saline. Saline was instilled into the left eyes. The animals were observed at 1, 12 and 24 h for presence or absence of irritation to the eye and conjunctiva.

Drugs

The following drugs were used: lignocaine hydrochloride (MacFarlan Smith Ltd; Laboratoires Roger Bellon), ouabain, acetylcholine chloride and aconitine (Sigma), which was dissolved in distilled water acidified with HCl to effect solution. Org. NA13 (3α-dimethylamino-5α-androstan-2β-ol-17-one) was kindly made available as the water soluble hydrochloride salt by Dr D.S. Savage, Organon Laboratories Ltd. All doses and results are expressed in terms of salt.

Results

Local anaesthetic activity

The aminosteroid NA13 was shown to possess more potent local anaesthetic properties than lignocaine in guinea-pigs. After intracutaneous application the effective concentration of NA13 to produce anaesthesia in 50% of test sites was 0.67 (s.e. mean 0.08) mg/ml, in contrast to lignocaine which had an effective concentration of 1.73 (s.e. mean 0.21) mg/ml indicating a potency ratio of 2.6 for NA13 (lignocaine = 1.0) in three determinations in guinea-pigs. As a surface anaesthetic NA13 was also more potent than lignocaine. In three determinations the effective concentration of NA13 to produce anaesthesia on 50% of sites was 1.33 (s.e. mean 0.23) mg/ml, whereas that for lignocaine was 2.55 (s.e. mean 0.47) mg/ml, from which a potency ratio of 1.92 was calculated for NA13 (lignocaine = 1.0).

The conduction blockade as demonstrated in the rat sciatic-nerve *in vivo* was similar for both NA13 and lignocaine (Table 1). The onset of the blockade was less rapid after NA13. At the higher concentration NA13 exhibited a longer duration of action than lignocaine, but the duration at lower concentrations was similar or shorter.

Anti-arrhythmic activity

Mice Against chloroform-induced ventricular arrhythmias both NA13 and lignocaine were effective after intraperitoneal injection in mice. The ED_{50} value for NA13 was 46 (39-52) mg/kg and for lignocaine 49 (43-53) mg/kg.

Rats NA13 was highly effective in delaying the onset of the first burst of ventricular arrhythmias induced by a slow intravenous infusion of aconitine to the anaesthetized animal. Table 2 illustrates the increase in aconitine tolerated after pretreatment with NA13. Significant increases are evident following doses of 25 and 50 mg/kg NA13 intravenously. Similarly lignocaine had a significant effect at 25 mg/kg, but increasing the dose to

Table 1 The activity of NA13 and lignocaine in producing sciatic nerve block in rats

Compound	*Concentration of drug (%)*	*Onset of local anaesthesia (min)*	*Duration of local anaesthesia (min ± s.e.)*
NA13	2	7	98 ± 6.3
NA13	1	5	65 ± 6.6
NA13	0.5	5	22 ± 0.3
Lignocaine	2	5	63 ± 6.6
Lignocaine	1	3	56 ± 4.2
Lignocaine	0.5	3	37 ± 3.5

Results from two series of experiments in which each animal received NA13 on one side and lignocaine on the other. Method of Åstrom & Persson (1961).

Table 2 The amount of aconitine by intravenous infusion required to induce ventricular arrhythmias in rats pretreated intravenously with NA13 and lignocaine

Drug	*Dose (mg/kg i.v.)*	*Number of animals*	*Aconitine tolerated (μg/kg ± s.e.)*	*% Increase in aconitine*	P *Value*
Control	–	14	32.3 ± 2.5	–	–
Lignocaine	6.25	3	35.7 ± 4.4	11	NS
	12.5	5	48.6 ± 15.6	20	NS
	25	5	50.9 ± 9.1	58	0.05
	50*	3	–	–	–
NA13	6.25	3	43.1 ± 7.5	33	NS
	12.5	3	59.1 ± 16.3	82	NS
	25	4	71.5 ± 6.7	121	0.05
	50	3	73.9 ± 9.3	128	0.05

* Toxic dose level.

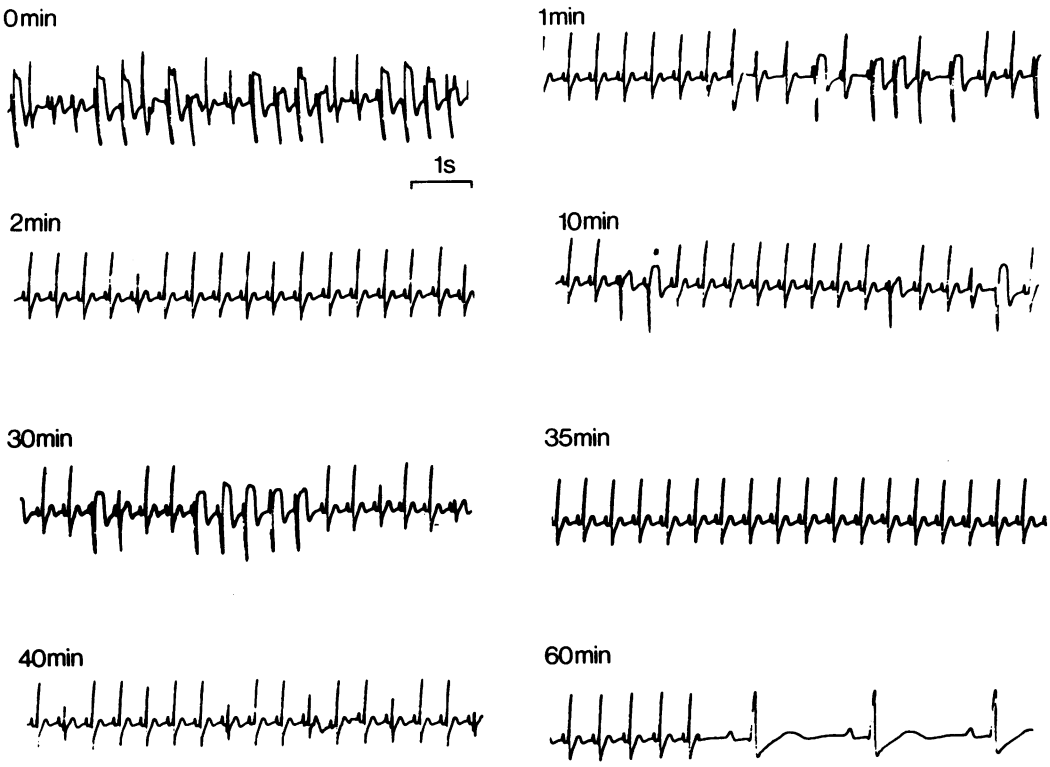

Figure 2 The action of NA13 in correcting the ventricular arrhythmia in a dog (No. 2) 20 h after coronary artery ligation (electrocardiogram, lead II). The figure above the record indicates time in min after the start of NA13 infusion 1 mg kg^{-1} min^{-1} for 0-10 minutes. At 30 min the infusion was repeated. Time scale = 1 second.

50 mg/kg was toxic in all animals. It is clear that NA13 is somewhat more active and less toxic than lignocaine in this test.

Rabbits The examination of the drugs on isolated atria of rabbits demonstrated the considerable increase in refractory period shown in Table 3. On a molar basis lignocaine was four times as active as NA13 in contrast to the results described above for local anaesthetic and anti-arrhythmic actions in rats and mice. Nevertheless evidence from Table 3 also suggests that lignocaine

Table 3 The effect of NA13 and lignocaine on the functional refractory period and on the contractions of spontaneously beating rabbit atria *in vitro*

Drug	*Concentration ($M \times 10^{-5}$)*	*Number of experiments*	*% Increase in refractory period (± s.e.)*	*Onset to 50% decrease in amplitude of contraction (min)*	*% Reduction in atrial beat frequency (± s.e.)*
NA13	1.65	6	13.0 ± 4.1		
NA13	3.3	6	18.4 ± 7.7	–	8.13 ± 0.6
NA13	6.6	6	43.4 ± 4.3	10	27.1 ± 13.0
NA13	13.2	6		6	23.4 ± 5.2
Lignocaine	0.42	5	21.1 ± 2.8		
Lignocaine	0.83	5	43.0 ± 10.4		
Lignocaine	1.65	5	43.5 ± 6.6		
Lignocaine	3.3	5		1.5	0
Lignocaine	6.6	5		1	0
Lignocaine	13.2	5		<1	100

Table 4 Effects of NA13 and lignocaine on the ventricular arrhythmia induced by artery ligation in dogs

Dog No.	Drug	Pre-drug heart rate		Heart rate after start of drug administration											
				0-1 min		1-5 min		5-10 min		10-15 min		15-20 min		20-30 min	
		V.R.	% Sinusal	V.R.	% S	V.R.	% S	V.R.	% S	V.R.	% S	V.R.	% S	V.R.	% S
1	NA13	171	0	168	3	161	87	180	100	180	50	168	50	180	30
2	NA13	135	27	132	69	135	74	135	88	136	100	136	100	135	40
2a	NA13	135	40	135	45	126	88	135	100	128	100	128	100	126	100
3	NA13	144	0	144	100	144	87	144	44	135	100	144	81	162	0
4	Lignocaine	234	0	225	4	216	4	180	0	180	10	189	9	180	23
5	Lignocaine	144	0	156	0	156	0	162	0	160	0	160	0	158	0
6	Lignocaine	162	0	156	0	156	0	156	100	144	100	144	91	138	91

The ventricular rate (V.R.) and percentage of sinus beats (% S) are given for before, during (0-10 min) and after (10-30 min) intravenous infusion of 1 mg kg^{-1} min^{-1} (total dose: 10 mg/kg) of NA13 and of lignocaine in dogs displaying ventricular arrhythmias due to coronary ligation 20-40 h previously.

may be more depressant on the myocardium when its effect on the amplitude of contraction of spontaneously beating atria is considered. For lignocaine the decrease to 50% of contraction occurred at similar times with only one tenth of the molar concentration of NA13 which was required for this effect. NA13 itself caused a small reduction in the rate of contraction at these concentrations.

Dogs NA13 in doses up to 50 mg/kg infused intravenously was completely ineffective in reverting ouabain-induced ventricular tachycardia to sinus rhythm in four anaesthetized dogs. In contrast lignocaine administered at 1 mg/kg increased the percentage of sinus beats from 5 ± 3.5 (±s.e.) before to 25 ± 14 immediately after intravenous infusion (four dogs). At 2 mg/kg lignocaine increased the percentage from 3 ± 2.9 to 79 ± 20 after infusion (four dogs).

Seven observations were carried out in six dogs 20-40 h after ligation of the anterior descending branch of the left coronary artery. NA13, administered by a slow intravenous infusion of 1 mg kg^{-1} min^{-1} over 10 min reverted the ventricular tachycardia (animals 1 and 3) or the polymorphous ventricular extrasystoles (animal 2) to sinus rhythm as shown in Table 4. The ECG from animal 2 is shown in Figure 2 which clearly demonstrates the corrective effect of NA13. In this case a second perfusion was carried out to effect full correction at 60 minutes.

Lignocaine at this dose level showed correction in one of the three dogs tested, whereas no corrective action on the remaining two dogs was observed possibly due to rapid metabolism of this compound after intravenous administration.

Toxicity

By intravenous and intraperitoneal routes in mice, NA13 was less toxic than lignocaine (Table 5). It was more toxic after subcutaneous administration. Both drugs exhibited similar toxic signs namely, tremor, excitation and convulsions. Neither compound showed toxicity after oral administration. After application of NA13 solutions to the rabbit eye, no mucosal irritation was observed up to 24 h later. Similarly the guinea-pigs used in the surface anaesthesia experiments did not show any evidence of irritation.

Discussion

Org. NA13 has been shown to be a potent local anaesthetic compound in three types of test designed to show conduction anaesthesia, topical or surface anaesthesia and infiltration anaesthesia

Table 5 The acute toxicity of NA13 and lignocaine by various routes in mice

Drug	*Route*	*LD_{50} mg/kg (95% limits of error)*
NA13	Intravenous	56.5 (37.5-71.2)
NA13	Intraperitoneal	156 (127-195)
NA13	Subcutaneous	201 (153-262)
NA13	Oral	>300
Lignocaine	Intravenous	28.5 (18.7-43.0)
Lignocaine	Intraperitoneal	117 (115-119)
Lignocaine	Subcutaneous	>300
Lignocaine	Oral	>300

after intracutaneous injection. The local anaesthetic properties of steroids have only previously been noted in steroidal alkaloids such as conessine, isoconessine and neoconessine (Stephenson, 1948). With these compounds local anaesthesia was found after intracutaneous injection in guinea-pigs, but the irritation after injection as well as undesirable pharmacological properties such as neuromuscular blockade and myocardial depression precluded any further interest.

Chemically NA13 bears little structural resemblance to the known local anaesthetics, nevertheless it possesses a large lipophilic nucleus and two hydrophilic groups at the 2 and 3 positions. These substitutions are probably optimal since analogues of NA13 do not show greater potency as local anaesthetic drugs (Buckett, 1972). The mode of action of NA13 has been confirmed in experiments with frog sciatic nerve *in vitro*, when the action potentials initiated by stimulation were blocked in passage along the nerve by solutions of the compound (Duff & Nicol, personal communication).

The increasing interest in the use of local anaesthetic drugs to control arrhythmias led us to study the anti-arrhythmic properties of NA13 in some detail. In the initial tests NA13 showed similar activity to lignocaine in chloroform-induced arrhythmias in mice and would thus be expected to be more active than procainamide and quinidine (Lawson, 1968). The action of NA13 in rats was clearly preferable to lignocaine, since the former compound produced anti-arrhythmic actions at doses below toxic levels whereas the standard drug was toxic at therapeutic dose levels against aconitine in this species. The results from experiments on the rabbit isolated atria also suggest some differences in the activity of the two compounds, with lignocaine being apparently more cardiotoxic, so it is clearly important to have confirmation of such actions in more than one species before clinical assessment of a new drug of this type.

NA13 also shows potent anti-arrhythmic actions in coronary artery ligated dogs but is unexpectedly inactive up to 50 mg/kg against ouabain-induced ventricular arrhythmias in the same species. This inactivity was also found in cats (Buckett, unpublished observations). However, our findings with lignocaine on ouabain arrhythmias are in agreement with Allen, Shanks & Zaidi (1971) who found a dose of 0.9 mg/kg of lignocaine intravenously produced a 50% reduction in the number of ventricular ectopic beats. The activity of other drugs possessing local anaesthetic properties, such as procaine, procainamide and quinidine has also been demonstrated against ouabain arrhythmias in dogs (Mosey & Tyler, 1954) so the profile of NA13 is indeed unusual. It is free from β-adrenoceptor blocking actions (Vargaftig, unpublished observations) so cannot be compared with propranolol which is active against ouabain-induced arrhythmias in the dog (Allen *et al.*, 1971) at low doses and whose activity is due partially, at least, to its local anaesthetic action (Barrett & Cullum, 1968).

In these experiments NA13 showed activity in correcting post-infarction arrhythmias to sinus rhythm in all dogs tested, whereas lignocaine was only effective under these conditions in one out of three dogs. This may well be due to the inactivation of lignocaine, since it has been emphasized (Allen *et al.*, 1971) that the rapid metabolism is responsible for the variability in its use and difficulty in using it as a standard. It is unlikely that NA13 is so rapidly metabolized and indirect evidence for this is given by the LD_{50} values of the two drugs. Lignocaine and NA13 show LD_{50} values of the same order after rapid intravenous administration, where there is no time for metabolic transformation, whereas after subcutaneous administration allowing metabolism then lignocaine is much less toxic than the aminosteroid. Foldes (1966) used the intravenous LD_{50} in mice as a guide to clinical potency and the subcutaneous LD_{50} as an indication of metabolic transformation of local anaesthetic drugs.

Our observations indicate that NA13 is a novel steroidal local anaesthetic drug possessing a profile of anti-arrhythmic action, inactive against ouabain-induced arrhythmias, a property which we are unable to explain at the present time.

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