(1,17). Studies of conversion of D>1.21 proteins to lipoproteins are inconclusive because of the necessity of making almost complete separation between these proteins and the lipoproteins. Furthermore, in the *in vivo* studies where these proteins are injected, relatively large amounts of labeled amino acids are present and these may well be reutilized in the synthesis of lipoproteins.

Summary. Alanine-1-C^{14} was administered to rabbits and its incorporation into the lipoproteins of D<1.063 and D 1.063 - 1.21 fractions and into the remaining serum proteins was measured. The D<1.063 fraction reached a higher specific activity than did the D 1.063-1.21 lipoprotein fraction or the D>1.21 fraction. Both lipoprotein fractions turned over at a faster rate than did the remaining serum proteins. C^{14}-labeled lipoprotein fractions were injected into rabbits and there was no significant interconversion between the two major density classes of lipoproteins studied. These data suggest that the D<1.063 and D 1.063 - 1.21 classes of lipoproteins are metabolically as well as chemically distinct.

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Received January 22, 1957. P.S.E.B.M., 1957, v95.

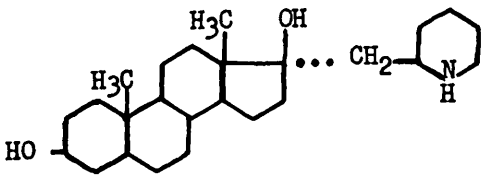
Anti-fibrillatory Activity of 17-(2-Piperidylmethyl)-3β, 17β-androstane diol. (23243)

W. SCHALLEK, F. W. ZABRANSKY, L. M. JAMPOLSKY, W. R. REHL AND M. W. GOLDBERG (Introduced by E. L. Severinghaus)

Pharmacological and Chemical Research Laboratories, Hoffmann-La Roche, Nutley, N. J.

A number of basic steroids have been found to possess cardiac activity. Krayer, Uhle and Ourisson(1) showed that 20-(5'-methyl-2'-piperidyl)-5-pregnene-3, 20 diol had anti-accelerator action on the heart-lung preparation of the dog. Margolin, Lu, Yelnosky and Makovsky(2) demonstrated both anti-accelerator and anti-fibrillatory activity for 16-cyclohexylamino-allopregnandiol in the dog, while Robson and Trounce(3) found that 16-dimethylaminomethyl - epi-dehydroisoandrosterone antagonized ventricular arrhythmias in the cat. The present report describes the anti-fibrillatory activity of 17-(2-piperidylmethyl)-3β, 17β-androstane diol (Ro 2-7302). This compound has the following

structure:



Methods. 1. *Chemical*: The 17-(2-piperidylmethyl)-3β, 17β-androstane diol used in this investigation was prepared by condensation of dehydroisoandrosterone with 2-picolyl lithium, followed by catalytic hydrogenation of the resulting 17-(2-pyridylmethyl)-5-androstene-3β, 17β-diol. This latter compound has recently been described by Heer and Hoffmann(4), who obtained it by a slightly different method. Our purified 17-(2-pyridylmethyl)-5-androstene-3β, 17β-diol melts at 179-183° (corr.), while Heer and Hoffmann (4) report a melting point range of 178-185°. Catalytic hydrogenation of the 17-(2-pyridylmethyl)-5-androstene-3β, 17β-diol was carried out as follows: To 17.2 g of 17-(2-pyridylmethyl)-5-androstene-3β, 17β-diol in 300 ml of methanol was added 8 ml of 6 N hydrochloric acid and the mixture hydrogenated in the presence of 1.6 g of platinum oxide catalyst at 100 lb./sq. in. and 50°C. After the hydrogen uptake had ceased, the catalyst was separated by filtration and the filtrate evaporated to dryness *in vacuo*. The residue so obtained was slurried with water, made alkaline and extracted with chloroform. Evaporation of the dried chloroform extract gave a residue, which on crystallization from ethyl acetate or from acetonitrile yielded 17-(2-piperidylmethyl)-3β, 17β-androstane diol, melting at 211-213°C. (corr.). Calcd. for $C_{25}H_{43}O_2N$: C, 77.07; H, 11.13. Found: C, 76.95; H, 10.84. Although this compound was not reported by Heer and Hoffmann(4), they do describe its 3-monoacetyl derivative. The sulfate (decomp.>320°), the d-camphor-10-sulfonate (decomp. 236-239°), the maleate (m.p. 126-160°) and the d-tartrate (decomp. 242-249°) all crystallize with one mole of water, whereas the hydrochloride (decomp.>320°) and the lactate (m.p. 120-123°) are hemihydrates. Only the lactate is soluble in water; this form (Ro 2-7302/4) was therefore used in the following experiments.

2. *Pharmacological*: As the technics have already been described in detail(5,6), only brief mention of them will be made here. *The isolated rabbit auricle test* (Dawes, 7) is based on measurement of the maximum rate of electrical stimulation which the auricles can follow; this rate is lowered by quinidine. Drug effects are indicated in terms of the greatest change in maximum frequency occurring during one hour's exposure to the drug. Only one drug test was conducted on each heart. *Auricular fibrillation in the dog* was induced by placing a cotton pledget soaked in 5% acetylcholine (ACh) on the area of the sinus node (Scherf and Chick, 8). After several control fibrillations (average duration 18 minutes), the drug being tested was injected, and its effect on duration of fibrillation noted. In addition to intravenous administration, absorption of drugs from the gastrointestinal tract was studied by injection into the duodenum. *Aconitine-induced arrhythmias* were studied in the dog by placing a pledget soaked in 5% aconitine on the sinus node area for 30 seconds (Prinzmetal *et al.*, 9). This produced a mixture of auricular flutter and auricular fibrillation with occasional ventricular ectopic beats. As the transition to and from these arrhythmias was gradual, it was difficult to determine their duration; however, the accompanying increase in heart rate was easily measured. The drugs being tested were injected 1 min. before application of aconitine; 3 control animals were injected with saline in the same manner. The figures in Table I show percentage increase in heart rate following aconitine. *Ventricular fibrillation* in the dog was induced by sensitizing the myocardium with chloroform, followed by epinephrine (Epi) 0.01-0.02 mg/kg I.V. (Melville, 10). This procedure resulted in ventricular fibrillation and death in 5 out of 8 control animals. Eight other dogs were injected with quinidine and 9 with Ro 2-7302/4, each animal receiving only a single dose of drug. *Electrocardiograms* were recorded in separate experiments. Three dogs received quinidine in successive doses of 5, 10, and 20

TABLE I. Anti-Fibrillatory Activity.

No. of animals per dose	Test	Dose of drug	Quinidine sulfate	Ro 2-7302/4
4	Isolated rabbit auricles	mg/l	% change in max frequency	
		.5	− 6 ± 6	−12 ± 4
		1	−22 ± 9	−19 ± 6
		5	−39 ± 4	−42 ± 11
3	ACh-induced auricular fibrillation in dog	mg/kg	% change in fibrillation duration	
	Intrav. admin.	.125		−19 ± 35
		.25		−88 ± 24
		.50		−93 ± 15
		2	0 ± 39	
		4	− 71 ± 29	
		8	− 97 ± 5	
	Intra-duodenal admin.	20	−100 ± 0	−96 ± 4
3	Aconitine-induced tachycardia in dog	mg/kg I.V.	% change in heart rate	
		10	+70 ± 70	+ 25 ± 40
			Saline controls,	+135 ± 75
2–4	Epi-induced ventricular fibrillation in dog	mg/kg I.V.	No. dogs fibrillated/No. dogs tested	
		1	1/2	
		2.5	0/2	0/2
		5	0/2	2/3
		10	0/2	3/4
		Total	1/8	5/9
		Saline controls		5/8

mg/kg I.V. with an interval of 1 to 2 hours between injections. Three other dogs received Ro 2-7302/4 by the same schedule. All experiments on dogs were performed under pentobarbital anesthesia; only one anti-fibrillatory drug was administered to each animal.

Results. The effects of quinidine sulfate and Ro 2-7302/4 on experimentally induced arrhythmias are compared in Table I. The drugs were equally active in depressing the maximum rate at which the isolated rabbit auricles can be driven. Ro 2-7302/4 was approximately 16 x as active as quinidine in antagonizing ACh-induced auricular fibrillation in the dog, since its action at 0.5 mg/kg I.V. was about the same as that of quinidine at 8 mg/kg. Both drugs antagonized ACh-induced auricular fibrillation when injected into the duodenum at a dose of 20 mg/kg. Dogs treated with Ro 2-7302/4 at 10 mg/kg I.V. showed less increase in heart rate following aconitine than did those treated with quinidine at the same dose. On the other hand, quinidine was more active than Ro 2-7302/4 in preventing Epi-induced ventricular fibrillation in the dog.

Effects of these drugs on the ECG of the dog are illustrated in Fig. 1. Blood pressure, heart rate and ECG data are given in Table II. Both quinidine and Ro 2-7302/4 at doses of 10 and 20 mg/kg I.V. produced bradycardia, hypotension, increase of P-Q, QRS, and Q-T durations, and decrease in amplitude of R wave. Quinidine increased amplitude of the S wave at all doses; Ro 2-7302/4 increased amplitude of this wave at 5 mg/kg I.V., had little effect at 10 mg/kg, and decreased amplitude at 20 mg/kg. Amplitude of the T wave was progressively increased with increasing doses of quinidine, whereas with increasing doses of Ro 2-7302/4 it was first decreased and then reversed.

Other cardiovascular effects: Neither quinidine nor Ro 2-7302/4 at doses of 1 and 4 mg/kg I.V. had any effect on the blood pressure changes induced by ACh, Epi, serotonin, or occlusion of the carotid artery.*

* We wish to thank Miss Tina Ferruggia for making these measurements.

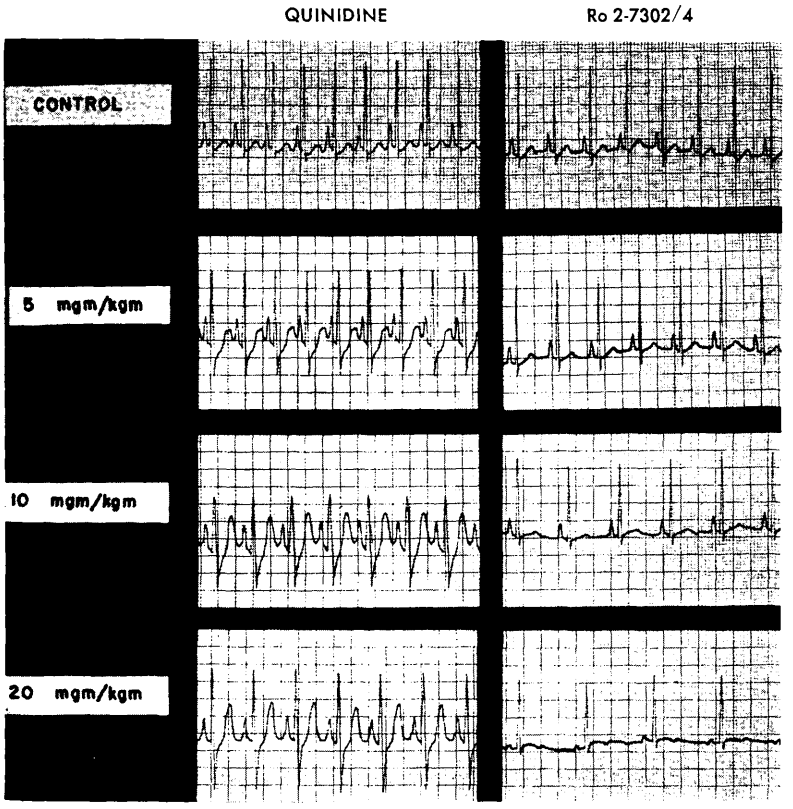


FIG. 1. Effects of drugs on electrocardiogram (Lead II) of dogs under pentobarbital anesthesia. Left: Dog. No. 589. Quinidine, 5, 10, and 20 mg/kg I.V. Right: Dog No. 643. Ro 2-7302/4, same dose levels.

Discussion. Ro 2-7302/4 showed activity equal or superior to that of quinidine on the isolated rabbit auricle, on ACh-induced auricular fibrillation and on aconitine-induced tachycardia in the dog. Both drugs produced side effects, as shown by marked blood pressure and ECG changes, at 10 and 20 mg/kg I.V. Whereas quinidine showed antifibrillatory activity at 4 and 8 mg/kg, Ro 2-7302/4 showed this activity at 0.25 and 0.50 mg/kg. This suggests that the safety margin of Ro 2-7302/4 is greater than that of quinidine. This compound merits further study as a potential anti-fibrillatory agent.

Summary. 17-(2-Piperidylmethyl) - 3β, 17β-androstane diol lactate (Ro 2-7302/4) has an activity equal to that of quinidine in diminishing maximum rate of the electrically

TABLE II. Effects of Drugs on Blood Pressure, Heart Rate and ECG (Lead II) of Dog. (Each figure is avg of 3 experiments; readings taken 1 min. after drug inj.)

Drug	Dose, mg/kg I.V.	Percentage change, relative to pre-drug value: Heart rate	Blood pressure: Systolic	Blood pressure: Diastolic	Duration: P–Q	Duration: QRS	Duration: Q–T	Amplitude: R	Amplitude: S	Amplitude: T
Quinidine sulfate	5	0	− 8	−19	+11	− 2	+ 7	−17	+46	+ 41
	10	− 9	−34	−41	+32	+ 27	+11	−32	+84	+118
	20	−24	−46	−66	+58	+108	+37	−42	+46	+164
Ro 2-7302/4	5	−16	−10	−12	+13	+ 26	+34	−10	+36	− 20
	10	−24	−21	−34	+24	+ 43	+48	−19	+14	− 80
	20	−47	−53	−72	+65	+100	+58	−33	−64	−240

driven isolated rabbit auricle. It is approximately 16 times as active as quinidine in antagonizing acetylcholine-induced auricular fibrillation in the dog. It is slightly more active than quinidine in antagonizing aconitine-induced tachycardia in the same species. The safety margin of this drug, as measured by the ratio between minimal anti-fibrillatory doses and those producing marked blood pressure and ECG changes, is greater than that of quinidine.

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Received March 1, 1957. P.S.E.B.M., 1957, v95.

Effect of Tolbutamide (Orinase) on Plasma Non-Esterified Fatty Acids. (23244)

EDWIN L. BIERMAN, THOMAS N. ROBERTS AND VINCENT P. DOLE
Rockefeller Institute for Medical Research and Department of Medicine, N. Y. Hospital.

Oral or parenteral administration of sulfonylurea derivatives produces hypoglycemia in normal subjects and in many diabetic patients(1,2). Since functioning pancreatic tissue is required for this action, it has been suggested that tolbutamide (1-butyl-3-p-tolylsulfonylurea) in some manner enhances the effect of endogenous insulin(3,4). On the other hand, there is evidence that tolbutamide may limit release of glucose from the liver into the blood stream(5,6) and thus reduce concentration of blood glucose in this manner rather than by promoting utilization. The close association between metabolism of carbohydrate and the level of plasma non-esterified fatty acids (NEFA) provides another way to test this question. Since an increased oxidation of carbohydrate appears to be associated with a fall of NEFA concentration, it should be possible to reveal any effect of tolbutamide on oxidation of carbohydrate by its action on the NEFA fraction of blood lipides.

Method. Tolbutamide was administered to 4 normal, 7 mild adult diabetic, and 3 severe juvenile-type diabetic patients, in a dose of 2 g dissolved in 20 cc saline and given intravenously over a 2-minute period. Venous blood samples, collected in duplicate before injection and at 20, 40, 60, and 120 minutes thereafter, were analyzed for glucose by a modification of the Nelson colorimetric procedure(7) and for NEFA by a method previously described(8). Urine samples for qualitative determinations of sugar and ketones were obtained at hourly intervals.

Results. Concentrations of blood glucose and NEFA observed in the normal and diabetic patients are given in Table I. In agreement with previous observations, tolbutamide produced hypoglycemia in normals and mild diabetics, but failed to lower blood sugar of the 3 diabetics with histories of recurrent ketosis. A decrease in NEFA occurred in every patient who responded with a reduction of blood sugar; no significant decrease was observed in the absence of a hypoglycemic effect. The correlation between changes in concentration of NEFA and blood sugar was highly significant ($p<0.001$).