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14β-Hydroxyprogesterone binds to the digitalis receptor, inhibits the sodium pump and enhances cardiac contractility

*†D. Bose, *D. Elliott, *T. Kobayashi, **J.F. Templeton, **V.P.S. Kumar & *[1]F.S. LaBella

*Department of Pharmacology and Therapeutics and †Department of Internal Medicine, Faculty of Medicine and **Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3

1 Certain derivatives of progesterone are potent inhibitors of high affinity, specific binding of ^{3}H-cardiac glycosides. The steroids interact at the cardiac glycoside site on Na,K-ATPase and inhibit the enzyme (the sodium pump) in cardiac and other tissues. However, the active congeners identified previously have been, unlike the cardiac glycosides, predominantly cardiodepressant.

2 Because a 14β-hydroxy substituent is an important determinant of activity of the cardiotonic cardiac glycosides, we synthesized 14β-hydroxyprogesterone. This derivative has about one-tenth the potency of the aglycone, ouabagenin, in a [^{3}H]-ouabain binding assay.

3 Like ouabagenin, but in contrast to the cardiodepressant congeners of progesterone, 14β-hydroxyprogesterone consistently elicited positive inotropy in isolated cardiac muscle and enhanced both the magnitude and frequency of fluctuations in scattered light (an index of oscillatory intracellular release of calcium).

4 Thus, at least one hydroxylated derivative (and putative endogenous metabolite) of progesterone, mimics the cardiac effects of cardiac glycosides including enhanced contractility.

Introduction

Certain 17-acetylated derivatives of progesterone, e.g. chlormadinone acetate, (CMA), chlormadinone acetate-3-hemisuccinate (CMA-S), medroxyprogesterone acetate, megestrol acetate, and cyproterone acetate, compete with cardiac glycosides for specific, high affinity binding sites, to inhibit Na,K-ATPase, and to impair ^{86}Rb uptake in isolated tissues (Chow *et al.*, 1979; Kim *et al.*, 1980; LaBella *et al.*, 1979; 1984; 1985; Wehling *et al.*, 1981; Temma *et al.*, 1983; Hnatowich & LaBella, 1984; Bihler *et al.*, 1985; Rafuse *et al.*, 1985). In a [^{3}H]-ouabain binding assay, CMA is three times more potent than the digitaloid aglycone, ouabagenin. Wehling *et al.* (1981) concluded that CMA binds to the same site on Na,K-ATPase as do the cardiac glycosides: CMA displaced radiolabel from the enzyme-[^{3}H]-ouabain complex at the same rate as did the cardiac glycosides; inhibition of Na,K-ATPase by CMA, as by ouabain, is non-competitive with ATP and competitive with potassium; and CMA inhibits purified cardiac Na,K-ATPase in the rank order of species sensitivity seen with the cardiac glycosides. Although CMA and related steroids may elicit transient or, occasionally, sustained enhancement of cardiac contractility, the major cardiac effect of these compounds, in contrast to that of the cardiac glycosides, is generally depressant. Indeed, progesterone itself has long been known to depress contractility in a variety of isolated cardiac tissues (see Tanz, 1963). Thus, these observations have given rise to the speculation that the binding site which mediates inhibition of the sodium pump, may not be identical to that which mediates positive inotropy, or that other actions of the steroids counteract and reverse the positive inotropic response (LaBella *et al.*, 1984; 1985).

Because the 14β-hydroxy function is an important determinant of biological activity of the cardiac glycosides (Guntert & Linde, 1981), we have synthesized 14β-hydroxyprogesterone (14β-OHP) (Templeton *et al.*, 1987a). This novel steroid shares with CMA the ability to inhibit both the sodium pump and the high affinity binding of [^{3}H]-ouabain; however, in contrast to CMA and its chemical relatives but like the cardiac glycosides, 14β-OHP

[1] Author for correspondence.

consistently produces a positive inotropic effect in isolated cardiac muscle.

Methods

[^{3}H]-ouabain radioligand binding assay

The [^{3}H]-ouabain binding assay was carried out as previously described (Hnatowich & LaBella, 1984). The steroids were added in 5 μl of 95% ethanol to reaction tubes; this volume of ethanol alone had no effect on specific binding of [^{3}H]-ouabain. Practically identical estimates of potencies were obtained for the steroids when the ethanolic solutions were evaporated to dryness in the reaction tube, tissue suspension added, and the tube vortexed for 1 min before addition of other components of the assay medium.

^{86}Rubidium uptake by human red blood cells

Human blood was collected from normal adult volunteers and placed on ice. Clotting was inhibited by 1 mM EGTA. The assay method was based on that of Belz (1981) with minor modifications. Following preincubation with steroids at 37°C for 60 min, RbCl, glucose and tracer amounts of ^{86}RbCl (approx. 2 mCi mg^{-1}; NEN, Montreal) were added and incubation proceeded at 37°C for 2 h. Duplicate assay tubes contained: 0.3 mM [RbCl + 0.5 μCi ^{86}RbCl], 5.6 mM glucose and drugs as required. Ouabain-insensitive ^{86}Rb uptake was determined in the presence of 0.1 mM ouabain. Cells were isolated by centrifugation, radioactivity determined, and the results expressed as a percentage of that ^{86}Rb uptake which is inhibited by 0.1 mM ouabain.

Tension measurements in cardiac trabecular muscle

An isolated right ventricular trabecula from dog heart was placed in a 10 ml vertical bath containing Krebs-Henseleit (KH) solution, bubbled with 95% O_2 and 5% CO_2 and maintained at 37.0 ± 0.2°C. The free end of the tissue was connected to a Grass FT-03C isometric force-transducer. A Pulsar 6i stimulator (F. Maer & Co.) connected to a custom built computer-controlled programmable pulse-generator (Boyechko & Bose, 1984) or a Grass SD5 stimulator provided square wave stimuli of 3 ms duration through a bipolar punctate platinum electrode to the trabecula. Stimulus amplitude was adjusted to about 10–20% above threshold and the muscle stretched to optimum length. The tissue was electrically stimulated at a basic cycle length of 2000 ms. When the magnitude of evoked contractions became constant, the preparations were treated with varying concentrations of ouabagenin, CMA, CMA-S, 14β-OHP or progesterone. The tissue was equilibrated for 1 h and contractions recorded on a Grass Polygraph. The KH solution had the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.4, $NaHCO_3$ 25 and glucose 11.1. K^+-free KH medium was prepared by replacing KCl with NaCl and KH_2PO_4 with NaH_2PO_4.

Measurement of scattered light intensity fluctuation (SLIF)

A strip of canine right ventricular trabecula 300 mm wide was mounted in an optically clear chamber for recording tension. A collimated beam of He-Ne laser (Spectraphysics; 7 mW) was directed at the edge of the muscle and scattered light collected at an angle of 30° by a PIN photodiode. The intensity of light was converted to a current by a low-noise amplifier. Small oscillations in sarcomere length of several cells stacked in the path of the coherent light cause a typical 'speckle pattern' which appears to the light sensor as SLIF. In order to minimize excessive changes in light intensity associated with the contractile response, light scattering was measured for 40 s immediately after cessation of stimulation. The recording, therefore, represents 'contractile behaviour' of a muscle in diastole. This mechanical response is due to asynchronous release of Ca^{2+} from the sarcoplasmic reticulum during diastole. The electrical transform of the optical signal was digitized by a Nicolet PC3901 oscilloscope and the data transferred from the serial port of the oscilloscope to an IBM PC-compatible microcomputer (MIND 2). The signal was analyzed with a Fast Fourier Transform Algorithm (ASYST; Macmillan Software) for its spectral content and magnitude. Frequencies above 10 Hz were filtered out digitally. Determination of the spectral power context of SLIF consisted of 4 trials, each on tissue from a different dog, for each drug. One-way Analysis of Variance in conjunction with Duncan's New Multiple Range Test was employed for testing statistical significance.

Potassium-mediated relaxation of noradrenaline-contracted isolated portal vein

Longitudinal strips of canine isolated portal vein (10 mm × 2–3 mm) were suspended in oxygenated K^+-free Krebs-Henseleit medium at 37°C and pH 7.4. Resting tension was adjusted to 1 g. Addition of noradrenaline (1 μM) resulted in contraction. Restoration of normal amounts of K^+ (5.4 mM) caused a prompt relaxation which was modified by pretreatment for 30 min with certain steroids or ouabagenin.

Synthesis of 14β-hydroxyprogesterone

14β-Hydroxyprogesterone was synthesized from 14α-

hydroxyprogesterone, the latter generated from the incubation of progesterone with *Mucor griseocyanus* (Templeton *et al.*, 1987b). The structures of the intermediates—14α-alcohol, C-14 unsaturated derivative, epoxide, and 14β-hydroxyprogesterone—were confirmed by ^{1}H and ^{13}C n.m.r. spectroscopic assignments of the respective atoms based on correlation with the reported spectra of related steroids (Habermehl *et al.*, 1985; Templeton *et al.*, 1987a). A detailed account of the synthesis of 14β-hydroxyprogesterone has been published elsewhere (Templeton *et al.*, 1987a).

Drugs

Noradrenaline, progesterone, ouabain, and ouabagenin were obtained from Sigma Chemicals, and chlormadinone acetate (CMA) from Ayerst Res. Labs. [^{3}H]-ouabain and ^{86}Rb were obtained from NEN, Canada Ltd.

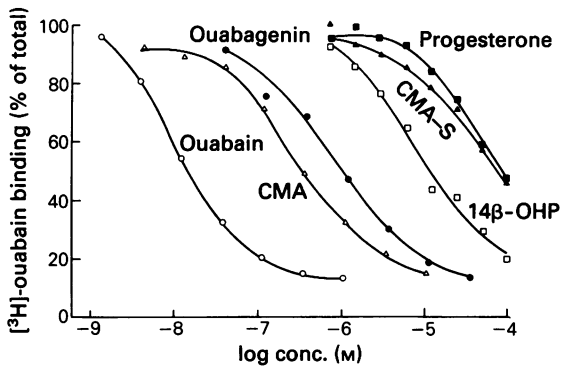

Figure 1 Inhibition by progesterone derivatives of specific binding of [^{3}H]-ouabain to membranes from dog heart. A typical experiment is shown. IC_{50}'s for ouabagenin, chlormadinone acetate (CMA), 14β-hydroxyprogesterone (14β-OHP) and progesterone were 670 nM, 269 nM, 10.5 μM, and 56 μM, respectively. CMA-S = chlormadinone acetate-3-hemisuccinate.

Results

Effect of 14β-hydroxyprogesterone on [^{3}H]-ouabain binding

The relative potencies in a cardiac glycoside radioligand binding assay of 14β-OHP and related steroids are shown in a typical experiment in Figure 1. In four experiments the mean (± s.e.mean) IC_{50} values for ouabagenin, CMA, 14β-OHP, and progesterone were: 670 ± 130 nM, 269 ± 46 nM, 10.5 ± 0.8 μM, and 56 ± 3.5 μM, respectively. The potency of CMA conforms to our previous determinations, i.e. about 1/20th the potency of unlabelled ouabain and three times more potent than the aglycone (ouabagenin). In attempts to increase the solubility of CMA we incorporated a hydrophilic moiety, 3-β-hemisuccinate, but the potency of CMA was reduced by about 200 fold (IC_{50} = 53 μM). Progesterone is only 1/250th as potent as CMA, but incorporation of the 14-β-OH substituent increases potency about 7 fold.

Effect of 14β-hydroxyprogesterone on ^{86}Rb uptake

Interaction with and inhibition of Na,K-ATPase by the digitaloid or pregnane steroids is manifested, at the cellular level, in diminished functioning of the sodium pump. Uptake of radioactive ^{86}Rb is a measure of the inward pumping of potassium, and this process was examined in fresh red blood cells from human subjects. The relative potencies among the various steroids in inhibiting ^{86}Rb uptake (Figure 2) correlates reasonably closely with their potencies in the binding assay with canine cardiac cell membranes. The IC_{50}s (mean ± s.e.mean) for progesterone, CMA, 14β-OHP, and ouabagenin were: 43 ± 12 μM, 450 ± 52 nM, 12.5 ± 0.5 μM, and 155 ± 34 nM respectively. The ratios of potency in the radioligand binding assay to potency to inhibit ^{86}Rb uptake were: 1.4, 0.57, 0.85 and 6.1, respectively. The lack of precise correlation between potencies in the binding assay and ^{86}Rb-uptake assays may reflect species differences in association

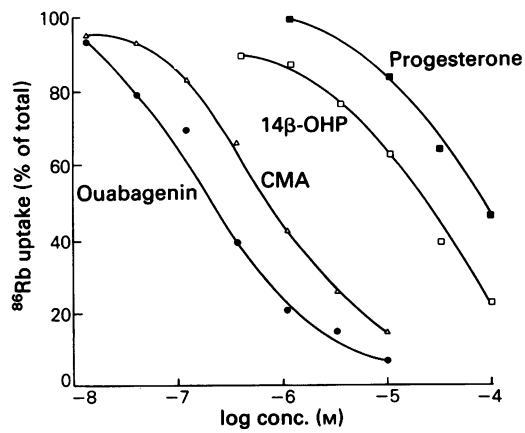

Figure 2 Inhibition by progesterone derivatives of ^{86}Rb uptake by human red blood cells. A typical experiment is shown. The mean IC_{50}'s for three experiments were as follows: ouabagenin, 155 nM; CMA, 450 nM; 14β-OHP, 12.5 μM; progesterone, 43 μM: abbreviations as in Figure 1.

and dissociation rates of individual steroids with sodium, potassium-ATPase as well as other tissue and species determinants (Schonfeld *et al.*, 1986).

Effect of 14β-hydroxyprogesterone on isolated cardiac tissue

Only ouabagenin and 14β-OHP caused an increase in active tension; however, at high concentrations both agents were cardiodepressant and elicited aftercontractions that were enhanced by increasing the stimulation frequency. The response to 14β-OHP is shown in Figure 3. CMA-S, CMA, and progesterone had mainly an inhibitory effect on contractility and produced no signs of the cardiotoxicity (aftercontractions) characteristic of digitalis-type agents. The results of the studies on cardiac contractility are summarized in Table 1. CMA at higher concentrations elicited positive inotropy in two instances. We reported previously that, although CMA and CMA-S are generally cardiodepressive, they occasionally elicit a transient increase in contractility but only rarely cause sustained positive inotropy in the intact perfused guinea-pig heart (LaBella *et al.*, 1984). Variability in the magnitude of the inotropic response, the onset of positive inotropic response to 14β-OHP and the time to attain maximum tension may reflect the poor water solubility of the compound, in addition to the variable contribution of endogenous noradrenaline resident in sympathetic nerves in the myocardium.

Mammalian myocardium contains stores of noradrenaline in nerve terminals; and inhibition of

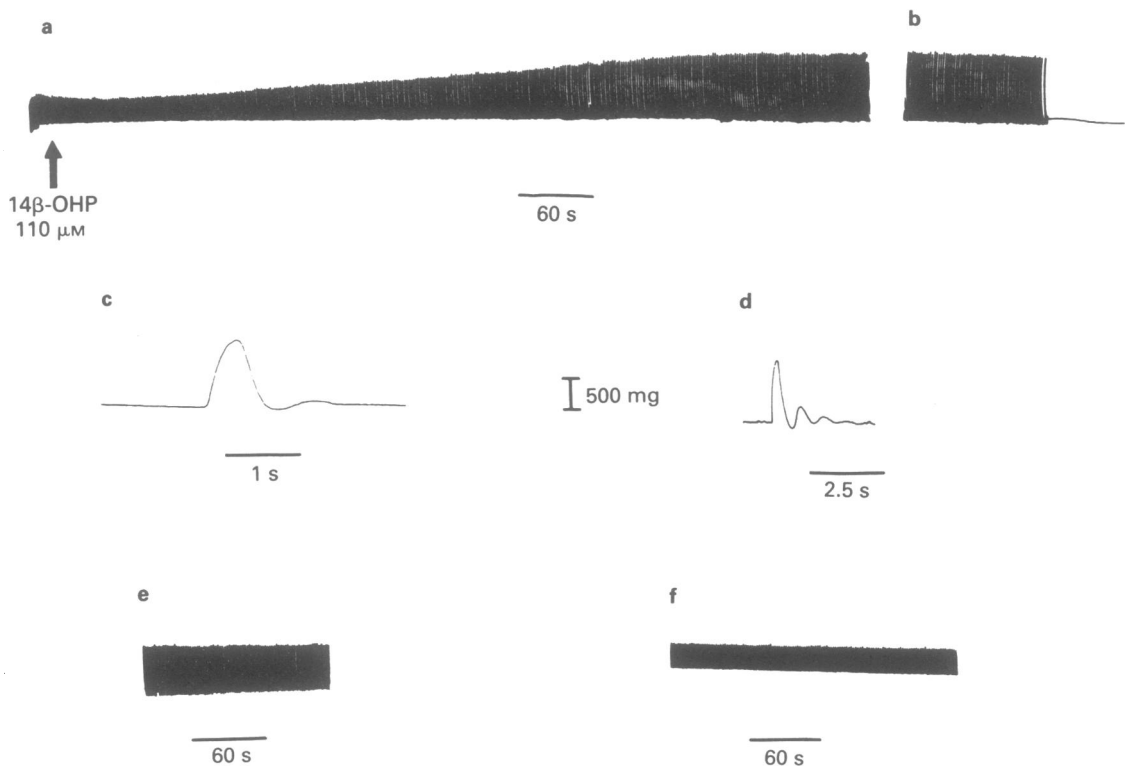

Figure 3 Effect of 14β-hydroxyprogesterone (14β-OHP) on tension of electrically driven trabecular muscle from the right ventricle of dog heart. (a) The time course of positive inotropy in response to 14β-OHP; slow recording speed. (b) Peak inotropic response and onset of toxicity about 25 min after addition of drug. The last beat is an aftercontraction occurring when stimulation was stopped. (c) Fast recording speed to show the complete trace of individual contraction; about 5 min after (b). The last driven contraction is followed by an aftercontraction. (d) Three min after (c). BCL of 750 ms. Note several aftercontractions which diminish in amplitude with time. (e) Forty min after (d) and (f) 20 min after (e), continued progression of toxicity with time. Contractile amplitude is diminished in conjunction with an increase in diastolic tension.

Table 1 Effects of progesterone derivatives on tension of the electrically driven cardiac trabecular muscle

Drug	*Muscle prep.*	*Conc.* (μM)	*Change in Tension (%)*
Ouabagenin	1	1	+150
	2	1	+115
Progesterone	1	1	−25
		10	−46
		100	−71
	2	100	−38
	3	100	−36
14β-OHP	1	40	+70
	2	50	+53
	3	50	+250
	4	100	+130
	5	100	+35
	6	200	+30
CMA-S	1	131	−40
	2	500	−40
CMA	1	3	−18
	2	10	−18
	3	100	−7
	4	300	+25
	5	300	+13

The % increase in contractility for each of several preparations represents the maximum tension recorded and was achieved between 10 and 40 min after addition of the steroid. 14β-OHP = 14β-hydroxyprogesterone; CMA-S = chlormadinone acetate-3-hemisuccinate; CMA = chloramidone acetate.

Na, K-ATPase in nerve terminals by cardiac glycosides is associated with noradrenaline release and inhibition of catecholamine reuptake (see Smith, 1984). The effect of the β-adrenoceptor blocking agent, sotalol, was studied on the positive inotropic response to 14β-OHP. In 7 experiments, concentrations of 14β-OHP between 150–300 μM caused a 36 ± 7.3% increase in contractile tension. In the presence of sotalol (10 μM) there was a 52.6 ± 12.7% decrease in tension. This change was statistically significant at the $P < 0.05$ level. The concentration of sotalol chosen completely abolished the positive inotropic effect of isoprenaline (0.1 μM) which was greater than that produced by 14β-OHP. These results indicate that part of the positive inotropic response of the trabecula to 14β-OHP is due to release of noradrenline from adrenergic nerve terminals. However, the significant residual effect indicates an additional direct effect of 14β-OH on cardiac contractility.

In separate studies on scattered light intensity fluctuation in strips of trabecular muscle (see below) concomitant measurements of tension were made. In four experiments 14β-OHP at 150 μM caused an increase in tension of 21.8 ± 3.2 (means ± s.e.mean) %, whereas progesterone at 200 μM caused inhibition of tension of 20.2 ± 2.3%.

14β-OHP was tested on two guinea-pig isolated hearts prepared for retrograde coronary perfusion by the Langendorff technique; at 100 μM there was a mean increase in tension of 30%. Similarly, in two preparations of cardiac trabecular muscle isolated from guinea-pig right ventricle, at 150 μM the steroid increased contractile strength by 33%.

Effect of 14β-hydroxyprogesterone on SLIF

Oscillations in the level of free intracellular calcium are reflected in asynchronous sarcomere contractions caused by calcium overloading of the sarcoplasmic reticulum and spontaneous release of calcium during diastole. Such release results in spontaneous slow contractile wave-like motion in the myocyte and this phenomenon is enhanced by increased Ca-loading of the sarcoplasmic reticulum especially by inhibition of Na-Ca exchange by, e.g. cardiac glycosides or low external Na concentration. This process can be estimated in thin bundles of cardiac muscle by measurement of scattered light intensity fluctuation (SLIF) (Lappe & Lakatta, 1980; Bose *et al.*, 1987).

Initially, upon mounting the trabecula in the chamber, the magnitude of SLIF is high. Gradually, as the muscle equilibrates and the force of contraction stabilizes at a higher plateau, SLIF also decreases to a stable level. Addition of a cardiac glycoside characteristically causes a marked increase in both the force of contraction and in SLIF. A similar effect is seen when extracellular sodium concentration is reduced. In contrast, forskolin, which increases cellular cyclic AMP and also cardiac contractility by mechanisms distinct from those through which the cardiac glycosides operate, does not affect SLIF (unpublished observations). Results from a representative experiment are shown for 14β-OHP in Figure 4. Addition of 14β-OHP increased the force of contraction (tension) and enhanced both the magnitude and frequency of SLIF. SLIF was especially prominent at higher concentrations of the steroid that induce toxicity, as indicated by after contractions. Compiled data on the effect of each of the steroids on spectral power content of SLIF are presented in Figure 5. Only 14β-OHP and ouabagenin, both of which promote a positive effect on contractility, elicit a significant increase in spectral power content at concentrations which enhance muscle tension. CMA had no significant effect on SLIF at concentrations below that which produced cardiodepression. Progesterone, at the lowest effective concentration, diminished the power content and depressed contractility.

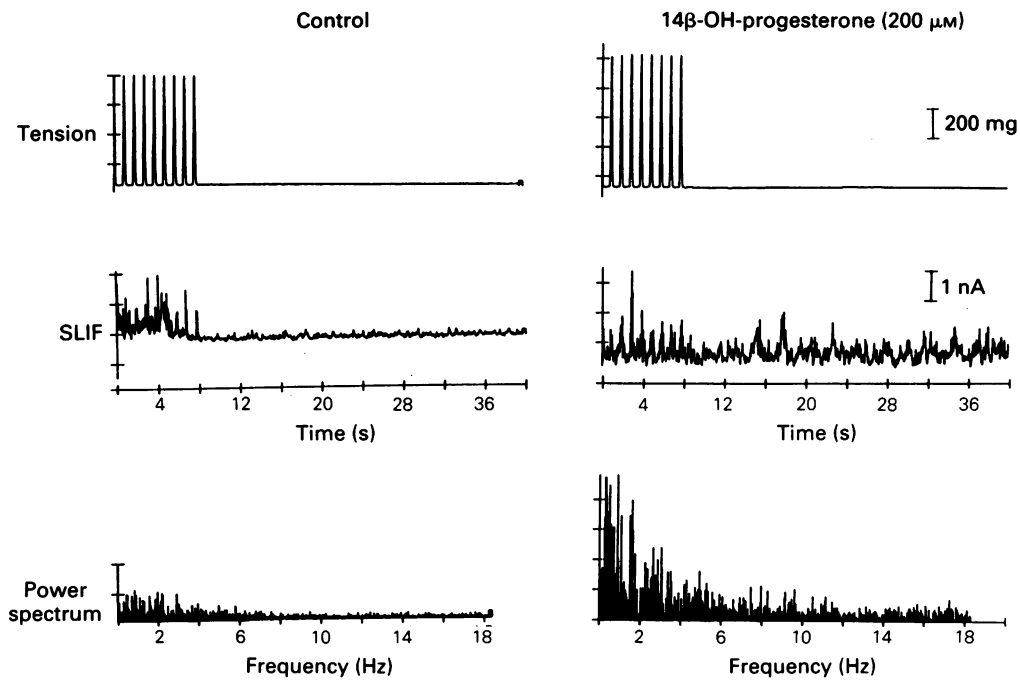

Figure 4 Scattered light intensity fluctuation (SLIF) and its power spectrum and muscle tension are shown before (left side) and after (right side) addition of 14β-hydroxyprogerterone.

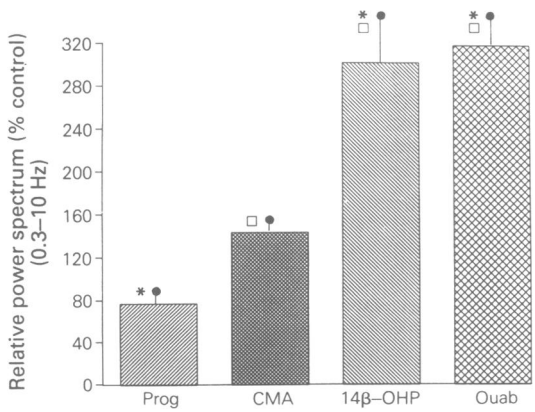

Figure 5 The spectral power content of SLIF in strips of trabecular muscle before and 40 min after addition of progesterone (Prog, 100 μM), chlormadinone acetate (CMA, 10 μM), 14β-hydroxyprogesterone (14β-OHP, 200 μM) or ouabagenin (Ouab 1 μM). The results are presented as the area of the magnitude spectrum in various frequency bands (0.3–10 Hz). □ Significantly different from Prog; * significantly different from CMA ($P < 0.05$).

Effect of 14β-hydroxyprogesterone on potassium-induced relaxation of portal vein

In potassium-deprived medium, noradrenaline induces a contraction of longitudinal strips of isolated portal vein. Restoration of potassium (5.8 mM) in the continued presence of noradrenaline causes rapid relaxation. Restoration of extracellular potassium is believed to re-establish the activity of the sodium pump of smooth muscle which has been sodium-loaded in potassium-free medium. Under these circumstances, the activated pump becomes electrogenic, inducing membrane hyperpolarization (Taylor *et al.*, 1970) and relaxation (Bose & Innes, 1973; Bonaccorsi *et al.*, 1977; Mallick *et al.*, 1987) of muscle cells. This relaxation response was blocked and replaced by a small contraction if the muscle was first exposed to ouabagenin or 14β-OHP (Table 2); presumably, reversal by the steroids of relaxation is mediated through Na,K-ATPase inhibition. In contrast, upon pretreatment of the muscle with CMA, CMA-S or progesterone, the potassium-induced relaxation- response was only minimally retarded or reduced in amplitude. As in the case of cardiac muscle, interference by these cardiodepressant steroids with calcium flux or

Table 2 Effects of steroids on K^+-mediated relaxation of isolated portal vein after contraction by noradrenaline in K^+-free medium

Steroid	*Conc.* (μM)	*% increase in t_i of relaxation*	n
Progesterone	50	305	2
	100	213	3
CMA-S	100	140	2
	125	150	2
	150	200	2
	200	96	3
	250	100	1
	300	260	2
	450	300	1
CMA	50	343	2
	100	131	5
	250	186	1
	300	201	2
14β-OHP	25	205	2
	50	380	4
	75	1594	2
	100	1160	2
Ouabagenin	0.1	795	2
	0.8	1780	2

The mean change in half-time (t_i) of relaxation after steroids or ouabagenin is expressed as a percentage of the (t_i) of relaxation in the untreated tissue. Abbreviations as in Table 1.

mobilization in venous smooth muscle can account for antagonism of relaxation. In high concentrations, progesterone by itself induced relaxation in the noradrenaline contracted vein.

Discussion

Solubility of steroids in aqueous buffers

Progesterone and its congeners are only slightly soluble in aqueous buffer. Hence, the potencies calculated on the basis of added steroid are probably considerably less than the true potencies. However, the biological assays seem to provide reasonable estimates of the relative affinities of these steroids, since the relative potencies of the steroids are quite similar, e.g. for the radioligand binding assay, ^{86}Rb uptake, and Na,K-ATPase inhibition. Furthermore, our structure-activity studies on a large number of synthetic steroids indicate that steric factors are of major importance in binding potency among compounds whose solubilities differ little or not at all. For example, in the radioligand binding assay the IC_{50} of one derivative with alpha-substituted halogen at the C-6 position is approximately 2 μM, whereas the beta-substituted congener is essentially inactive ($IC_{50} > 200$ μM). Similar differences in potencies are seen with other steroid enantiomers where individual members would be expected to vary only slightly, if at all, in their partition coefficients. Furthermore, both the 3-hemisuccinate (Figure 1) and 3-rhamnoside (Repke, 1985) of CMA are much more water soluble than CMA, yet their potencies in the [^{3}H]-ouabain binding assay are only a fraction to that of CMA.

Mechanisms of steroid-induced positive and negative inotropy

In previous studies CMA and CMA-S were shown to inhibit purified Na,K-ATPase, to decrease ^{86}Rb uptake by cardiac and skeletal muscle, and to inhibit high affinity, specific binding of [^{3}H]-ouabain (Chow *et al.*, 1979; Kim *et al.*, 1980; LaBella *et al.*, 1984; 1985; Hnatowich & LaBella, 1984; Bihler *et al.*, 1985). These agents either had no apparent effect on the contractions of guinea-pig isolated atria (LaBella *et al.*, 1979) or were mainly depressant to the guinea-pig heart perfused by the Langendorff technique (LaBella *et al.*, 1984). The cardiodepressant action of CMA has been confirmed (Weiland *et al.*, 1987). Progesterone itself has been shown in many studies to be cardiodepressant on a wide variety of isolated cardiac preparations from several species (reviewed by Tanz, 1963). Based on these findings we initially proposed that the site responsible for the positive inotropic effect of cardiac glycosides may be distinct from that responsible for the inhibition of sodium, potassium-ATPase. Subsequently, this proposal was modified to include the possibility that these steroids have a direct depressant effect on the myocardium which counteracts the positive inotropy resulting from inhibition of the sodium pump (LaBella *et al.*, 1984; 1985). A prevalent hypothesis specifies that the positive inotropic effect of the cardiac glycosides results from enhanced levels of free intracellular calcium arising from the inhibition of Na,K-ATPase; elevated levels of intracellular sodium resulting from membrane pump inhibition favour net accumulation of free intracellular calcium via a sodium/calcium exchange process (Akera, 1981). That CMA, progesterone and certain structurally related steroids inhibit Na,K-ATPase but fail to influence SLIF, suggests that the predominant cardiodepression seen with these agents is mediated by an extra-Na,K-ATPase site of action that suppresses the elevation in free cytoplasmic calcium normally associated with cardiac systole.

The most significant finding of the present study is the demonstration that 14β-OHP exerts a consistent positive inotropic effect on cardiac muscle. In cardiodepressant molecules like progesterone and CMA, the steroid moiety differs from that of the cardiac

glycosides, specifically, in the configuration of the A/B and C/D ring junctions; both junctions are *cis* (bent) in the cardiac glycosides and *trans* (flat) in progesterone and CMA. Introduction of a 14β-OH moiety into progesterone converts the C/D junction to the *cis* configuration and confers a positive inotropic action to that steroid. In this regard the work of Gelbart & Thomas (1978) is particularly relevant. They found that 17β-formyl-guanylhydrazone derivatives of a series of non-digitalis-like steroids (C/D *trans*) inhibited myocardial Na,K-ATPase but had only a depressant effect on myocardial contractility. In contrast, the corresponding 14β-hydroxylated guanylhydrazone was digitalis-like structurally (C/D *cis*) and elicited a positive inotropic response.

Does 14β-hydroxyprogesterone or a related steroid occur endogenously?

14β-OHP is about one order of magnitude less potent than the digitalis-derived aglycones (genins) and two orders of magnitude less potent than the cardiac glycosides. However, potency of the genins can be enhanced up to several hundred fold upon glycosidation; other modifications, such as additional hydroxylation, also contribute to increased potencies (Guntert & Linde, 1981). Weiland *et al.* (1987) reported that CMA-3-arabinofuranoside produces positive inotropy and is 'non-arrhythmogenic', If, indeed, appropriate hydroxylation and glycosidation of progesterone occur in the animal organism, a physiological basis exists for the elaboration of at least one type of potent regulator of Na,K-ATPase. Confirmation of the reportedly low cardiotoxicity of CMA-glycosides could lead to the availability of agents superior to the digitalis family of drugs.

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