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GLYCOSIDATION OF CHLORMADINOL ACETATE ALTERS ITS ACTIONS ON Na^+/K^+-TRANSPORTING ATPase AND CARDIAC CONTRACTILITY: A CONTRIBUTION TO THE ENDOGENOUS DIGITALIS PROBLEM

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Compared to the progesterone derivative chlormadinol acetate **1**, the arabinofuranoside **2**, rhamnoside **3** and glucoside **4** of **1** are less potent in the Na/K-ATPase assay, but evoke, contrary to **1**, positive inotropy *in vivo*. In anaesthetized cats the circulation effects of **2** and **3** appear to be more favourable than those of the digitalis glycoside digitoxin. Hence, the progestin **1** is transformed through glycosidation into an interesting cardioactive steroid.

KEY WORDS: Chlormadinol acetate; chlormadinol acetate glycosides; Na^+/K^+-transporting ATPase; cardiac contractility.

ABBREVIATIONS: CMA, chlormadinone acetate; CMLA, chlormadinol acetate = 17α-acetoxy-6-chloro-3β-hydroxy-pregna-4,6-dien-20-one; Na/K-ATPase, Na^+/K^+-transporting ATPase (EC 3.6.1.37).

INTRODUCTION

As shown by LaBella and coworkers (reviewed in reference[1]), CMA and CMLA develop among 150 hormonal steroids checked the highest affinity for Na/K-ATPase which is the target enzyme (receptor) of digitalis glycosides.[2,3] These glycosides selectively inhibit the enzyme and thus elicit the therapeutically beneficial, positive-inotropic action on cardiac muscle.[4,5] Depending on test conditions, the inhibitory potency of CMA lies between 1/20 and 1/50 the potency of the digitalis prototype ouabain.[1] Wehling *et al.*[6] concluded that CMA binds to the same site on Na/K-ATPase as ouabain, competitively inhibits the enzyme like ouabain with respect to K^+ and in a rank order of species sensitivity as typical for cardiac glycosides. CMA, however, causes cardiodepression, although it inhibits the Na^+/K^+ pump,[1] that with cardiac glycosides invariably leads primarily to increased cardiac contractility. The authors[1] concluded that CMA weakens cardiac contractility by some unknown mechanism and that the cardiodepressing action overrides any positive effects on contractility that otherwise result from inhibition of the Na^+/K^+ pump.

LaBella *et al.*[1] believe that CMA and similar synthetic progesterone derivatives are unlikely to occur in animal tissues, but that a sequence of enzymatic processes can

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transform the *planar* progesterone geometry into the strongly *bent* geometry of cardiac glycosides. Therefore, researchers[1] have announced attempts to synthesize 14β-hydroxyl derivatives of 5β-pregnanes to test the hypothesis that these derivatives, due to the strongly bent junctions of the rings A/B and C/D, should exert all of the cardiac glycoside-like actions, including the pronounced positive inotropy that is lacking in the CMA actions.

Starting from a hypothesis on the mechanism of the cardiodepressing action of CMA and CMLA summarized in the discussion part, we have synthesized several CMLA glycosides which, as described in the present paper, inhibit Na/K-ATPase and elicit positive-inotropic actions *in vivo* on heart contractility.

METHODS

The synthesis of the CMLA glycosides, the structure of which is shown in Figure 1, has been performed by an improved method for the use of the Fétizon's reagent in the synthesis of cardiac glycosides[7] as described in detail elsewhere.[8] The preparation and determination of Na/K-ATPase from human cardiac muscle as well the determination of steroid potency in the Na/K-ATPase assay has been carried out exactly as reported earlier.[9] The *in vivo* inotropic effects of digitoxin, CMLA **1**, the arabinofuranoside derivative **2**, and the rhamnoside derivative of CMLA **3** were determined on cats[10] under hexobarbital pentobarbital anaesthesia by measuring the maximum

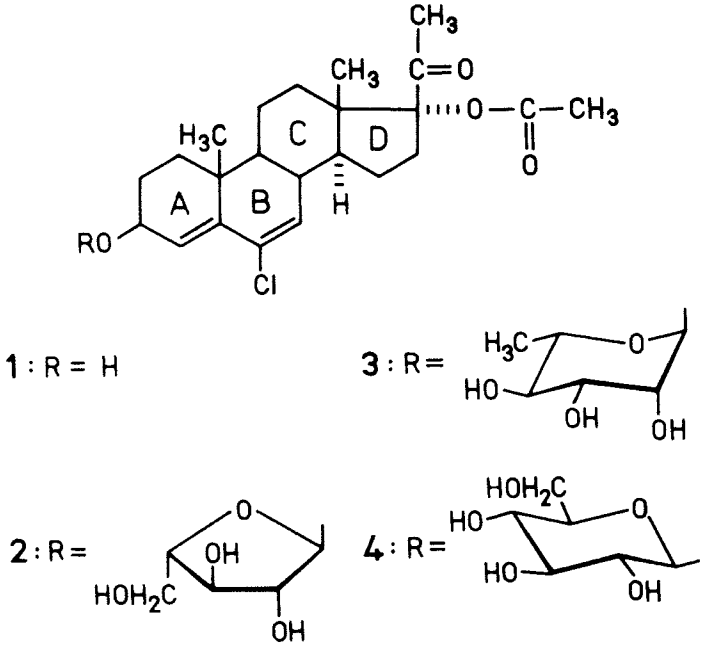

FIGURE 1 Structural formulae of **1**: chlormadinol acetate, **2**: 3β-O-arabinofuranosyl-chlormadinol acetate, **3**: 3β-O-rhamnosyl-chlormadinol acetate and **4**: 3β-O-glucosyl-chlormadinol acetate. The concentrations required for half-maximum inhibition (IC_{50}) of Na/K-ATPase from human cardiac muscle/human brain cortex are for **1**: 4.1/7.2 μM, **2**: 27/87 μM, **3**: 33/50 μM and for **4**: 26/44 μM.

rate of rise of ventricular pressure (dP/dt_{max}) reflecting negative or positive inotropic effects as reviewed in reference.[11] Stock solutions of the three compounds in ethanol were rapidly injected at 10 min intervals into the tube of a continuous saline infusion. Besides dP/dt_{max}, the frequency and rhythmicity of heart beating as well the peripheral systolic and diastolic blood pressure were recorded 10 min after each injection.

RESULTS

Na/K-ATPase Inhibition

Measured on the human cardiac enzyme, the inhibitory potency of the glycosides of CMLA (**2**, **3**, **4**) is about 7 times lower than that of the aglycone (**1**) as listed in Figure 1. Contrary to the favourable action of glycosidation of digitalis steroids with an A/B *bent* ring junction, glycosidation of steroids with an A/B *flat* ring junction as exemplified here by CMLA **1** brings about a decrease in potency because the sugar residues cannot reach the sugar binding subsite, and also interferes with the interaction of the steroid nucleus with its binding subsite on Na/K-ATPase; cf. reference[9]. The susceptibility to inhibition by the compounds **1**–**4** is somewhat higher with the cardiac enzyme than with the cerebral enzyme. The rate constants for the formation and dissociation of the inhibitory complexes with the cardiac enzyme are much higher with **1**–**4** than with digitoxin (not shown).

In vivo Inotropic Action

Figure 2 depicts the influence of CMLA **1**, its arabinofuranoside **2** and the digitalis glycoside digitoxin on cardiac contractility and circulation parameters in anaesthetized cats. Compound **1** depresses at high doses cardiac contractility, measured in terms of dP/dt_{max}, i.e., it produces negative inotropy, which is not associated with changes of blood pressure as well as frequency and rhythmicity of heart beating. Contrary to **1**, its glycoside **2** amplifies dP/dt_{max} up to 70 %. At a lower dose, which enhances contractility by 50 %, heart frequency is but slightly increased and the blood pressure not significantly raised. The positive inotropic response after the cessation of application of **2** continues for at least one hour. Even at the highest doses, arrhythmias are not evoked and no animal dies. Compound **3** changes all the above-mentioned circulation parameters quantitatively and qualitatively in a manner similar to **2**. Digitoxin, however, produces at concentrations, which enhance dP/dt_{max} by 40 to 70 %, arrhythmias and eventually death in an increasing proportion of the animals used. Moreover, digitoxin raises blood pressure much more than **2** does in the range of positive-inotropically acting doses. In conclusion, the effects of **2** on cardiac contractility and other circulation parameters appear to be more favourable than those of digitoxin, though their comparative therapeutic range remains to be settled. As an obvious drawback, the molar effect of **2** and **3** on inotropy is about ten times lower than that of digitoxin.

DISCUSSION

As long ago as 1953, Szent-Györgyi[12] came to the conclusions that normal serum contains substances which have a digitalis-like action, and that the chemical properties of these substances are indicative of a steroid structure. As reviewed by Wilkins,[13]

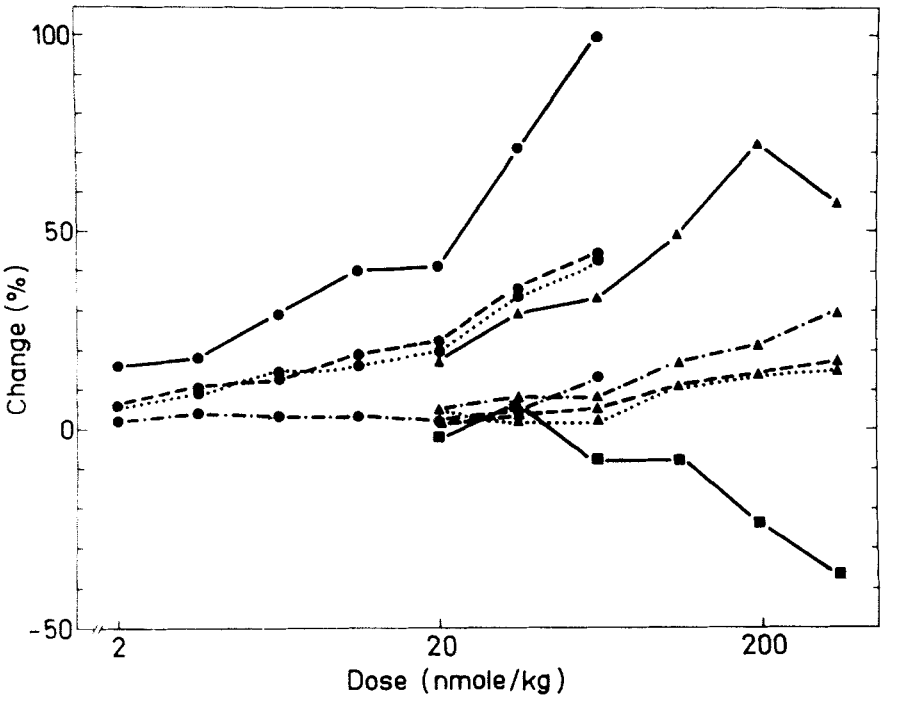

FIGURE 2 Average actions of cumulatively increased doses of digitoxin (●), chlormadinol acetate (■) or 3β-O-arabinofuranosyl-chlormadinol acetate (▲) on cardiac contractility, dP/dt_{max} (solid lines), heart frequency (dots and dashes), systolic (dashed lines) and diastolic (dotted lines) peripheral blood pressure measured in six anaesthetized cats each and expressed in per cent change of base values. In the digitoxin assay, arrhythmias appeared at 1.12×10^{-8} mole/kg in 2 of 6 animals, and at 6.32×10^{-8} mole/kg in 5 of 6 animals followed by death. The glycoside of chlormadinol acetate, however, did not produce arrhythmias and deaths even at the highest doses applied. 3β-O-rhamnosyl-chlormadinol acetate produced circulation changes similar to those shown here for the arabinofuranoside derivative. The negative inotropic action of chlormadinol acetate (shown) is not associated with alterations of heart frequency, systolic and diastolic peripheral blood pressure (not shown).

the numerous reports which have appeared since provide impressive circumstantial evidence for an endogenous digitalis-like substance, but the inhibitor has remained elusive and its precise nature is unknown.

Dehydroepiandrosterone sulfate (DHEAS), an important circulating androgen, has been identified by Diamandis *et al.*[15] in a chromatographic fraction from cord and digitalis-like factors (measured by digoxin radioimmunoassay) in plasma of healthy adults which inhibit hog brain Na/K-ATPase and displace ouabain from its binding site on the enzyme. However, the reported[14] inhibitory potency of DHEAS is very weak and is, according to our determinations in the human cardiac Na/K-ATPase assay, almost absent in a concentration as high as 600 μM (not shown). Progesterone has been identified by Diamandis *et al.*[15] in a chromatographic fraction from cord and maternal blood serum which contains a potent inhibitor for the Na^+/K^+ pump in human erythrocytes, but progesterone cannot be the inhibitor responsible because its potency is far too low.[15] Progesterone (IC_{50} near 110 μM) although at least ten times

more inhibitive-effective than DHEAS when assayed on human cardiac Na/K-ATPase[9] can nevertheless be safely excluded as the potential digitalis-like factor since it evokes in isolated cardiac preparations mostly a depression or only a transient increase in contractile force as reviewed by Tanz.[16]

The negative-inotropic action of progesterone and the progesterone derivatives CMA[1] and CMLA (Figure 2) appears to be related to the *flat*-junction of the rings C and D as the following pieces of evidence indirectly suggest. 3β, 14-Dihydroxy-17β-formyl-5β,14β-androstane-17-guanylhydrazone is like the digitalis aglycone digitoxigenin (both with a C/D *bent*-junction) positive-inotropically active, whereas 3β-hydroxy-17β-formyl-5β,14α-androstane-17-guanylhydrazone (with a C/D *flat*-junction), although a similarly strong inhibitor of Na/K-ATPase, produces but transiently positive inotropy which subsequently converts to negative inotropy.[17,18] It was not possible to measure the time for maximum inotropy to develop, since the effect was overridden by the development of negative inotropy as also occurs with progesterone, CMA and CMLA all with C/D *flat*-junction. Probably, all the steroids mentioned previously easily cross the plasma membrane of cardiac muscle cell, but only the derivatives with a C/D *flat*-junction inhibit mitochondrial respiration thus causing negative inotropy.[18] Although the absence of this harmful action on energy generation appears to require a C/D *bent*-junction, we hypothesized that it should be possible to circumvent the side-effect by the application of glycosides of CMLA, which may not easily cross the plasma membrane and thus not interfere with mitochondrial function. This idea appears to have proved correct as the positive-inotropic effects of the glycosides of CMLA, depicted in Figure 2, suggest. Clearly, our findings do not exclude the possibility that the CMLA glycosides slowly penetrate the plasma membrane and interfere with mitochondrial function thus reducing the inotropic efficacy of the glycosides.

The most important conclusion emerging from the present study is that the C/D *bent*-junction, occurring in steroids of vegetable origin only, is, contrary to the current dogma, not an indispensable requirement for cardiotonic action. For this reason, animal hormone steroids with a C/D *flat*-junction may be able to elicit digitalis-like, positive inotropic actions when, through conjugation with glucuronic or sulfuric acid, their uptake by the cell and interference with mitochondrial respiration is cut off or made difficult.

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