

Biosynthesis of Endogenous Cardiac Glycosides by Mammalian Adrenocortical Cells: Three Steps Forward

2003 was the 50th anniversary of the isolation and identification of the mineralocorticoid aldosterone—a momentous event if only because aldosterone has been widely regarded as the last major metabolically active steroid to be described in the mammalian adrenal gland (1). In this issue of the journal, Qazzaz et al. (2) describe studies that support the possibility of *de novo* biosynthesis, in the adrenal, of a material whose overall physicochemical, immunocrossreactive, and chromatographic characteristics are indistinguishable from those of the cardiac glycoside (CG) digoxin and its dihydro analog (in which the lactone ring is fully saturated). Using a mouse tumor cell line derived from adrenocortical cells, the authors show that the ^{14}C label from acetic acid, as well as that from labeled cholesterol, becomes incorporated into the two digoxin-like materials. Moreover, inhibition of hydroxymethylglutaryl (HMG)-CoA reductase with Mevastatin reduced the ^{14}C labeling of both products while leaving the issue of side chain cleavage unanswered. Although sophisticated analytical methods were not used in the study, the fingerprinting of the endogenous products and the behavior of the ^{14}C labeling are entirely consistent with the presence of digoxin and dihydrodigoxin or their respective isomers and, therefore, with the conclusion that these steroids are natural products of mammalian adrenocortical metabolism. This work joins an accumulating body of evidence suggesting that the mammalian adrenal cortex mediates the biosynthesis and secretion of not only the common corticosteroids but also a variety of CG-like entities, including an endogenous ouabain and proscillaridin-like materials (3–8).

The current observations are noteworthy for several reasons. The first reason is that the authors used ^{14}C -labeled substrates, including ^{14}C -cholesterol, which is stably ring-labeled. Thus, the presence of radiolabel from the latter substrate in the digoxin-like product could have arisen only from further metabolism of cholesterol itself and not, for example, from a less interesting mechanism involving isotope exchange. For this reason, the results show beyond a reasonable doubt that the steroid moieties of the digoxin-like products became labeled from further metabolism of cholesterol itself. The second reason is that it appears that the number of biosynthetic steps necessary to produce digoxin-like materials is relatively small. Only three major enzyme reactions may be needed for the conversion of pregnenolone or progesterone to the simplest steroid with CG activity. These steps include the inversion of the configuration at carbons 5 and 14 (to form the *cis-trans-cis* configuration), addition of a lactone ring at carbon 17, and the addition of the three sugars. Although the enzymes that mediate these transformations have yet to be described, the obvious brevity of the pathway is of great interest because it implies that the exact sequence of the key reactions should be comparatively easy to determine. Moreover, a similar sequence of

early reactions will likely apply to the biosynthesis of endogenous ouabain and other endogenous CGs. The third reason concerns the three sugars on the endogenous digoxin, which appear to be digitoxose moieties. The plant sugar is in the diet, but its biosynthesis in mammals has not been described, and further work will be required to identify the sugars conclusively as well as their mechanism of their addition. The fourth reason is that digitalis glycosides are present in plants scattered throughout farmland in North America and Europe. The plants are occasionally consumed by humans (as herbal remedies) and browsing livestock, and the less polar materials are readily absorbed into the circulation. This led to lingering concerns that the “endogenous” CGs originated from the diet despite extensive evidence to the contrary. The present results are therefore significant because they address an endogenous source that, in principle, may account for some or most of the digoxin-like immunoreactive materials in the circulation and urine (9–12).

The popular CGs (digoxin and ouabain) interact with astounding specificity with all known isoforms of the sodium–potassium pump (Na-pump). The initial cellular mechanism of action of therapeutic concentrations (1–3.5 nmol/L) of digoxin (and ouabain) is thought to involve the inhibition of a small fraction of surface membrane α -2 or α -3 isoforms of the Na-pump. In addition to the classic effects of CGs on ion transport, new work shows that the formation of CG–Na-pump complexes triggers a multifaceted intracellular signaling cascade (13). The relative physiologic and therapeutic significance of these two mechanisms of CG action is not known. This is, however, an exciting development. The data on biosynthesis and regulated secretion combined with earlier findings of a family of highly conserved and specific receptors and multiple downstream signaling events point to the endogenous CGs as new mammalian hormones (14).

The issue of biosynthesis of CGs by mammalian cells has implications for the therapeutic use of digoxin and digitalis preparations. Worldwide, digitalis remains among the most frequently prescribed agents for the treatment of heart failure and atrial arrhythmias. In the heart failure setting, CGs augment cardiovascular contractility as well as neural reflex control of the circulation and renin secretion. However, the therapeutic usefulness of digoxin is controversial: some patients benefit from the drug, whereas others have adverse reactions. We have suggested that digoxin should not be given to patients in whom the ambient concentrations of endogenous CGs are increased (15). This highlights a need for the development of analytical methods that use clinically relevant volumes of blood for the routine determination of endogenous CGs so that the appropriate information concerning optimal therapy can be obtained (12).

In summary, Qazzaz et al. (2) provide important evidence of biosynthesis of digoxin-immunoreactive steroids

in mammalian cells. Much challenging critical work, including the identification of the three key enzymes alluded to above as well as the major intermediates and end products, remains to be accomplished. Nevertheless, the results reawaken interest in the remarkable nature of mammalian adrenocortical metabolism as the source of a new class of metabolically active steroid hormones.

References

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John M. Hamlyn

*Department of Physiology
School of Medicine
University of Maryland, Baltimore
Baltimore, MD 21201*

DOI: 10.1373/clinchem.2003.029017
