Cardenolides and bufadienolides as hormones: what is missing?

David Lichtstein,1 Haim Rosen,2 and Moran Dyela1

1Department of Medical Neurobiology and 2Departments of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

According to the Medical Subject Heading (MeSH), hormones are defined as “chemical substances having a specific regulatory effect on the activity of certain organs. The classical definition of hormones limits them to the domain of chemical signaling molecules produced by endocrine glands and secreted directly into the bloodstream. By binding to receptors, hormones trigger various responses in tissues/cells containing cognate receptors.” Hence, the requisite components for a compound to be accepted as a hormone are presence in the human body, synthesis in and release from usually specialized cells, possession of functional receptors on target cells, and, following interaction with its receptor, induction of a specific effect on the activity of the target cells.

Cardenolides such as ouabain and bufadienolides such as bufalin are steroids originally identified in plants (Digitalis, Strophanthus) and toads (Bufo), which have been used for hundreds of years in Western and Eastern medicine to treat heart failure, arrhythmias, and other maladies. In the past 20 years, the cardenolides ouabain and digoxin and the bufadienolides 19-norbufalin, marinobufagenin, and cinobufagenin were identified independently by seven laboratories as normal constituents of human plasma, eye lens, and placenta and in bovine adrenals and hypothalamus (9). The identification consisted of purification of the compounds to homogeneity, followed by nuclear magnetic resonance and mass spectroscopy analyses (9). Hence, the presence of these steroids in mammalian tissues is beyond reasonable doubt. Although cardenolides and bufadienolides are referred to by some as hormones, they, as yet, are not accepted as such by the scientific community.

Cardenolides and bufadienolides have an established specific receptor, the α subunit of the Na+-K+-ATPase. This integral enzyme of cell membranes catalyzes the active transport of sodium and potassium ions against their electrochemical gradients. Consequently, the Na+-K+-ATPase plays an important role in regulating cell volume, the electric potential of the plasma membrane, as well as cytoplasmic pH and Ca2+ levels, through the Na+/H+ and Na+/Ca2+ exchangers, respectively, and several secondary transport systems of organic molecules. In the past 10 years, it has been recognized that in addition to pumping ions, Na+-K+-ATPase has parallel roles in activating signaling complexes in cardiac myocytes, renal epithelial cells, neuronal and several other cell types. The signaling variously activates Src, phospholipase C, MAPK, Akt, and reactive oxygen species, slow Ca2+ oscillations, and consequent nuclear factor-κB activation (1, 8). The binding of cardenolides and bufadienolides to the α subunit of the Na+-K+-ATPase results in the inhibition of Na+-K+-ATPase ion transport activity and also induces the activation of the signaling cascades mentioned above (8). Importantly, the activation of the intracellular signaling reactions occurs at cardenolide and bufadienolide concentrations similar to those present in the human circulation. Furthermore, at the systemic level, cardenolides and bufadienolides have been implicated in many physiological and pathophysiological mechanisms, including cell growth and cancer, body or organ weight gain, mood disorders, vascular tone homeostasis, blood pressure, hyperten-

The NaCl sensitivity of blood pressure is thought to be due, at least in part, to the compromised ability of the kidneys to excrete sodium, which is mediated by a variety of factors, both genetic and environmental. A vast amount of literature supports the notion that one of these factors are cardenolides and bufadienolides, which serve as “natriuretic hormones” involved in the regulation of sodium excretion by the kidney (5).

According to this concept of “natriuretic hormones,” the primary role of these endogenous steroids is to promote natriuresis via inhibition of the ion-transporting activity of the Na+-K+-ATPase and hence sodium reabsorption in the renal proximal tubules. Other mechanisms involving the release of hypothalamic endogenous ouabain, leading to increased sympathetic nerve activity and consequent vasconstriction and natriuresis, were also postulated (3). In a paper by Arnaud-Batista and colleagues (2), published in an issue of the American Journal of Physiology-Renal Physiology, the authors show that ouabain and especially, bufalin induce diuresis, natriuresis, and kaliuresis in the isolated intact rat kidney. A Src family kinase inhibitor, PP2, and UO126, a highly selective inhibitor of both MEK1 and MEK2, blunted the steroid effects. These results demonstrate for the first time the relevance of the signaling effects of cardenolides and bufadienolides in their natriuretic influence on isolated intact kidney function, pointing to the possible physiological role of these compounds as natriuretic hormones. Expansion of these findings, using in vivo experimental systems, should definitively establish the role of the steroids as regulators of natriuresis. The currently available literature, some of which is cited above, clearly indicates that these steroids completely fulfill the requirement of possessing a specific cellular receptor which, following interaction, results in an alteration in the target cell’s response. The huge diversity in cardenolide and bufadienolide actions (4) can be attributed to the large number of α, β, and FXXY subunits of the Na+-K+-ATPase, which may establish different receptors for the steroids or to different abilities for activating specific transduction pathways, a characteristic now widely recognized as functional selectivity (10). However, the possibility of the existence of additional receptors for these steroids cannot be ruled out and should be addressed experimentally. Furthermore, differences in the response to the steroids might be attributable to the membrane protein interacting with the Na+-K+-ATPase complex in a particular cell.
The biosynthetic pathway for these steroids in mammalian tissue has not been established. Indeed, numerous studies support the notion that cardenolides and bufadienolides, in particular ouabain, are synthesized in and released from the adrenal gland and hypothalamus (6, 9). Furthermore, results of experiments using a radioactive tracer chase support the notion that cholesterol is the substrate for the synthesis of cardenolides and that cholesterol side-chain cleavage and 3β hydroxylation are the first reactions in their synthesis (7). However, none of the more distal reactions in the biosynthetic pathway has been worked out. Studies based on substrate utilization, inhibitors, and tracer methods, in combination with chromatographic and mass spectral analyses, are crucial. Such studies are expected to establish cardenolides and bufadienolides as hormones and to shed a new perspective on the different effects of these compounds.

DISCLOSURES

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REFERENCES