Signaling through the Na/K-ATPase: implications for cardiac fibrosis

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THE CARDIOTONIC EFFECTS of digitalis have been exploited in clinical practice for many years. However, while the existence of endogenous digitalis-like substances (also called cardiotonic steroids) has been accepted for some time, their exact chemical nature has been only recently demonstrated (1). Specifically, both cardenolid (e.g., ouabain) and bufodienelide (e.g., marinobufagenin) have been unequivocally identified and quantified in humans as well as mammalian animal models of disease (2). Moreover, the concentrations of these cardiotonic steroids have been found to be elevated in a variety of disease states ranging from preeclampsia to congestive heart failure (2). The molecular mechanisms by which these cardiotonic steroids signal have also undergone reevaluation. The classic or ionic signaling model proposes that the binding of cardiotonic steroids to the Na/K-ATPase results in the inhibition of its pumping activity and, as a consequence, the increase in the concentration of cytosolic Na\(^+\) within some compartment of the cell. This increase in cytosolic Na\(^+\) then leads to increases in alterations in Na\(^+\)/Ca\(^2+\) exchange and elevations in cytosolic calcium mediating a number of signaling events (2). Alternatively, it has also been shown by Xie and colleagues that these chemicals can induce a signal cascade involving a non-pumping pool of the plasmalemmal Na/K-ATPase, which requires caveolar structure, association with the signaling proteins Src and the EGFR, as well as the generation of reactive oxygen species (8, 11, 21). The relevance of this model has been demonstrated in a number of systems including cell-free systems (13). The role that cardiotonic steroids play in different animal models of disease has been explored using the measurement of the circulating levels of these endogenous cardiotonic steroids, pharmacological administration, as well as the administration of neutralizing antibodies raised against these steroids (2).

Lingrel and colleagues (12) have approached this issue from a different perspective. Rather than simply alter the circulating levels of these endogenous cardiotonic steroids, this laboratory has produced genetic manipulations of the different Na/K-ATPase isoforms to alter their affinity for endogenous cardiotonic steroids in mice (4). Under wild-type conditions, the \(\alpha_1\)-isoform of the rodent Na/K-ATPase is resistant to both ouabain and marinobufagenin, whereas the \(\alpha_2\)-isoform is considerably more sensitive. By point mutations in a specific region, these investigators have made \(\alpha_1\)-sensitive and \(\alpha_2\)-resistant variants (4). Using these novel, genetically manipulated animals, this laboratory has demonstrated that the \(\alpha_1\)-sensitive animals have greater cardiac contractility in response to marinobufagenin infusion than wild-type animals, whereas the \(\alpha_2\)-resistant animals appeared to be resistant to ACTH-induced hypertension (3, 15, 20). This group has also demonstrated that the \(\alpha_1\)-sensitive animals develop more natriuresis in response to a saline load than wild-type animals (14).

In the current study, Wansapura and coworkers (19) examined the effects of manipulations of the \(\alpha_1\) and \(\alpha_2\)-isoforms on the susceptibility of animals to develop cardiac hypertrophy and fibrosis in a pressure overload model. The results are extremely interesting. First, under basal conditions, there were no substantial differences among the wild-type, \(\alpha_2\)-resistant and \(\alpha_1\)-sensitive strains. Four weeks following aortic banding, the degree of hypertension appeared to be greater in the wild-type than either the \(\alpha_1\)-sensitive or \(\alpha_2\)-resistant animals subjected to aortic constriction. However, the amount of cardiac hypertrophy and cardiac fibrosis was far more in the \(\alpha_1\)-sensitive animals than either the \(\alpha_2\)-resistant or wild-type animals, both of which expressed an \(\alpha_1\)-resistant isoform. These \(\alpha_1\)-sensitive animals also displayed left ventricular dilatation and decreased systolic function following 4 wk of aortic banding (19). As discussed in this article, an \(\alpha_1\)-dependent signal cascade has been described that involves PLC activation and PKC\(\delta\) translocation, leading to the phosphorylation of Fli-1, a known negative regulator of collagen synthesis (6). It is, therefore, quite likely that the greater sensitivity of the \(\alpha_1\)-sensitive animals to endogenous cardiotonic steroids allowed for greater degrees of fibrosis to result from increases in cardiotonic steroids by the aortic banding procedure. Unfortunately, the authors did not report endogenous cardiotonic steroid concentrations under basal and aortic banding conditions in the different mice strains. However, the authors did report that treatment with the Fab fragment of an ovine antibody to digoxin (Digibind) prevented the development of the cardiac changes in \(\alpha_1\)-sensitive animals, supporting the central role of endogenous cardiotonic signaling in the pathological processes (19).

While many aspects of this report are consistent with previous work demonstrating the role of signaling through the Na/K-ATPase-Src-EGFR cascade in the cardiomyopathy of renal failure (5, 9, 10), a major inconsistency should be mentioned. Kennedy et al. (9) clearly demonstrated the activation of Src, ERK, and evidence for oxidant stress in the hearts of mice subjected to experimental renal failure, whereas Wansapura and coworkers (19) did not find increases in phosphorylated Src in the hearts of the \(\alpha_1\)-sensitive animals and did not measure ERK phosphorylation or seek evidence for oxidant stress. While this may be related to technical difficulties with activated Src measurements in the current article, one must also consider the possibility that the classic or ionic signaling pathway may be a better model than the Na/K-ATPase-Src-EGFR cascade to explain the pathological changes observed in the \(\alpha_1\)-sensitive mice subjected to aortic banding (2).

Regardless of which mechanism is involved, the implications for clinical medicine are quite profound. With the understanding that cardiac fibrosis develops far faster in murine models than in clinical subjects (18), we should note that the human \(\alpha_1\)-isoform of the Na\(^+\) pump is quite sensitive to cardiotonic steroids compared with that of rodents (12). More-
over, circulating concentrations of both ouabain and marinobufagenin appear to be similar in humans and rodents under basal conditions as well as with disease (2). On this background, humans would be more analogous to the \( \alpha_1 \)-sensitive strain than the wild-type mice studied by Wansapura et al. (19). Therefore, we might expect humans to demonstrate considerable deleterious effects where cardiotic steroid concentrations are elevated such as hypertension, renal failure, and congestive heart failure (2). Does this mean that blocking the cardiotic steroids will ameliorate the progressive cardiac injury seen in these conditions? Frankly, this will require controlled studies with appropriate immunotherapy or pharmacotherapy. Regarding immunotherapy, studies of Digibind in patients with end-stage renal disease are needed. As far as pharmacotherapy, some evidence has perhaps inadvertently already been accumulated. Pitt and coworkers (16) demonstrated a beneficial effect of spironolactone in patients with advanced congestive heart failure in the Randomized Aldactone Evaluation Study (RALES). Although the anti-aldosterone effects of spironolactone were postulated to account for this beneficial effect, it should be noted that both spironolactone and its major metabolite, canrenone, have been shown to be competitive antagonists of cardiotic steroid binding to and signaling through the plasmalemmal Na/K-ATPase (17). Therefore, it is quite possible that much of the antifibrotic effects attributed to this aldosterone antagonist are actually due to the blockade of cardiotic steroid signaling through \( \alpha_1 \) of Na/K-ATPase. That said, the development of more specific antagonists to cardiotic steroid signaling such as Rostafuroxin and their ultimate study in randomized clinical trials will be necessary to comprehensively address this issue (7). Alternatively, if the Na/K-ATPase-Src-EGFR pathway can be definitively implicated, this cascade has a number of potential therapeutic targets that might be clinically exploited (2).

Like all good studies, the article of Wansapura et al. (19) raises a lot of questions. We still do not know whether the cardiotic steroids mediate their effect through changes in ion flux, the Na/K-ATPase-Src-EGFR cascade, or some combination of these pathways with possibly other undiscovered mechanisms. We also do not know whether the same phenomenon seen in the murine model is truly applicable to humans. However, it does appear to be clear that signaling through the \( \alpha_1 \)-subunit of the Na/K-ATPase could be an important pathway leading to cardiac hypertrophy, fibrosis, and failure in a relevant murine model of hypertension.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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