Commentary

Acute Stimulation of Na/K Pump by Cardiac Glycosides in the Nanomolar Range

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Digitalis glycosides are major tools for the identification of the Na/K ATPase as well as the Na/K fluxes mediated by this transport system. Since its discovery 50 yr ago (Schatzmann, 1953), the inhibitory effect of these compounds on the Na/K pump has been confirmed repeatedly. There has been evidence to contrary effects, although. Thus, Hougen et al. (1981) reported a stimulatory effect of nanomolar concentrations of ouabain on ^{86}Rb uptake in guinea pig atria. This effect could be suppressed by propranolol, or by prior depletion of endogenous pools of norepinephrine in the tissue, and it was regarded as an indirect effect elicited by norepinephrine released by an action of ouabain on nerve endings in the heart.

Several others have reported stimulating effects of low concentrations of cardiac glycosides on active Na/K transport in multicellular cardiac preparations, and also in the squid axon, it was noted that the inhibitory effect of ouabain on ^{22}Na efflux was preceded by an up to 20% stimulation possibly related to the low occupancy of the receptors in the early phase of exposure (Baker and Willis, 1972).

Now, in this issue of *The Journal*, Gao et al. (2002), using whole-cell patch-clamp technique, have demonstrated that dihydro-ouabain and ouabain in the concentration range 10^{-10}–10^{-7} M stimulate the Na/K pump–mediated current in single cardiac myocytes obtained from guinea pigs, dogs, and human subjects. The major advantage of this approach is that effects arising from localized changes in the ionic composition of the interstitial space or the release of endocrine factors from endogenous pools can be excluded. That this stimulatory effect is exerted directly on the Na/K pump is demonstrated by the observations that it is abolished by the omission of Na^+ from the pipette solution or of K^+ from the buffer surrounding the cells as well as by the addition of the Na/K pump inhibitor vanadate to the pipette solution.

Thus, there is good evidence that cardiac glycosides at low concentrations within a few minutes elicit up to 35 ± 10% stimulation of the Na/K pump in cardiac myocytes. Moreover, there is indirect evidence that this reflects an even larger (107 ± 30%) stimulation of the α_2 subunit isoform of the Na/K ATPase. Does this imply that during digitalization, where the plasma concentration of digoxin is ~1 nM, the Na/K pumps in the heart are stimulated rather than inhibited? Probably not, as it would be in conflict with a number of observations. For example, intravenous infusion of ouabain in a therapeutic dose induces a significant net loss of K^+ from the human myocardium within 2–4 min, which is an effect that is associated with significant increases in systolic ejection rate and left ventricular dp/dt (Brennan et al., 1972). More recently, it was shown that in atrial tissue obtained from patients in standard digoxin treatment, the electrogenic effect of the Na/K pump was significantly reduced (Rasmussen et al., 1990). In addition, there is strong evidence that the inotropic action of cardiac glycosides is related to an inhibition of the Na/K pump (Levi et al., 1994). Moreover, standard digitalization increases exercise-induced hyperkalemia, reflecting a marked increase in the net loss of K^+ from the working muscles, most likely arising from reduced Na/K pump capacity (Schmidt et al., 1995). In biopsies obtained from the heart and skeletal muscle of digitalized patients, 24 and 13%, respectively, of the Na/K pumps are occupied by digoxin (Schmidt et al., 1993). There is no evidence that standard digitalization is associated with stimulation of the Na/K pump. Finally, it should be noted that in digitalized subjects, no significant increase in the content of [^{3}H]ouabain binding sites could be detected, neither in skeletal muscle nor heart (Schmidt et al., 1993). Thus, in the intact organism, it has not been possible to discern the compensatory upregulation of Na/K pumps taking place in cell cultures during long-term exposure to ouabain. Hence, stimulation of active Na/K transport arising from an increased population of Na/K pump seems to be an unlikely response to low concentrations of cardiac glycosides in vivo.

What could be the physiological significance of the stimulating effect of dihydro-ouabain and ouabain on the Na/K pump? The relative increase in active Na^+ transport is of the same order of magnitude as that elicited by catecholamines or insulin in resting skeletal muscle (Clausen, 1986), but far below the acute 10–20-fold increase in active Na/K transport elicited by elec-

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trical stimulation in skeletal muscle (Juel, 1986; Nielsen and Clausen, 1997). Gao et al. (2002) suggest that the stimulation could represent another mechanism of isoform-specific regulation, possibly exerted by endogenous glycoside like compounds.

Unfortunately, however, despite decades of efforts in numerous laboratories, endogenous cardiac glycoside compounds have not yet been isolated, completely purified, and chemically identified. It was proposed that ouabain secreted from the adrenals is present in human plasma. However, later studies were not able to confirm these observations (Lewis et al., 1994). A quantitative analysis revealed that with the proposed rate of ouabain secretion from the adrenals, it would take 26 d to occupy only 1% of the entire pool of ouabain binding sites present in skeletal muscle (Hansen, 1994). Moreover, the metabolic pathways required for the synthesis of ouabain are specific to plants and have not been described in normal mammalian cells (Goto et al., 1992). The evidence suggesting the existence of endogenous cardiac glycoside compounds is indirect and based on studies with antibodies and incompletely purified preparations (for review see Goto et al., 1992). As concluded in a recent review (Therien and Blostein, 2000), the chemical properties of these compounds must be characterized more precisely before a consensus can be reached about their physiological significance.

Perspectives

In spite of these reservations, the observations of Gao et al. (2002) provide further incitements to the research on endogenous digitalis-like factors. There is a pressing need to characterize the effects of long-term exposure to ouabain and to search for the existence of similar effects of small concentrations of cardiac glycosides in other preparations, not to mention to search for possible effects on translocation and the affinity of the Na/K pump for Na^+ and K^+. More importantly, if such phenomena are general, they may reveal new molecular information about how the Na/K pump is working and regulated. For instance, it should now be considered that regulation of the Na/K pump may be exerted via an action on the outer surface of the plasma membrane at the digitalis receptor, in addition to the traditional view the Na/K pump is regulated, for instance, via cAMP and protein kinase A from the cytoplasmic side of the plasma membrane.

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