LETTERS TO THE EDITOR

Comments on
"Active Transport and Inotropic State in Guinea Pig Left Atrium"
which appeared in
Circ. Res. 52: 411-422, 1983

This paper addresses the question of whether or not the positive inotropic effects in heart of cardiac glycosides (ouabain) result from an inhibition of the sarcolemmal Na-K pump. Such Na-K pump inhibition could increase the intracellular sodium concentration ([Na\(^+\)], which, via Na-Ca exchange, could produce an increase in intracellular calcium (Baker et al., 1969). The rise of calcium might then be responsible for the increase in developed tension. However, in agreement with the results of other workers (see Noble, 1980), Lechat et al. (1983) find that, at moderate concentrations (10^{-7} M), ouabain can increase force with no apparent effect on the Na-K pump, as measured by \(^86\)Rb uptake which remains unchanged. Furthermore, at lower concentrations, ouabain actually stimulates \(^86\)Rb uptake, suggesting a stimulation rather than an inhibition of the Na-K pump. Such results therefore appear to indicate that the positive inotropic effect is not necessarily related to changes in [Na\(^+\)]. In this letter, however, we show that even if \(^86\)Rb uptake remains unchanged, it is still possible that a significant number of Na-K pumps have been inhibited and that [Na\(^+\)] has risen. This can occur because, when some of the pump sites are inhibited, [Na\(^+\)] will increase, and this will stimulate the remaining pump sites so that, in the steady state, the total Na-K pump activity (as measured by Rb or K uptake) will be unchanged.

Consider a simple case in which [Na\(^+\)], is determined solely by the balance between a passive Na\(^+\) leak into the cell of constant amplitude (J) and a Na\(^+\) influx via the Na-K pump. If pump activity is proportional to [Na\(^+\)], (e.g., Eisner and Lederer, 1980; Eisner et al., 1981), then

\[
\text{Na-K pump rate} = k[\text{Na}^+] \\
\text{where } k \text{ is a constant.}
\]

Hence, \(d[\text{Na}^+]/dt = J - k[\text{Na}^+]\).

In the steady state
\[d[\text{Na}^+]/dt = 0 \text{ so that}\]
\[\text{Na-K pump rate} = J.\]

In other words, inhibiting (or stimulating) some of the pumps (equivalent to decreasing or increasing k, respectively) will have no effect on pump rate in the steady state. This is because—under these conditions—the active Na\(^+\) efflux must equal the net passive Na\(^+\) influx, J. Hence, as long as J remains unchanged, active Na\(^+\) efflux and, hence, \(^86\)Rb uptake will also remain unchanged. Nevertheless, [Na\(^+\)] will have risen to a new steady level, and this will lead via Na-Ca exchange to a rise of [Ca\(^{2+}\)], and, hence, a positive inotropic effect. In the work of Lechat et al., \(^86\)Rb uptake was measured 50 minutes after ouabain was added. Direct measurements of [Na\(^+\)], in sheep Purkinje fibers indicate that, 50 minutes after a cardiac glycoside has been added, [Na\(^+\)] is approaching a new steady state level (Deitmer and Ellis, 1978a, 1978b). Hence, a steady state analysis of the experiments of Lechat et al. probably is justified.

One complication is produced by the fact that another process (possibly Na-Ca exchange) can extrude Na ions from the cell when the Na-K pump is completely inhibited (Deitmer and Ellis, 1978a). If this process is also stimulated by a rise in [Na\(^+\)], then it is possible that, after partial inhibition of the Na-K pump, the accompanying rise of [Na\(^+\)] will be insufficient to restore Na-K pumping to its initial rate. In this case, \(^86\)Rb uptake in the steady state will indeed decrease. However, this other Na extrusion process does not appear to be important in the physiological range of [Na\(^+\)] (Deitmer and Ellis, 1978a), and its effect can probably be ignored for concentrations of glycosides which inhibit a small fraction (<20%) of the pumps. Even allowing for this other Na extrusion system, a given concentration of ouabain will be expected to produce a much larger fractional increase of [Na\(^+\)], than the fractional reduction of K or Rb uptake.

Measurements of [Na\(^+\)] and tension have shown that tension depends steeply on [Na\(^+\)] (Eisner et al., 1981, 1983), and, therefore, significant positive inotropy could be produced by concentrations of ouabain that produce only a small rise of [Na\(^+\)], and (following the above analysis) an undetectably small decrease of Rb uptake. Therefore, the finding of positive inotropy unaccompanied by changes of Rb uptake is perfectly consistent with the inotropic effects being due to an increase of [Na\(^+\)], produced by Na-K pump inhibition.

A related point concerns the demonstration by Lechat et al. of a stimulation of Rb uptake by low (10^{-9}-10^{-8} M) concentrations of ouabain. The authors found that this effect was abolished by propranolol, and suggested that it could result from direct stimulatory effects of ouabain, and from release of norepinephrine producing Na-K pump stimulation. On this topic, we would simply point out that an agent that stimulated the Na-K pump, only, would not be expected to affect the steady state Na-K pump rate and, hence, \(^86\)Rb uptake. This is because [Na\(^+\)] would decrease until pump rate again balanced the Na leak. The possibility should therefore be considered that the increase of Rb uptake is produced by an increase of passive Na entry into the cell. This
would elevate the steady state [Na\(^+\)], and, hence, would lead indirectly to a stimulation of the Na-K pump.

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Reply to the Preceding Letter

Eisner and colleagues make a valid and important observation in their comments on our study of the effects of ouabain on monovalent cation transport and inotropic state in guinea pig left atrium (Lechat et al., 1983). We agree that our measurements of Rb\(^+\) uptake were made under steady state conditions, and indeed our experiments were designed so that this would be the case. We further agree that blockade of a fraction of pump sites with cardiac glycoside would not be expected to alter the rate of active K\(^+\) (or K\(^+\) analog) transport under steady state circumstances if [Na\(^+\)]\(_i\) is the rate-limiting entity in pump turnover and [Na\(^+\)]\(_i\) is determined principally by a passive Na\(^+\) leak into the cell. Whether other processes such as Na-Ca exchange can extrude Na\(^+\) from guinea pig atrial cells has not been studied under the conditions of our experiments, and so cannot be dismissed as a possibility. In any event, it is entirely plausible that our findings of positive inotropy unaccompanied by increases in [Na\(^+\)]\(_i\) produced by partial pump inhibition.

The final point of Eisner and colleagues regarding possible effects of ouabain on passive Na\(^+\) entry into the cell brings into focus an important limitation of ion flux studies in stimulated preparations of intact myocardium. Because complete blockade of the Na-K pump by saturating ouabain concentrations (usually 10\(^{-7}\) or 10\(^{-4}\) M) is necessary to estimate the "passive" component of unidirectional monovalent cation fluxes, and because these high concentrations of glycoside cause cessation of beating and contracture, there is no practical means available using isotopic flux measurements to assess accurately passive transsarcolemmal movements of Na\(^+\) or K\(^+\) in beating preparations. It may be assumed, however, that such fluxes are substantial in experiments such as ours at 3.3 Hz. As emphasized by Lechat et al. (1983), responses to glycoside exposure are clearly affected by interventions that alter adrenergic and/or muscarinic cholinergic effects, and these interventions are known to alter transsarcolemmal Na\(^+\), K\(^+\), and Ca\(^{2+}\) movements by mechanisms other than direct modulation of the Na-K pump.

Because of the limitations inherent in measurements of ion fluxes and contents in preparations of intact myocardium, we have studied the relations of [Na\(^+\)] to contractile state and to rapidly exchangeable [Ca\(^{2+}\)] during cardiac glycoside exposure in spontaneously beating monolayers of cultured chick embryo ventricular cells (Biedert et al., 1979; Barry et al., 1981, 1982). Our findings indicate that the positive inotropic effects of cardiac glycosides in this preparation are accompanied by increases in [Na\(^+\)] and in Ca\(^{2+}\) entry, probably via Na-Ca exchange. The recently published studies of Lee and Dagostino (1982) and of Wasserstrom et al. (1983) add additional direct evidence based on measurements of intracellular Na\(^+\) activity with Na\(^+\)-selective microelectrodes, indicating that the positive inotropic effects of cardiac glycosides are closely correlated with increased [Na\(^+\)].

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