Active Transport and Inotropic State in Guinea Pig Left Atrium

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SUMMARY. Although the positive inotropic effect of cardiac glycosides correlates well with inhibition of Na+ pump activity in many preparations, digitalis at low concentrations (10^-9 to 10^-8 M) may produce an apparent stimulation of monovalent cation transport in isolated intact myocardium or produce an inotropic effect that does not correlate with pump inhibition. Digitalis is known to modify tissue metabolism of endogenous neurotransmitters that may affect inotropic state, Na,K-ATPase activity, and K+ permeability. We examined the interactions of low concentrations of ouabain with adrenergic and cholinergic influences in isolated guinea pig left atria stimulated at 3.3 Hz in which inotropic state and monovalent cation transport (measured as 86Rb+ uptake) were assessed simultaneously. Ouabain (10^-9 M) stimulated Rb+ transport (+25%) without an inotropic response; the stimulatory effect on transport was abolished by propranolol or atropine pretreatment. In atria pretreated with atropine, 10^-8 M ouabain produced a small positive inotropic effect (+10%) without measurable associated Na+-K+ pump inhibition. This inotropic response was abolished in catecholamine-depleted atria. Ouabain (10^-7 M) always produced a positive inotropic response (about +25%) independent of catecholamine depletion, β-adrenergic blockade, or muscarinic blockade, but Rb+ uptake inhibition was observed only in β-adrenergically-blocked atria. In all preparations, ouabain concentrations greater than 10^-7 M caused an inotropic response associated with pump inhibition. At concentrations 3 × 10^-7 M and higher, mechanical toxicity was observed in all preparations except those pretreated with propranolol. Incubation with low concentrations of ouabain did not modify the inotropic response to isoproterenol. At concentrations of isoproterenol sufficient to stimulate Rb+ transport by 25%, there was a large (+80%) inotropic response. We conclude first, that, in guinea pig atria exposed to ouabain, the mechanism as well as the extent of inotropic response and of monovalent cation transport modification is concentration dependent, second, that at low concentrations (1-10 × 10^-9 M), in vitro inotropic and monovalent cation transport responses are in part mediated by an effect of ouabain on endogenous neurotransmitters; and third, that in this preparation at concentrations between 10^-9 and 10^-7 M ouabain, monovalent cation transport as measured by tissue 86Rb+ uptake does not correlate with inotropic response. (Circ Res 52: 411-422, 1983)

The digitalis glycosides produce in cardiac tissue a positive inotropic effect that correlates closely with inhibition of Na+ pump activity in many preparations (Akera and Brody, 1978; Biedert et al., 1979; Lee et al., 1980; Noble, 1980; Barry et al., 1981). These observations support the hypothesis that an initial step in the action of cardiac glycosides is binding to and inhibition of the monovalent cation active transport enzyme, sodium- and potassium-stimulated adenosine triphosphatase (Na,K-ATPase) (Repke, 1963; Langer and Serena, 1970). The consequent rise in intracellular Na+ is thought to be linked to the inotropic response via Na+-Ca2+ exchange (Baker et al. 1969; Glitsch et al., 1970; Biedert et al., 1979; Horackova and Vassort, 1979). However, this hypothesis does not address two properties of cardiac glycoside responses observed at low concentrations (10^-9 to 10^-8 M). First, some studies have shown a positive inotropic effect at low concentrations of cardiac glycosides without measurable inhibition of Na,K-ATPase (Murthy et al., 1974; Okita, 1977; Rhee et al., 1981) or change in intracellular Na+ content (Bentfeld et al., 1977; Busse et al., 1979). These observations conflict with the view that the inotropic effect of digitalis is mediated solely by an increase in bulk intracellular Na+. Second, in low concentrations, digitalis has been reported to stimulate Na+ pump activity in certain cardiac preparations as well as to stimulate Na,K-ATPase in some crude enzyme preparations (Bonting et al., 1964; Cohen et al., 1976; Peters et al., 1974; Ellis, 1977), although the only demonstrable effect of digitalis in highly purified Na,K-ATPase preparations is inhibition of enzyme activity (Matsui and Schwartz, 1966; Schwartz, 1975; Steckhoven and Bonting, 1981). Further, stimulation of Na+-K+ pump activity has been reported in association with a positive inotropic effect (Ghysel-Burton and Godfraind, 1979; Noack et al., 1979; Godfraind and Ghysel-Burton, 1980; but also see Grupp et al., 1982).

Based on these observations, alternative hypotheses have been advanced. Akera and Brody (1978) have suggested that a prolonged Na+ transient due to pump
inhibition affects Na+ concentration in a subsarcolemmal pool that may regulate Na+-Ca++ exchange and thereby modify contractile state without changing bulk Na+ content in the cell at the end of a contractile cycle. Lüllmann and Peters (1979) and Gervais et al. (1977) have proposed that during excitation-contraction coupling digitalis may enhance Ca++ release from sarcoplasmic reticulum associated with Na,K-ATPase. Neither hypothesis explains the apparent stimulation of Na+ pump activity by low glycoside concentrations observed by some workers, as noted above.

The implicit assumption of each proposal is that all effects of digitalis in myocardial preparations are due to an interaction with sarcoplasmal Na,K-ATPase of contractile cells. However, digitalis has long been known to influence another important aspect of myocardial function: response to autonomic stimulation. As summarized by Gillis and Quest (1979), substantial evidence indicates that, under certain conditions, digitalis may sensitize cardiac tissue to the effects of neurotransmitters as well as alter tissue turnover of those neurotransmitters (Sharma et al., 1980; Godfraind and Godfraind-DeBecker, 1965; Toda and West, 1966).

These observations are relevant because both β-adrenergic and muscarinic agonists, and probably α-adrenergic agonists as well, influence inotropic state. Furthermore, β-adrenergic agonists stimulate K+ uptake in cardiac tissue (Stafford, 1962; Hougen et al., 1981) and modify K+ currents in specialized conducting tissue (Hauswirth et al., 1968). Previous workers concluded that because the peak inotropic response to digitalis is not modified by β-adrenergic blockade, the effect of digitalis on tissue norepinephrine turnover is not reflected in the inotropic state of the tissue. However, the relevant studies such as those of Koch-Weser (1971) focus on relatively high concentrations of digitalis. In our view, studies to date have not excluded the possibility that, at low concentrations (in the range producing Na+ K+ pump stimulation or positive inotropic response without pump inhibition), the response to digitalis may reflect a combination of direct effects of myocardial Na,K-ATPase plus an interaction with endogenous neurotransmitters.

To elucidate these complex interrelationships, we tested the hypothesis that in isolated, electrically driven guinea pig left atria, the inotropic response, as well as Na+-K+ pump activity after exposure to low concentrations of ouabain, is modulated by interactions with endogenous neurotransmitters.

**Methods**

Two hundred thirty-three albino guinea pigs (Hartley strain, Elm Hill Breeding Labs) of either sex weighing 300-400 g were stunned by a blow on the neck and the hearts removed rapidly. Brief immersion and gentle shaking of the beating heart in buffered physiological medium at room temperature removed visible blood from the atria. The whole left atrium was dissected free, placed between two metal clamps, and suspended in a 200-ml organ bath thermostatically maintained at 30°C. The initial resting tension was adjusted to 500 mg. The atria were stimulated at 3.3 Hz through two punctate platinum electrodes by a Grass S88 stimulator, using rectangular pulses with a duration of 3 msec, and a voltage between 2 and 4 V (about twice threshold). The standard buffered medium contained (in mm): Na+, 145; K+, 4.0; Ca++, 1.8; Mg++, 1.2; HCO3-, 24; Cl-, 130; H2PO4-, 1.1; and glucose, 5.5. The medium was equilibrated with a mixture of 95% O2 and 5% CO2, which yielded a pH of 7.4, Po2 greater than 500 mm Hg, and Pco2 about 35 mm Hg. Isometric contractile tension was measured with either a Sanborn FTA 10 or Grass FT.03 force-tension transducer, and recorded on a Gould Brush 2200 strip chart recorder. In all experiments, atria were stimulated for 90 minutes before baseline tension measurements were made. Total systolic tension, total diastolic tension, and developed tension were recorded in milligrams, and later expressed as mg tension/100 mg of wet weight of tissue between the holding clamps.

Ouabain (g-strophanthin, Sigma Chemical Co.) was added to the bath at final concentrations noted in individual experiments after the 90-minute stabilization period. Tensions were recorded 60 minutes later, and were expressed as a percentage of the baseline developed tension or diastolic tension.

In the first series of experiments, we measured the uptake of the K+ analogue Rb+ using 86Rb+ as tracer as previously described (Hougen et al., 1981). Rb+ uptake was measured over the same time period and in the same tissue preparation in which the inotropic effects of ouabain were assessed. Under the conditions used in these experiments (K+, 4.0 mm; Rb+, 0.1 mm), we have shown that guinea pig atrial myocardium handles Rb+ in a manner indistinguishable from K+ (Hougen et al., 1981). We determined in preliminary experiments that Rb+ uptake in electrically stimulated
atria was linear for at least 30 minutes. We selected a 20-minute incubation time to obtain a sufficiently large Rb\(^+\) uptake with minimal scatter in the results. In agreement with Hougen et al. (1981), Rb\(^+\) uptake was found to be proportional to the wet weight of tissue over the range of tissue weights used. Three groups of atria were studied: atria without pretreatment, atria pretreated with atropine [atropine sulfate, \(2 \times 10^{-6}\) M (Sigma Chemical Co.)], added after 30 minutes, during the stabilization period, and atria pretreated with propranolol [DL-propranolol, \(10^{-6}\) M (Sigma Chemical Co.)], added after 60 minutes during the stabilization period. Each group was studied using the following schedule:

- **0-90 min**: stabilization period. Atropine or propranolol added if appropriate.
- **90 min**: measure baseline tension and add ouabain; atropine or propranolol exposure continued if appropriate.
- **140 min**: add Rb\(^+\).
- **150 min**: record inotropic state.
- **160 min**: remove, count, and weigh atria.

Fifty minutes after the addition of ouabain, RbCl was added to achieve a final Rb\(^+\) concentration of \(0.1\) mM at a \(^{85}\)Rb specific activity sufficient to yield 100,000 counts/min per ml of medium at a counting efficiency of 10\% (Cerenkov radiation, measured in a Beckman model LS-133 scintillation counter). Twenty minutes after Rb\(^+\) was added, atria were removed and rinsed immediately in buffer. The atrial tissue between the clamps was immediately placed in vials containing 1 ml of standard physiological medium for counting. Tissue samples were blotted and weighted immediately after counting. Total Rb\(^+\) uptake was expressed in nanomoles of Rb/mg wet weight tissue per 20 minutes of incubation time.

Rb\(^+\) active uptake was calculated as the difference between uptake in the presence and absence of \(10^{-3}\) M ouabain. The nonspecific uptake of Rb\(^+\) after incubation with \(10^{-3}\) M ouabain according to the above schedule was \(0.095 \pm 0.001\) (SEM) nmol/mg wet weight (n = 12). Active transport measurements reported are means of at least five atria in each group.

For the second group of experiments, we used atria depleted of endogenous catecholamines. As previously reported (Hougen et al., 1981), depletion of greater than 95\% of endogenous catecholamines was produced by administering 6-OH-dopamine (Sigma Chemical Co.) 50 mg/kg intraperitoneally every 12 hours. The animals were killed 12 hours after the third injection and hearts were removed for study.

In a third set of experiments, we determined the concentration-effect curve of isoproterenol after incubation with \(10^{-9}\) M, \(3 \times 10^{-9}\) M, and \(10^{-8}\) M ouabain. Exposure to ouabain was begun after the first 30 minutes of the 90-minute stabilization period. After 90 minutes, a cumulative concentration-effect curve of isoproterenol was determined. The inotropic response of each atrium was compared to its own baseline developed tension. The maximum effect of isoproterenol, which occurred 2-3 minutes after the drug was added, was recorded and results were expressed as percent increase in developed tension. A minimum of four experi-
Fig. 2. Developed tension in atropine-treated atria after exposure to ouabain. In these atria, both $10^{-9}$ and $10^{-8}$ M ouabain produced a small but significant positive inotropic effect that was manifest as a reduction in the rate of decline of developed tension. Symbols are the same as in Figure 1, with the addition of O, $1.5 \times 10^{-7}$ M.

**Results**

**Inotropic Effects of Ouabain**

**Untreated Atria**

To establish the relationships among Rb$^+$ uptake, inotropic state, and ouabain concentration under our experimental conditions, we examined the response of atria to a wide range of ouabain concentrations: zero, $10^{-9}$, $10^{-8}$, $10^{-7}$, $3 \times 10^{-7}$, and $6 \times 10^{-7}$ M. In control atria during the 60 minutes of tension recording, there was a spontaneous decline in developed tension ($-5.7 \pm 1.3\%$), and of diastolic tension ($-7.8 \pm 1.6\%$) as summarized in Figure 1 and Table 1. Neither $10^{-9}$ nor $10^{-8}$ M ouabain produced any detectable positive or negative inotropic effect. The threshold concentration for any positive inotropic effect of ouabain was about $3 \times 10^{-8}$ M. During the 60-minute observation period, $10^{-7}$ M ouabain produced a sustained and progressive positive inotropic effect (+29.6 $\pm$ 1.8%) without any increase in diastolic tension. Increasing the concentration of ouabain further increased the maximum inotropic effect, but this increase was not sustained. The maximum increase in developed tension in response to ouabain concentrations $3 \times 10^{-7}$ M and above was invariably followed by a decrease of developed tension and a progressive increase in diastolic tension.

**Atria Treated with Muscarinic Antagonist**

Cardiac glycosides have been shown to modify the response of certain preparations to cholinergic agonists (Toda and West, 1966). In addition, stimulation of presynaptic muscarinic receptors on adrenergic neurons is known to inhibit norepinephrine release (Muscholl, 1980; Vanhoutte and Levy, 1980). Therefore, to assess a possible cholinergic influence on the response of these atria to ouabain, we studied atria exposed to atropine throughout the stabilization and observation periods. In atropinized atria (as shown in Fig. 2), both $10^{-9}$ M and $10^{-8}$ M ouabain produced a small but significant positive inotropic effect which was manifest as a reduction in the rate of spontaneous decline of developed tension. If correction for multiple comparisons is applied, only the $10^{-8}$ M effect is statistically significant (Table 2). Otherwise, all inotropic effects were similar to those observed in atria not exposed to atropine. The maximum concentration of ouabain producing a positive inotropic effect without increased diastolic tension was $10^{-7}$ M. A slightly higher concentration ($1.5 \times 10^{-7}$ M) produced a non-sustained positive inotropic effect with an increase in diastolic tension. Atropine itself, when added during the initial period of stabilization, produced either no effect or a slight and transient (less than 10 min) positive inotropic effect. The positive inotropic effect of $10^{-8}$ M ouabain in a separate series of control and atropinized atria (see 6-OH-dopamine experiments) was indistinguishable from these data.

**Atria Treated with β-Adrenergic Antagonist**

Because ouabain has been shown to alter tissue metabolism of norepinephrine, we also addressed the question of indirect β-adrenergic effects of ouabain in a third group of atria pretreated with propranolol. The positive inotropic effect of ouabain in β-blocked atria was indistinguishable from that found in untreated atria. Interestingly, a higher concentration of ouabain ($6 \times 10^{-7}$ M) was necessary to increase diastolic tension compared to controls ($3 \times 10^{-7}$ M) (Fig. 3 and Table 3). In contrast to atria exposed to atropine, $10^{-8}$ M ouabain did not produce any positive inotropic effect in atria exposed to propranolol. Propranolol $10^{-6}$ M in itself produced on average a 10% decline in developed tension during the 30-minute period after addition to the bath (i.e., at time 60 min during stabilization).

**Effects of Ouabain on Rubidium Transport**

To correlate monovalent cation transport with inotropic state in response to ouabain, we measured Rb$^+$ uptake as well as inotropic response in the experimen-
TABLE 2
Inotropic Response and Simultaneous Rb+ Transport in Atropine-Treated Atria Exposed to Ouabain

<table>
<thead>
<tr>
<th></th>
<th>Initial systolic tension (mg)</th>
<th>Initial diastolic tension (mg)</th>
<th>% change in developed tension</th>
<th>% change in diastolic tension (60 min)</th>
<th>Rb+ uptake (nmol/mg per 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>1517 ± 113</td>
<td>341 ± 34</td>
<td>-2.8 ± 0.3</td>
<td>-10.5 ± 1.8</td>
<td>0.464 ± 0.040</td>
</tr>
<tr>
<td>Ouabain 10^-9 M</td>
<td>1453 ± 141</td>
<td>337 ± 39</td>
<td>-0.7 ± 0.15f</td>
<td>-5.6 ± 0.6*</td>
<td>0.466 ± 0.030</td>
</tr>
<tr>
<td>Ouabain 10^-8 M</td>
<td>1151 ± 185</td>
<td>307 ± 42</td>
<td>-1.0 ± 0.5*</td>
<td>-2.0 ± 1.0f</td>
<td>0.464 ± 0.055</td>
</tr>
<tr>
<td>Ouabain 10^-7 M</td>
<td>1146 ± 110</td>
<td>275 ± 46</td>
<td>8.7 ± 3.2%</td>
<td>+21.0 ± 6.0‡</td>
<td>0.443 ± 0.044</td>
</tr>
<tr>
<td>Ouabain 1.5 X 10^-7 M</td>
<td>1265 ± 140</td>
<td>300 ± 20</td>
<td>10.3 ± 3.0†</td>
<td>+25.6 ± 6.7‡</td>
<td>0.369 ± 0.030</td>
</tr>
<tr>
<td>Ouabain 3 X 10^-7 M</td>
<td>1166 ± 132</td>
<td>247 ± 38</td>
<td>43 ± 2%</td>
<td>+49.5 ± 4.2†</td>
<td>0.246 ± 0.033†</td>
</tr>
<tr>
<td>Ouabain 6 X 10^-7 M</td>
<td>1198 ± 167</td>
<td>314 ± 24</td>
<td>+58.2 ± 7.4%</td>
<td>-49.0 ± 9.4‡</td>
<td>0.138 ± 0.010‡</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
* P < 0.05 compared to control.
† P < 0.01 compared to control.
‡ P < 0.001 compared to control.

ments described above. In atria not exposed to atropine or propranolol, 10^-9 M ouabain stimulated Rb+ transport with an average increase of 22% in total Rb+ uptake and of 28% in active (ouabain-sensitive) uptake. At concentrations of 10^-8 and 10^-7 M, ouabain did not significantly alter the Rb+ uptake rate, but clearly produced a positive inotropic effect at 10^-7 M (Fig. 4 and Table 1). Significant inhibition of Rb+ uptake was found with 3 X 10^-7 and 6 X 10^-7 M ouabain (54% and 88% inhibition of active Rb+ uptake, respectively).

In atropinized atria, the control Rb+ uptake was not significantly different from untreated atria (Fig. 5 and Table 2). In contrast to the results from control atria, no stimulation of Rb+ uptake was observed with 10^-9 M ouabain. At ouabain concentrations greater than 10^-7 M, Rb+ uptake was inhibited to an extent similar to that observed in untreated atria.

In β-adrenergically-blocked atria, the control Rb+ uptake was not significantly different from untreated atria (Fig. 6 and Table 3). Again in contrast to control atria, no stimulation of Rb+ uptake was observed with 10^-9 M ouabain. However, inhibition of Rb+ uptake was observed at concentrations of ouabain (10^-7 M) lower than was the case in untreated atria (3 X 10^-7 M). This effect just escaped statistical significance (p = 0.06) if correction for multiple comparisons was made.

Catecholamine Depletion

Since cardiac glycosides under appropriate conditions may alter norepinephrine metabolism, it is possible that the positive inotropic effect of low concentrations of ouabain (10^-9 to 10^-8 M) may be an indirect effect dependent upon endogenous catecholamines. We tested this hypothesis by examining the inotropic effects of low ouabain concentrations in guinea pig atria depleted of endogenous catecholamines by in vivo administration of 6-OH-dopamine.

The lowest ouabain concentration producing a significant positive inotropic effect was 10^-8 M in atropinized atria, and a sustained positive inotropic effect was observed under all conditions at 10^-7 M ouabain. Muscarinic blockade with atropine is known to en-
hance norepinephrine release from sympathetic nerve terminals in cardiac tissue (Muscholl, 1980). Therefore, we studied the effects of both of these concentrations of ouabain in six groups of 9 to 10 atria: untreated control atria, untreated atria plus ouabain, atropinized control atria, atropinized atria plus ouabain, 6-OH-dopamine-pretreated atropinized atria, and 6-OH-dopamine-pretreated atropinized atria plus ouabain. For this series of experiments, the two concentrations of ouabain were tested consecutively. Ouabain (10^{-8} M) was applied at 90 minutes (after the usual stabilization period). Tension was recorded after 60 minutes. The ouabain concentration was then raised to 10^{-7} M, and tensions were again recorded after a second 60-minute period. All tension modifications were referenced to those measured at the end of the 90-minute stabilization period.

Results from this series of experiments are summarized in Table 4. Ouabain (10^{-8} M) again produced a positive inotropic effect in atropinized atria of control animals (+1.3 ± 3.7% increase in developed tension compared to -9.1 ± 1.6% decline in control atropinized atria, P < 0.001). This positive inotropic effect of 10^{-8} M ouabain was not observed in atropinized atria of 6-OH-dopamine-treated animals (−3.2 ± 2.5% compared to −6.4 ± 1.9%). The inotropic effect of 10^{-7} M ouabain was identical in each series of atria, and no increase in diastolic tension was observed with this concentration in catecholamine-depleted animals.

These results suggest that the small positive inotropic effect of 10^{-8} M ouabain in atropinized atria is at least in part mediated by endogenous catecholamines. Whether this effect is presynaptic or postsynaptic (for example, by sensitizing atria to β-adrenergic effects of norepinephrine) was not addressed in this experiment.

**Isoproterenol Concentration-Effect Curves in the Presence of Ouabain**

Godfraind and Godfraind-DeBecker (1968) showed that incubation of atria in low concentrations of ouabain will potentiate the contractile response to norepinephrine. To assess the possibility that low concentrations of ouabain may sensitize atria to the inotropic effects of β-adrenergic stimulation, we determined the concentration-effect curve of isoproterenol after incubation with low concentrations of ouabain. Since the inotropic response as well as calcium-dependent exocytic norepinephrine release may be modified by calcium concentration in the medium, each concentration-effect curve was performed at three calcium concentrations: 0.9 mM, 1.8 mM, and 2.5 mM. As summarized in Table 5, the inotropic response to isoproterenol was not significantly altered by preincubation with low concentrations of ouabain at any of the calcium concentrations tested. Isoproterenol did not increase diastolic tension under the conditions of these experiments.

**Influence of Isoproterenol on Rubidium Transport**

Because previous experiments indicated that the stimulation of Rb transport was dependent upon the presence of tissue catecholamines (Hougen et al., 1981), we also measured the influence of isoproterenol on Rb uptake. A 25% increase of active Rb uptake was observed at 3 × 10^{-8} M isoproterenol, a concentration producing an 80% increase in developed tension. Isoproterenol 10^{-7} M produced a striking 50% increase in active Rb uptake, as summarized in Table 6. Atropine pretreatment did not modify this effect.

**Discussion**

**The Inotropic Effects of Low Concentrations of Ouabain**

The threshold concentration for a positive inotropic effect of ouabain in atria without pretreatment was about 3 × 10^{-8} M. Lower concentrations (10^{-9} or 10^{-8} M) produced a detectable inotropic effect only in atropinized atria. Since this effect was not observed in atria from catecholamine-depleted animals, it appears that this effect is dependent on endogenous catecholamines. Since the concentration-effect curve of isoproterenol on contractile state remained unchanged after incubation with either 10^{-9} or 10^{-8} M ouabain at Ca^{++} concentrations from 0.9 to 2.5 mM, any effect of ouabain mediated via endogenous catecholamines appears to be postsynaptic.

Cardiac glycosides have been reported to stimulate norepinephrine release from adrenergic nerve terminals in several preparations (Garcia and Kirpekar, 1973; Paton, 1973), including the spontaneously beating guinea pig heart (Seifen, 1974). However, the
TABLE 3

Inotropic Response and Simultaneous Rb+ Transport in Propranolol-Treated Atria Exposed to Ouabain

<table>
<thead>
<tr>
<th>Glycoside Concentration</th>
<th>Initial Systolic Tension (mg)</th>
<th>Initial Diastolic Tension (mg)</th>
<th>% Change in Developed Tension</th>
<th>% Change in Diastolic Tension (60 min)</th>
<th>Rb Uptake (nmol/mg per 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1265 ± 89</td>
<td>290 ± 10</td>
<td>−1.5 ± 0.5</td>
<td>−8.8 ± 1.0</td>
<td>0.547 ± 0.052</td>
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<td>(n = 10)</td>
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<td></td>
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<tr>
<td>Ouabain 10−9 M</td>
<td>1436 ± 151</td>
<td>391 ± 46*</td>
<td>−2.4 ± 0.5</td>
<td>−9.4 ± 1.6</td>
<td>0.525 ± 0.050</td>
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<td>(n = 6)</td>
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<tr>
<td>Ouabain 10−8 M</td>
<td>1265 ± 85</td>
<td>282 ± 43</td>
<td>−1.9 ± 0.3</td>
<td>−7.1 ± 1.6</td>
<td>0.521 ± 0.019</td>
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<td>(n = 5)</td>
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<tr>
<td>Ouabain 10−7 M</td>
<td>1365 ± 75</td>
<td>312 ± 22</td>
<td>12.7 ± 2.6†</td>
<td>−5.0 ± 2.0</td>
<td>0.390 ± 0.035</td>
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<td>(n = 8)</td>
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<tr>
<td>Ouabain 3 × 10−7 M</td>
<td>1331 ± 133</td>
<td>347 ± 27*</td>
<td>40.0 ± 5.0‡</td>
<td>3.2 ± 5.0</td>
<td>0.385 ± 0.024‡</td>
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<td>(n = 5)</td>
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<tr>
<td>Ouabain 6 × 10−7 M</td>
<td>1358 ± 116</td>
<td>312 ± 24</td>
<td>+76.6 ± 4.0‡</td>
<td>+254 ± 24‡</td>
<td>0.175 ± 0.006‡</td>
</tr>
<tr>
<td>(n = 6)</td>
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<td></td>
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</tbody>
</table>

Results are expressed as mean ± SEM.
* P < 0.05 compared to control.
† P < 0.01 compared to control.
‡ P < 0.001 compared to control.

glycoside concentration at which these effects were observed was generally equivalent to or greater than 10−7 M ouabain. Inhibition of norepinephrine uptake in guinea pig ventricle has been reported at 10−7 M ouabain (Sharma et al., 1980). Furthermore, stimulation of presynaptic muscarinic receptors inhibits norepinephrine release, and muscarinic antagonists such as atropine increase norepinephrine release from cardiac adrenergic nerve terminals (Vanhouffe and Levy, 1980; Muscholl, 1980). Thus, our findings suggest that a small effect of low ouabain concentrations on norepinephrine turnover may be potentiated under conditions such as muscarinic blockade that potentiate norepinephrine release. At concentrations higher than 10−7 M, we found no evidence of interaction with endogenous neurotransmitters.

The therapeutic serum concentration of cardiac glycosides is equivalent to approximately 1-3 × 10−9 M ouabain (Smith and Haber, 1970; Hougen and Smith, 1978). It should be emphasized that the concentration of 10−8 M ouabain at which we observed a small inotropic effect in the presence of atropine cannot be correlated in any simple way with therapeutically relevant concentrations in man, for at least two reasons. First, human cardiac Na,K-ATPase is approximately 10 times more sensitive to ouabain than the guinea pig myocardial enzyme (DePover and Godfraind, 1979). It should be recognized that species specificity may also be influenced by the existence of more than one receptor affinity (Wellsmith and Lindenmayer, 1980) so that quantitatively exact comparisons are difficult. Second, tissue-to-medium concentration ratios of cardiac glycosides are considerably less than tissue-to-plasma ratios in vivo (Hougen and Smith, 1978; Busse et al., 1979). Thus, it may well be that a concentration of ouabain corresponding roughly to 1 to 3 × 10−9 M in man is equivalent to a higher concentration in our preparation in terms of Na+ -K+ pump inhibition. The effects of ouabain in vitro at medium concentrations between 10−9 and 10−8 M are of uncertain clinical relevance, as are our observations of effects that appear to be catecholamine dependent at 10−8 M ouabain.

Influence of Ouabain on Rubidium Transport

We found that 10−9 M ouabain, but not 10−8 M, stimulated active Rb+ uptake, and that this effect was abolished by exposure of atria to either propranolol or atropine. At concentrations greater than 10−7 M, the expected inhibitory effect of ouabain on monovalent cation active transport was reflected by inhibition of Rb+ uptake.

β-Adrenergic agonists are known to stimulate in-
ward K+ transport in quiescent cardiac muscle (Staf-ford, 1962; Hugen et al., 1981), and our finding that isoproterenol stimulates Rb+ transport in beating guinea pig atrial myocardium (Table 6) confirms these findings. Suppression of the ouabain-induced stimulation of Rb+ transport by β-adrenergic blockade could be explained by increased local norepinephrine concentration due to ouabain. It should be emphasized, however, that in our experiments, the concentration of isoproterenol necessary to cause stimulation of Rb+ uptake equivalent to 10−9 M ouabain also caused an inotropic response substantially in excess of that observed with 10−9 M ouabain. It appears unlikely that the concentration of norepinephrine in the synaptic cleft could be enhanced by ouabain sufficiently to stimulate Rb+ transport 25%, as we found. We therefore conclude that the ouabain effect probably is not due solely to an increase in local norepinephrine concentration. An alternative hypothesis is that ouabain and catecholamines act additively or synergistically in this concentration range to modify potassium permeability and thereby increase Rb+ uptake by a mechanism at least in part independent of direct stimulation of Na,K-ATPase.

As summarized in Figure 5 and Table 2, the muscarinic antagonist atropine, like propranolol, also abolished the stimulatory effect of ouabain on monovalent cation transport. Of relevance is the observation that digitalis enhances some cholinergic effects by increasing acetylcholine release, as well as directly potentiating its effects (Gillis and Quest, 1979). Furthermore, several groups have reported that muscarinic stimulation enhances K+ permeability (Hutter, 1961; Ten Eick et al., 1976; Musso and Vassalle, 1977; Galper et al. 1982). Thus, it is conceivable that an indirect effect of ouabain mediated by acetylcholine may be necessary for stimulation of Rb+ transport.

These observations suggest that enhancement of Rb+ uptake may reflect summation of the influence of low glycoside concentrations on effective levels of both acetylcholine and norepinephrine. Further, the stimulatory effect on monovalent cation transport is unrelated to any direct positive inotropic response, and is unrelated, as well, to inhibition of the Na+–K+ pump in myocardial cells observed at higher concentrations. Our findings indicate that pump stimulation is to be expected only in preparations with intact autonomic effector mechanisms, a prediction borne out in our studies of cultured chick embryo ventricular cells lacking adrenergic and cholinergic input (Barry et al., 1981; Biedert et al., 1979).
Relation of the Positive Inotropic Effect of Ouabain to Inhibition of Na⁺ Pump Activity

These experiments suggest that the influence of ouabain on inotropic state and Na⁺-K⁺ pump activity is mediated by different mechanisms at different ouabain concentrations. In the remaining discussion, Na⁺-K⁺ pump activity will be considered synonymous with monovalent cation active transport as measured by the ouabain-inhibited component of myocardial uptake of the K⁺ analogue Rb⁺. We recognize that Rb⁺ uptake as determined in these experiments is at best a relatively crude approximation to Na,K-ATPase-mediated pump activity, even after correcting for residual "nonspecific" Rb⁺ uptake in the presence of 10⁻⁶ M ouabain.

The response of the guinea pig atrial preparation to low concentrations (10⁻⁹ to 10⁻⁸ M) of ouabain is sensitive to autonomic influences. Ouabain can produce stimulation of the Na⁺-K⁺ pump without an associated inotropic response, and it can cause an inotropic response without measurable Na⁺-K⁺ pump inhibition. We did not observe simultaneous stimulation of pump activity and a positive inotropic effect. For reasons discussed by Noble (1980), differences in K⁺ concentration in the medium may be responsible for this difference between our results and those reported by Ghysel-Burton and Godfraind (1979).

At an intermediate concentration (10⁻⁷ M), ouabain under all circumstances produced a sustained positive inotropic effect without evidence of rhythm disturbances or mechanical toxicity, and without measurable inhibition of Na⁺-K⁺ pump activity. These results are in agreement with reports from Lüllmann and colleagues (Bentfeld et al., 1977; Busse et al., 1979), who found a sustained inotropic effect in guinea pig atria without evidence of pump inhibition. At 10⁻⁷ M ouabain, we found that—in contrast, under conditions of β-adrenergic blockade—the inotropic response correlated closely with inhibition of Na⁺ pump activity. Taken together, these findings suggest that at a critical subtoxic glycoside concentration, direct inhibitory and indirect, adrenergically mediated stimulatory influences of ouabain on K⁺ or Rb⁺ uptake may offset one another. In any event, the inotropic response to ouabain at 10⁻⁷ M or greater concentrations was not appreciably influenced by catecholamine depletion, β-adrenergic blockade, or muscarinic blockade, and is therefore not mediated by endogenous neurotransmitter effects.

At high concentrations (greater than 1.5 × 10⁻⁷ M), ouabain always inhibited Na⁺ pump activity and al-
TABLE 5
Positive Inotropic Response to Graded Isoproterenol Concentrations after Incubation in the Presence of Ouabain (Cumulative Percent Increase in Developed Tension)

<table>
<thead>
<tr>
<th>Isoproterenol concentration (M)</th>
<th>10^-9</th>
<th>3 x 10^-9</th>
<th>10^-8</th>
<th>3 x 10^-8</th>
<th>10^-7</th>
<th>10^-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca = 0.9 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 4)</td>
<td>17 ± 5</td>
<td>48 ± 14</td>
<td>99 ± 17</td>
<td>126 ± 19</td>
<td>161 ± 29</td>
<td>178 ± 37</td>
</tr>
<tr>
<td>Ouabain 10^-8 M (n = 4)</td>
<td>9 ± 4</td>
<td>29 ± 13</td>
<td>70 ± 28</td>
<td>115 ± 36</td>
<td>156 ± 27</td>
<td>194 ± 17</td>
</tr>
<tr>
<td>Ouabain 10^-7 M (n = 4)</td>
<td>11 ± 2</td>
<td>33 ± 4</td>
<td>98 ± 14</td>
<td>146 ± 16</td>
<td>170 ± 13</td>
<td>182 ± 8</td>
</tr>
<tr>
<td>Ca = 1.8 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>15 ± 5</td>
<td>29 ± 8</td>
<td>59 ± 12</td>
<td>79 ± 11</td>
<td>89 ± 6</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>Ouabain 10^-8 M (n = 4)</td>
<td>10 ± 3</td>
<td>29 ± 7</td>
<td>70 ± 13</td>
<td>94 ± 12</td>
<td>107 ± 10</td>
<td>111 ± 11</td>
</tr>
<tr>
<td>Ca = 2.5 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>11 ± 1</td>
<td>29 ± 5</td>
<td>55 ± 6</td>
<td>94 ± 12</td>
<td>109 ± 14</td>
<td></td>
</tr>
<tr>
<td>Ouabain 3 x 10^-9 M (n = 6)</td>
<td>11 ± 2</td>
<td>19 ± 2</td>
<td>47 ± 5</td>
<td>56 ± 3</td>
<td>73 ± 10</td>
<td></td>
</tr>
<tr>
<td>Ouabain 10^-8 M (n = 5)</td>
<td>9 ± 2</td>
<td>20 ± 3</td>
<td>52 ± 6</td>
<td>64 ± 7</td>
<td>87 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

ways produced a positive inotropic response. The strong correlation emphasized in studies of other preparations (Aker and Brody 1978; Biedert et al., 1979) was observed in this concentration range. Mechanical toxicity also began to emerge at this concentration, with one interesting exception. No increase in diastolic tension after 3 x 10^-7 M ouabain was observed in β-adrenergically blocked atria. Since mechanical toxicity is probably dependent on intracellular calcium load, it seems likely that β-adrenergic blockade diminishes the calcium load of the atria under the conditions of these experiments, possibly by reducing the Ca^{++} conductance of slow channels.

The studies reported here serve to emphasize the complexity of the mechanisms of myocardial response to cardiac glycosides. This is also evident in the recent report of Grupp and colleagues (1982). We would add the final caveat that these results pertain to guinea pig atrial myocardium; ventricular tissue (or for that matter atrial tissue from another species) might respond differently in terms of endogenous neuroeffector-mediated phenomena and/or Na^{+}-K^{+} pump effects. We

TABLE 6
Rb Uptake in Control and Atropinized Atria Exposed to Isoproterenol

<table>
<thead>
<tr>
<th>Rb uptake:</th>
<th>Rb uptake: atropinized atria (nmol/mg per 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control atria (nmol/mg per 20 min)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>Before isoproterenol</td>
<td>0.492 ± 0.017</td>
</tr>
<tr>
<td>Isoproterenol 10^-8 M</td>
<td>0.530 ± 0.070</td>
</tr>
<tr>
<td>Isoproterenol 3 x 10^-8 M</td>
<td>0.598 ± 0.040*</td>
</tr>
<tr>
<td>Isoproterenol 10^-7 M</td>
<td>0.676 ± 0.043†</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* P < 0.05 compared to control.
† P < 0.01 compared to control.
conclude that other preparations less subject to diffusion limitations and interstitial space uncertainties, and without endogenous neurotransmitter stores, may be better suited for working out details of cellular mechanisms of direct inotropic action of cardiac glycosides (Barry and Smith, 1982). The full elucidation of inotropic and toxic mechanisms in the conscious patient (or experimental animal) with intact neural pathways remains the greatest challenge of all.

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INDEX TERMS: Digitalis • Ouabain • Inotropy • Cation transport • Neuroeffector
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