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SHORT COMMUNICATIONS

The Binding of Tritiated Ouabain to Sodium- and Potassium-Activated Adenosine Triphosphatase and Cardiac Relaxing System of Perfused Dog Heart

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SUMMARY

Isolated dog hearts were perfused *in situ* with ouabain; control hearts were perfused for similar lengths of time without the addition of ouabain. After the onset of a significant positive inotropic effect, the hearts were removed, and $(Na^+ + K^+)$-ATPase and cardiac relaxing system were isolated. The $(Na^+ + K^+)$-ATPase activity was significantly decreased in the ouabain-treated hearts. The binding *in vitro* of ^{3}H-ouabain to the $(Na^+ + K^+)$-ATPase of the treated hearts was significantly lower than binding to control enzyme. A small extent of binding of ^{3}H-ouabain to the cardiac relaxing system was observed and may be attributed to the presence of $(Na^+ + K^+)$-ATPase.

We have previously demonstrated a direct relationship between ^{3}H-ouabain binding *in vitro* and inhibition of cardiac $(Na^+ + K^+)$-ATPase (1, 2). These results suggest that the $(Na^+ + K^+)$-ATPase may be the pharmacological receptor for cardiac glycosides, a concept first proposed by Repke (3). In order to correlate $(Na^+ + K^+)$-ATPase activity more directly with the positive inotropic effect, ouabain was infused into dog hearts, and when partial or peak positive inotropic effects had been produced (by varying the concentration of drug and duration of infusion), the hearts were removed and three separate membrane fractions were isolated: cardiac relaxing system, mitochondria, and $(Na^+ + K^+)$-ATPase (4). In animals in which a positive inotropic effect had occurred, the $(Na^+ + K^+)$-ATPase was the only one of the enzymatic systems consistently affected (4).

These studies *in situ* suggest that ouabain attaches to its locus of action on the enzyme [i.e., $(Na^+ + K^+)$-ATPase] and remains bound throughout the isolation procedure.

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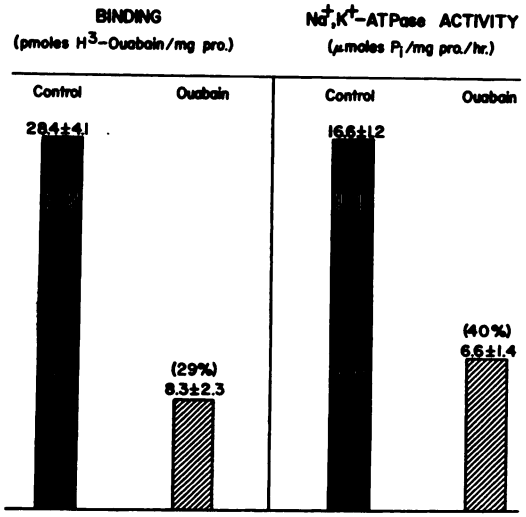

FIG. 1. *³H-Ouabain binding to cardiac (Na^+ + K^+)-ATPase isolated from control and ouabain-perfused dog hearts*

The control animals were perfused with Krebs-Henseleit solution, and the experimental animals were perfused with the same solution plus 10^{-6} M ouabain. The hearts were removed for isolation and assay of (Na^+ + K^+)-ATPase. The various enzyme preparations were then labeled with ³H-ouabain in the presence of 1.25 mM Tris-ATP and 1.25 mM $MgCl_2$, with or without 50 mM Na^+ (7, 8). The values in this figure represent the Na^+-stimulated binding of ³H-ouabain. The control values were set at 100% in each case. The values are followed by their standard errors of the mean.

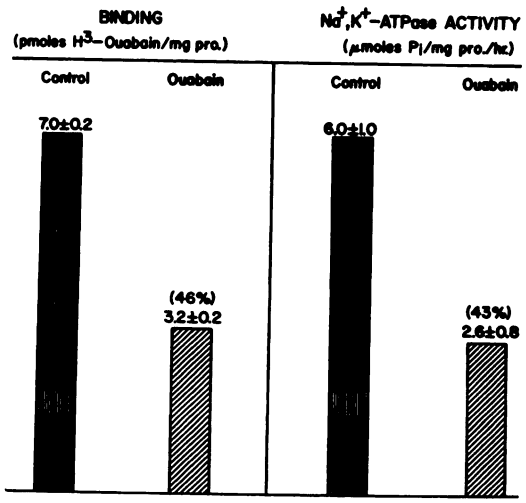

FIG. 2. *Relation between ³H-ouabain binding and (Na^+ + K^+)ATPase activity of cardiac relaxing system isolated from control and ouabain-perfused dog hearts*

Cardiac relaxing system was isolated as described previously (4). The preparations were assayed for (Na^+ + K^+)-ATPase activity and then labeled with ³H-ouabain under conditions maximal for binding (1, 7, 8). The control values were set at 100% in each case. The values are followed by their standard errors of the mean.

In addition, evidence has previously been presented that the ouabain–(Na^+ + K^+)-ATPase complex resulting from interaction *in vitro* between the drug and the enzyme system isolated from glycoside-sensitive species is stable (2, 5). If ouabain administered *in vivo* binds firmly to receptor sites and produces inhibition of enzyme activity, fewer sites should in theory be available for further reaction with ouabain administered *in vitro*. To test this hypothesis, the binding of ³H-ouabain to (Na^+ + K^+)-ATPase obtained from hearts perfused *in situ* with unlabeled ouabain was compared with the binding of ³H-ouabain observed in hearts subjected to perfusion without the glycoside.

The procedures for the isolation and assay for determination of ³H-ouabain binding to the (Na^+ + K^+)-ATPase preparation have all been described in detail (1, 6–8).

The activity of the (Na^+ + K^+)-ATPase isolated from dog hearts perfused with ouabain was 40% of the control value, as has been reported previously (4). Moreover, ³H-ouabain binding *in vitro* to this (Na^+ + K^+)-ATPase preparation was only 29% of the control value (Fig. 1). These observations indicate that some of the ouabain administered *in vivo* is firmly bound and, therefore, fewer sites are available for further interaction with ouabain *in vitro*. The problem of possible artifactual distortion of these results has been discussed in detail (4). In essence, we have shown that glycoside binding and its effects most probably did not occur during the homogenization and isolation procedures (4).

Published data suggest that the cardiac relaxing system ("sarcoplasmic reticulum") may also be *directly* affected by cardiac glycosides. For example, glycosides have been shown to alter Ca^{++} uptake (9–11). Furthermore, ³H-glycoside administered by perfusion *in situ* appears to accumulate in

the "microsomal" fraction, and some binding *in vitro* of labeled glycoside to this fraction, which has been presumed to represent the relaxing system, has been found (12, 13). The dog cardiac relaxing system that we have isolated exhibited a Mg^{++}-stimulated ATPase activity of approximately 60 μmoles of P_i per milligram of protein per hour. The fraction contained a small amount of ouabain-sensitive (Na^+ + K^+)-ATPase, which constituted about 10% of the total activity. The canine cardiac relaxing system bound ^{3}H-ouabain *in vitro* to an extent that corresponded quantitatively (8) to the level of (Na^+ + K^+)-ATPase activity present (Fig. 2). When ouabain was infused into the dog heart, this amount of (Na^+ + K^+)-ATPase activity was decreased (Fig. 2), just as was the subsequent binding of ^{3}H-ouabain *in vitro*. Furthermore, the absolute amount of (Na^+ + K^+)-ATPase activity of the "sarcoplasmic reticulum" was more than one-third that of the "purified" (Na^+ + K^+)-ATPase fraction, and only about one-fourth as much ^{3}H-ouabain was bound as in the (Na^+ + K^+)-ATPase fraction. This is further indication of a correlation between binding and enzyme activity. Moreover, relaxing system preparations obtained from rabbit and beef heart, which possess no measurable (Na^+ + K^+)-ATPase activity, also did not bind ^{3}H-ouabain or ^{3}H-digoxin *in vitro* (1, 4). The present data represent further evidence suggesting that the (Na^+ + K^+)-ATPase is the pharmacological receptor for the cardiac glycosides. The binding of ^{3}H-ouabain to other subcellular fractions, such as relaxing system, can be attributed to the presence of small quantities of (Na^+ + K^+)-ATPase that sediment with the particulate preparation.

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