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A Positive Correlation between Natriuresis and Inhibition of Renal Na–K–Adenosine Triphosphatase by Ouabain* (33963)

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A variety of natriuretic agents has been shown to selectively inhibit the Na–K-activated portion of the renal microsomal adenosine triphosphatase (ATPase) system *in vitro* (1–5). The data suggest that inhibition of this enzyme could be the mechanism whereby drugs inhibit renal sodium transport. Establishment of Na–K–ATPase as the renal receptor for natriuretic agents would be advanced if these drugs inhibited the enzyme when administered *in vivo*. Jones *et al.* (2) found that renal Na–K–ATPase from rats treated with diuretic mercurials was depressed. Hook and Williamson (6) obtained comparable results with furosemide and ethacrynic acid, even though ethacrynic acid did not produce natriuresis. However, in both studies the doses employed were sufficiently high that enzyme inhibition could have represented a toxicological, rather than pharmacological, effect. When smaller, diuretic doses of mercurials and ethacrynic acid were studied in dogs, Nechay *et al.* (5) could find no enzyme inhibition *in vitro* when the kidneys were removed and assayed. Thus, these authors could not implicate Na–K–ATPase as the renal receptor for these drugs.

The digitalis glycosides are powerful natriuretic compounds and are specific inhibitors of Na–K–ATPase *in vitro* (7). Both natriuresis and enzyme inhibition produced by digitalis can be reversed by addition of postassium [see Ref. (5)]. Furthermore, Palmer and Nechay (4) demonstrated that varying doses of ouabain, in the chicken, produced a biphasic effect on sodium transport that was mirrored by a similar biphasic effect on Na–K–ATPase when ouabain was

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added *in vitro*. Therefore, from the available data it appears that if renal Na–K–ATPase is the natriuretic receptor for any group of compounds, it would be the digitalis glycosides. However, to our knowledge, no attempt had been made to demonstrate inhibition of renal Na–K–ATPase following *in vivo* administration of a digitalis glycoside. In the present study, ouabain was administered to dogs and during the natriuresis a kidney was removed and enzyme activity was determined *in vitro*. The data demonstrate a significant correlation between the effect of ouabain on sodium excretion *in vivo* and Na–K–ATPase *in vitro*.

Methods. Mongrel dogs of either sex, weighing 14–21 kg, were anesthetized with pentobarbital sodium, tracheotomized, and infused intravenously, at 0.5 ml/kg/min, with 0.9% NaCl containing inulin (0.4%). The right kidney was removed through a retroperitoneal incision. The left ureter was cannulated, and after approximately 30 min, urine was collected every 10 min and a blood sample was taken every 20 min. After urine flow stabilized, ouabain was infused directly into the renal artery in doses of 5, 10, or 20 μg/min. After 30 min of infusion the left kidney was also removed.

Upon removal, the kidneys were immediately placed in cold saline. Each individual kidney was dissected into medulla and cortex and placed in a solution (pH 6.8) containing 0.25 *M* sucrose, 5 m*M* EDTA, 30 m*M* histidine, and 0.1% deoxycholic acid (DOC), homogenized, and centrifuged at 9000*g* for 20 min. The supernatant was decanted and refrigerated for 30–48 hr. The microsomal fraction was prepared by centrifuging the 9000*g* supernatant fraction at 100,000*g* for 30 min. The pellet formed was resuspended in a media of 0.25 *M* sucrose, 1 m*M* EDTA, and 30 m*M* Tris (pH 7.4).

The ATPase activity was measured in 5 m*M* ATP, 30 m*M* Tris, 5 m*M* $MgCl_2$ at pH 7.4. Half the beakers contained 115 m*M* NaCl and 10 m*M* KCl (total ATPase) and half the beakers contained no NaCl or KCl (Mg–ATPase) in a total volume of 3 ml. The reaction was started by adding 0.1 ml of the microsomal suspension (approx 1 mg of protein/ml) to the beakers and was terminated by the addition of 1 ml of cold TCA. Incubation time was 15 min at 37°. After centrifugation the supernatant was analyzed for phosphate (P_i) and the microsomes for protein (8, 9). Enzyme activity was expressed as μmoles of P_i released/15-min incubation/mg of mirosomal protein. Urine and plasma samples were analyzed for sodium (flame photometry) and inulin (10). Statistical analyses employed are described by Goldstein (11).

TABLE I. Effect of Ouabain (10 μg/min) on Renal ATPase in a 15-kg Dog.[a]

		(μmoles of P_i/mg of protein/15 min)	
		Control kidney	Treated kidney
Cortex	Total	7.3	4.4
	Mg	2.1	2.3
	Na^+K^+	5.2	2.1
Medulla	Total	12.3	11.6
	Mg	2.4	2.3
	Na^+K^+	9.9	9.3

[a] The right kidney was removed and used as the control. Ouabain was infused for 30 min into the renal artery before removing the left kidney. Total activity was determined in the presence of Na, K, and Mg; Mg activity was obtained with no Na or K present.

Results. Infusion of ouabain (5, 10, or 20 μg/min) into the left renal artery of dogs for 30 min produced a dose-related natriuresis and diuresis with no significant change in inulin clearance or systemic blood pressure. Concomitantly, there was a dose-related depression of Na–K–ATPase obtained from the renal cortex (Table I, Fig. 1). In the experiment illustrated in Table I, infusion of ouabain (10 μg/min) markedly reduced total ATPase in the cortex without altering Mg–ATPase, indicating a specific inhibition of Na–K–ATPase. As would be expected, Na–K–ATPase activity was higher in the medulla than in the cortex. However, no effect of ouabain was seen on the medullary enzyme in this animal (Table I), nor was any consistent pattern seen at any dose studied

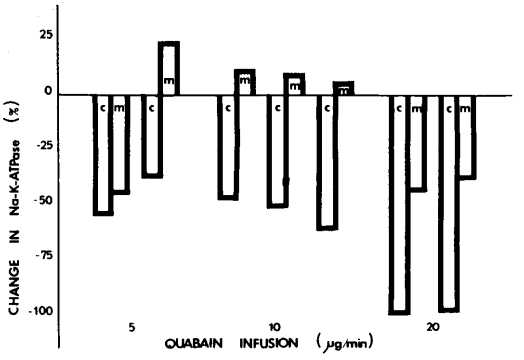

Fig. 1. Effect of increasing doses of ouabain *in vivo* on Na–K–ATPase activity: Na–K–ATPase was determined in cortex (c) and medulla (m) of the right kidney. Following 30 min of ouabain infusion the left kidney was removed and assayed for ATPase. Change in Na–K–ATPase represents difference in activity between the kidneys as a percentage of control (right) activity.

(Fig. 1). This could be attributed to a site of action of ouabain only in the cortex. However, it is more likely that the absence of ouabain effect in the medulla merely reflects the low blood flow to this region which would result in less drug being delivered to the medulla than the cortex.

Inasmuch as ouabain had no effect on Mg–ATPase, the effect of the drug could be represented as the percentage change in Na–K–ATPase. When the effect of ouabain on Na–K–ATPase was plotted in this manner as a function of dose a positive relationship is shown (Fig. 1). Furthermore, when these changes in enzyme activity were plotted against the drug-induced increase in sodium excretion (Fig. 2), the relationship between *in vivo* and *in vitro* effects of the drug was obtained was statistically significant ($p < .05$).

Discussion. The data from this study support the premise that renal Na–K–ATPase is the natriuretic receptor for ouabain. However, several basic assumptions must be made in a study of this type. It is assumed that the resting level of enzyme activity in the 2 kidneys is the same so that the difference between the two kidneys truly represents a drug-induced decrease in enzyme activity. Secondly, it is assumed that after preparation of the enzyme the drug remains bound in the same manner and at the same sites as it was *in vivo*. The first assumption has been justified with the observation that no differences in Na–K–ATPase activity between kidneys were found in four animals. The second assumption, however, is not so easily handled. The data presented clearly demonstrate that ouabain is tightly bound to the tissue, but clarify little regarding the specificity of this binding. It does seem unlikely, however, that an agent would switch binding sites during enzyme preparation. Therefore, these data suggest that ouabain simultaneously inhibits renal Na–K–ATPase and sodium transport *in vivo* and support the premise that Na–K–ATPase could be the renal receptor for ouabain. Nechay *et al.* (5) suggested that ouabain may share a common, or at least similar, mechanism with the organomercurial diuretics, yet no *in vitro* inhibition was observed when diuresing kidneys were assayed for Na–K–ATPase. Quite possibly natriuretic agents other than ouabain inhibit Na–K–ATPase *in vivo* but do not have sufficient binding capacity and are "washed out" of the tissue during preparation of the enzyme. Confirmation of this point requires more sophisticated techniques than are presently available. Lastly, it should be emphasized

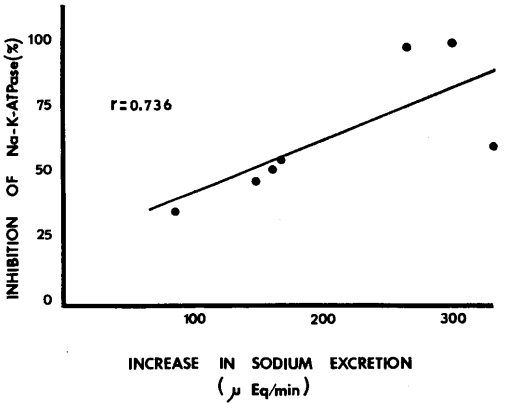

FIG. 2. Correlation between the effect of ouabain on sodium excretion ($U_{Na}V$) and Na–K–ATPase from renal cortex: difference in sodium excretion before and during ouabain infusion in the left kidney is plotted against the difference in enzyme activity between the 2 kidneys. The correlation coefficient ($r = 0.736$) was statistically significant ($p < .05$).

that even though the positive correlation between the effects of ouabain on sodium excretion and enzyme inhibition are highly suggestive of a cause and effect relationship, the evidence remains circumstantial. The possibility still remains that ouabain inhibits sodium transport by some other means and Na–K–ATPase inhibition occurs during the enzyme preparation.

Summary. Ouabain was infused into the left renal artery of anesthetized dogs after removal of the right kidney. After 30 min of infusion the left kidney was removed and assayed for Na–K–ATPase in both cortex and medulla. Ouabain inhibited the enzyme from the cortex in a dose-dependent manner, while having no consistent effect on the enzyme from the medulla. The inhibition of cortical enzyme activity was positively correlated with increased sodium excretion and this correlation was statistically significant. These data are consistent with, but not conclusive proof of, the premise that Na–K–ATPase is the renal receptor for the natriuretic action of ouabain.

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