INTRARENAL DISTRIBUTION AND ATPase INHIBITING ACTIVITY OF OUABAIN IN DOGS

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Abstract—Experiments were undertaken to substantiate the hypothesis that the mechanism of the direct effect of ouabain on the renal excretion of electrolytes is the result of inhibition of the transport enzyme, \((\text{Na}, \text{K})\)-ATPase. In dogs hydrated with saline, an injection of \(^3\text{H}\)-ouabain into the unilateral renal artery produced a continuing marked increase in excretion of water and sodium from the kidney, but not from the counter kidney. At maximal diuresis—90 min after ouabain injection, both kidneys were removed to assay microsomal ATPase activity and determine radioactivity distributed in subcellular structures. It was demonstrated that \(^3\text{H}\)-ouabain was deposited in the microsome fraction obtained from the injected kidney in concentrations ranging from \(10^{-7}\) to \(10^{-6}\) M/kg wet weight, and \((\text{Na}, \text{K})\)-ATPase activity of this fraction was inhibited as compared with that of the microsomal fraction obtained from control kidneys. Since \((\text{Na}, \text{K})\)-ATPase activity of renal microsomes was significantly inhibited in vitro by more than \(10^{-7}\) M of ouabain, ouabain concentration in microsomes obtained from the injected kidney was considered to be sufficient to inhibit ATPase activity. These findings indicate that ouabain diuresis under the present condition is closely related to direct inhibitory effect of ouabain on \((\text{Na}, \text{K})\)-ATPase activity of microsomes in tubular cells.

Previously Tanabe et al. (1) presented experimental evidence that when ouabain is administered into the unilateral renal artery, a remarkable diuretic effect on the administered side together with an increase in urinary sodium excretion was seen. On the other hand, based on in vitro experiments it is generally known that cardiac glycosides inhibit transport ATPase. However, regarding the direct effect of ouabain on the renal excretion of electrolytes, no direct scientific evidence is available regarding relationship with the inhibition of transport ATPase. The present experiments were undertaken to substantiate the hypothesis that the mechanism of the direct effect of ouabain on the renal excretion of electrolytes is the result of inhibition of the transport enzyme, \((\text{Na}^+, \text{K}^+)\) ATPase.

MATERIALS AND METHODS

In vivo experiments

Mongrel dogs of both sexes, weighing about 10 kg were anesthetized with pentobarbital. In the present \(^3\text{H}\)-ouabain experiments, six mongrel dogs were used. The midline of the suprapubic part of the abdomen was incised and polyethylene catheters were inserted into both ureters. Isotonic saline was continuously infused into the jugular vein at a rate of 7 ml per min throughout the experiment. When the urine volume of the right and left ureters
became constant, both ouabain 0.04 mg/kg and $^3$H-ouabain 30 μci were injected into the left renal artery. Urine samples were then collected separately from each kidney at 15 min intervals and urine volume, electrolytes, and radioactivity were measured. At maximal diuresis, namely at 90 min after ouabain injection both left and right kidneys were removed, and after macroscopic separation into the cortex, outer and inner zone of the medulla, the respective radioactivity was measured. In addition, the above mentioned 3 layers were rapidly cooled and homogenized. Fractionation into debris, mitochondria, microsome and soluble fraction were conducted and their respective radioactivity was measured. As for the radioactivity measurement, the samples were prepared using Packard’s sample oxidizer and measurements were made with a liquid scintillation counter. The concentration of ouabain was expressed by $10^{-6} \text{M/kg wet weight of tissue}$. Since it is presently accepted that ouabain is not metabolized, it was assumed that all radioactivity represents the original compound.

At 5 min, 90 min, 5 hr and 24 hr after ouabain injection, both kidneys were removed. ATPase activity was measured in the cortex, outer and inner zone of medulla. After treatment with 0.2% DOC, the microsomal fraction was separated by centrifugation. The ATPase activity was measured by the method of Miyasaka (2). The inorganic phosphate was measured by the method of Martin and Doty (3) and the protein by the method of Lowry et al (4).

**In vitro experiments**

Using the microsomal fraction separated from non-treated dog renal cortex, both ouabain $10^{-6} \text{M}$ and $^3$H-ouabain 1 μci were added and incubation was conducted in physiological saline at 38 °C. After incubation for 5, 10, 20, 30 and 90 min, centrifugation was conducted and the radioactivity of the microsomal fractions was measured. To the microsomal fraction separated in the same manner as described above, ouabain $10^{-6} \text{M}$ was added and the inhibitory action of (Na+, K+) ATPase activity was studied after incubation for 5, 10, 20, 30 and 90 min.

**RESULTS**

**Intrarenal distribution of $^3$H-ouabain in dogs**

Both ouabain 0.04 mg/kg and $^3$H-ouabain 30 μci were injected into the left renal artery. At maximal diuresis, that is 90 min after ouabain injection, both kidneys were removed to assay microsomal ATPase activity and radioactivity distributed in cortex, outer and inner zone of the medulla. As shown in Fig. 1, it was noted that a high concentration of ouabain was seen in the outer zone of the medulla on the injected side. Ouabain, however, showed a poor distribution in the inner zone of the medulla. On the other hand, it was also noted that large amounts of ouabain were seen in the counter kidney. The results were essentially the same in all 6 experiments.

**Distribution at a subcellular level following $^3$H-ouabain administration in dogs (refer to Fig. 2)**

When ouabain was administered under the same conditions as described above, a high
Fig. 1. Intrarenal distribution of $^3$H-ouabain in dogs.

Fig. 2. Subcellular distribution of $^3$H-ouabain in dog kidneys.

centration of ouabain was seen in the microsome fraction of the outer zone of the medulla. Thus it was demonstrated that ouabain was deposited in the microsome fraction obtained from the outer zone of medulla on the injected side in a concentration of $6 \times 10^{-6}$ M/kg wet weight. In this case, the microsome fraction on the injected side showed a higher concentration as compared to the control side. In the subcellular distribution of ouabain in the outer zone of the medulla, the debris, mitochondria and soluble fraction showed lower values compared with the microsomal fraction. Further, with regard to the microsome fraction, the distribution in the cortex and inner zone of the medulla was small compared with that in the outer zone of the medulla.
Fig. 3. Time-action relationships for inhibitory effect of ouabain on microsomal ATPase activity in dog kidneys in vivo.

On the time course observation of the inhibitory effect of ouabain on the (Na\(^+\), K\(^+\)) ATPase activity of the microsomal fraction in dog kidney in vivo

Ouabain was administered under the same conditions as described above. At 5 min, 90 min, 5 hr and 24 hr after injection of ouabain, the ATPase activity was measured in the microsomal fraction of the cortex and outer zone of the medulla. When maximal diuresis was seen 90 min after ouabain administration, a remarkable inhibition of ATPase activity compared with other periods was seen. Further, the ATPase activity of the injected side as compared with that of the control kidney showed a remarkable inhibition. However, at 5 min, 5 hr and 24 hr after ouabain administration, the ATPase activity was the same in the injected side and the control side (Fig. 3).

Effect of ouabain on (Na\(^+\), K\(^+\)) ATPase activity in vitro

When the incubation time was 90 min, (Na\(^+\), K\(^+\)) ATPase activity of renal cortex

Fig. 4. The relationship between concentration of ouabain and the inhibitory effect of (Na, K) ATPase activity in vitro.
Microsomes was inhibited in vitro by more than $10^{-9}$ M of ouabain. When the incubation time was 20 min, transport ATPase was inhibited by $10^{-6}$ M of ouabain. In this experiment, the microsomal fraction obtained from the cortex was used rather than that of the outer zone of the medulla as that of the cortex has a larger mass and the microsomal fraction can be more readily separated (Fig. 4).

In vitro experiments on the time course observation of the inhibitory effect of ouabain on the $(Na^+, K^+)$ ATPase activity of the microsomal fraction of dog kidney

Even at 5 min after the addition of ouabain $10^{-6}$ M a significant inhibition compared with the control was seen and the inhibition by ouabain became more pronounced with the lapse of time. At 90 min after, an intense inhibition of ATPase activity by ouabain was evident (Fig. 5).

In vitro experiments on ouabain binding in the microsome fraction of dog kidney

To the microsome fraction separated from the renal cortex and outer zone of the medulla of healthy dogs, both ouabain $10^{-6}$ M and $^3$H-ouabain 1 µCi were added. This was immedi-

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![Fig. 5](image-url)  
**Fig. 5.** Time-action curve for inhibitory effect of ouabain on $(Na, K)$ ATPase activity in dog kidneys in vitro.

![Fig. 6](image-url)  
**Fig. 6.** Time course observation of the binding of ouabain to the microsomal fraction in dog kidneys.
ately cooled and centrifuged. After division into the supernatant and precipitate, the respective radioactivity was measured. It was noted that up to the first 20 min the radioactivity of the microsome fraction showed a rapid increase and was followed thereafter by a gradual slowing of the increase. Maximal values were seen around 90 min after the addition of ouabain. In contrast, the radioactivity in the incubation medium showed a decrease with the lapse of time.

**DISCUSSION**

One of the most interesting observations in the present experiment is that after the injection of ouabain, the microsome fraction of the outer zone of the medulla showed a particularly high concentration of ouabain. Namely, the concentration of ouabain in the outer zone of the medulla was $6.0 \times 10^{-3}$ M/kg wet weight. It was demonstrated that $(\text{Na}^+, \text{K}^+)$ ATPase activity of the microsomal fraction obtained from the injected kidney was inhibited as compared with that of the microsomal fraction obtained from the counter kidney. Accordingly, the concentration of ouabain which brought about inhibition of ATPase activity was on the order of $10^{-6}$ M in vivo. With in vitro experiments, the ouabain concentration which brought about a marked inhibition of ATPase activity of the renal microsome fraction was also on the order of $10^{-6}$ M. Thus, these findings indicate that ouabain concentration in microsomes obtained from the injected kidney was sufficient to inhibit ATPase activity. Further purification of the microsomal fraction and investigation of the cell membranes and endoplasmic reticulum are now being done.

Determination of the time-action relationship between the diuretic action induced by ouabain and its inhibitory effect on $(\text{Na}^+, \text{K}^+)$ ATPase activity is also required. In in vivo experiments, it was noted that there was no diuretic effect at 5 min after ouabain administration. At the same time, no inhibition was seen in the ATPase activity of the microsome fraction. At maximal diuresis, that is at 90 min after ouabain injection, the $(\text{Na}^+, \text{K}^+)$ ATPase activity of the renal microsome fraction was remarkably inhibited. At 5 and 24 hr after ouabain administration, there was no difference between the $(\text{Na}^+, \text{K}^+)$ ATPase activity of the renal microsome fraction obtained from injected side and that of the control side. It was also noted that the diuretic effect had definitely disappeared at this time.

In in vitro experiments, a good parallel was seen with the above in vivo experimental results. The inhibitory effect of the $(\text{Na}^+, \text{K}^+)$ ATPase activity induced by ouabain was intensified with the lapse of time. Further, $^3$H-ouabain showed a high concentration deposit in the renal microsome fraction. A good parallel was also seen between the time course change in the binding of ouabain to the microsomal fraction and the time course change of the above described $(\text{Na}^+, \text{K}^+)$ ATPase activity inhibition. Such evidence may substantiate the time-action relationship between the diuretic effect of ouabain and its inhibitory effect of $(\text{Na}^+, \text{K}^+)$ ATPase.

**REFERENCES**