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The Effect of 6-Hydroxydopamine on Specific [^{3}H]Ouabain Binding to Some Sympathetically Innervated Organs of the Cat

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SUMMARY

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To determine the distribution of (Na^+ + K^+)-ATPase at the sympathetic nerve endings of various organs of the cat, specific [^{3}H]ouabain binding to microsomal preparations of different tissues was measured in either NaCl-treated or 6-hydroxydopamine-treated adult cats. Specific [^{3}H]ouabain binding to microsomal preparations either decreased (salivary glands, heart, nictitating membrane, and vas deferens), increased (brain), or showed no change (kidney and spleen) following administration of 6-hydroxydopamine. The dissociation constants of [^{3}H]ouabain, determined by Scatchard analysis, either decreased (heart and nictitating membrane), increased (salivary glands and vas deferens), or did not change (brain) as a result of the administration of 6-hydroxydopamine. More than 80% of the total number of specific [^{3}H]ouabain binding sites in salivary glands, heart, nictitating membrane, and vas deferens were localized at the sympathetic nerve endings. These results are consistent with the hypothesis that (Na^+ + K^+)-ATPase located on the heart muscle cell membrane may be a part of the "pharmacological receptors" for ouabain and that the inhibition of (Na^+ + K^+)-ATPase found on the sympathetic nerve endings of heart muscle may be involved, in part, in the development of cardiac glycoside-induced cardiac arrhythmia.

INTRODUCTION

One of the current theories of the mode of the positive inotropic action of cardiac glycosides is based on the ability of these steroids to inhibit cardiac (Na^+ + K^+)-ATPase (1). The first convincing demonstration of correlation *in vitro* between (Na^+ + K^+)-ATPase inhibition and the inotropic action of digitalis was the remarkable similarity in species sensitivity of enzyme inhibition by cardioactive steroids as well as the pharmacological action of this class of drugs (2). More recently, several workers have reported a direct correlation between (Na^+ + K^+)-ATPase inhibition and the inotropic action of ouabain from experiments with intact dog hearts *in situ* (3-5). This observation has been questioned, however, on the basis that administration of the high concentrations of ouabain used in these investigations could induce not only the maximum positive inotropic effect but also arrhythmia, and therefore any conclusion regarding a cause-and-effect relationship based on kill-

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ing the dog at the maximum positive inotropic effect produced by toxic or lethal concentrations of ouabain is not valid (6). When a dose regimen of ouabain was used that would allow only positive inotropy and maintain it for a long period of time, without leading to arrhythmia in intact dog heart preparations, no significant inhibition of cardiac ($Na^+ + K^+$)-ATPase in comparison with control dogs could be observed at two different time intervals (6–8). Using short-acting cardiac steroids in drug washout experiments in isolated rabbit hearts, ($Na^+ + K^+$)-ATPase isolated from such preparations was still significantly inhibited, whereas the positive inotropic effect was no longer present (9).

Although, on biochemical as well as immunological evidence, ($Na^+ + K^+$)-ATPase derived from a variety of tissues and species appears to be a single enzyme system, its sensitivity toward cardiac glycosides shows species and tissue differences (2, 10, 11). This difference in tissue sensitivity of ($Na^+ + K^+$)-ATPase toward cardiac glycosides may affect the interpretation of results obtained by previous workers. For example, ($Na^+ + K^+$)-ATPase derived from the peripheral organs of the rat appears to be nearly insensitive to inhibition by cardiac glycosides, but the enzyme system obtained from rat nervous tissue shows high sensitivity to inhibition by ouabain (12, 13). Therefore, in terms of ouabain sensitivity as well as morphological localization, the peripheral organs of the rat and possibly of other species may be assumed to contain two types of ($Na^+ + K^+$)-ATPase. One type may be localized on the nerve endings of the organ, and the second type may be found on the plasma membrane of the non-neuronal cells in that organ. Since the ouabain sensitivity of rat brain ($Na^+ + K^+$)-ATPase is markedly different from that of other rat tissues (13), it is possible that the neuronal ($Na^+ + K^+$)-ATPase of a peripheral organ such as the heart will have a different sensitivity to cardiac glycosides than ($Na^+ + K^+$)-ATPase located on the muscle cell membranes. We have examined this possibility by measuring specific [^{3}H]ouabain binding to microsomal fractions of several organs obtained from NaCl- and 6-hydroxydopamine-treated cats.

MATERIALS AND METHODS

Adult male cats were used in all experiments and were divided into two groups. In the first group, catecholaminergic nerve endings were effectively destroyed with intravenous injections of 6-hydroxydopamine hydrobromide, two doses of 20 mg/kg on the first day and two doses of 50 mg/kg 1 week later, as described by Thoenen and Tranzer (14). The second group, which served as a control, received equal volumes of NaCl instead of the drug. The animals were killed 1 week after the last dose of 6-hydroxydopamine or NaCl. Cats were anesthetized with sodium pentobarbitol (35 mg/kg; Nembutal, Abbott). Organs of interest—salivary glands, heart, spleen, kidney, nictitating membrane, brain, and vas deferens—were removed immediately and placed on ice.

The procedure for the preparation of the microsomal fraction of the salivary glands, heart, spleen, kidney, and brain was similar to that described by Schwartz *et al.* (15). The tissue was chopped into small pieces and placed in 9 volumes (w/v) of medium I (1 mM Tris-EDTA in 0.25 M sucrose). The mixture was homogenized for 35–40 sec at medium speed in a Macro-Waring Blendor. The homogenate was passed first through four and then through eight layers of cheesecloth and was centrifuged for 15 min at 10,000 × g. The supernatant was discarded, and the pellet was homogenized in 8 volumes of medium II (0.1% sodium deoxycholate and 1 mM EDTA in 0.25 M sucrose) at medium speed in the Macro-Waring Blendor for six 30-sec periods in ice. The mixture was then centrifuged at 10,000 × g for 30 min. The supernatant was separated from the bulk of the loosely packed precipitate by decantation through eight layers of cheesecloth and centrifuged at 100,000 × g for 45 min. The supernatant fluid was decanted, and the pellet was suspended in 1.5 volumes of medium III (0.05% sodium deoxycholate and 1 mM EDTA in 0.25 M sucrose) with a glass homogenizer and Teflon pestle and centrifuged at 20,000 × g. The supernatant

was collected by decantation through eight layers of cheesecloth and centrifuged further at 100,000 × *g* for 45 min. The pellet was suspended with a glass homogenizer and Teflon pestle in 1 mM EDTA to give a protein concentration of 2–5 mg/ml. The suspension was then brought to 1.5 volumes with the addition of 0.5 volumes of NaI solution containing 6 M NaI, 15 mM EDTA, 75 mM $MgCl_2$, and 150 mM Tris. After 30 min the almost clear solution was diluted to 2.5 volumes by the addition of 1.5 volumes of 1 mM EDTA. The resulting suspension was then centrifuged at 35,000 × *g* for 30 min (Sorvall SS34 rotor). The supernatant was discarded, the pellet was loosened from the centrifuge tube with a glass rod, and the tube was rinsed thoroughly into a glass homogenizer with small amounts of 1 mM EDTA. The particulate fraction was washed thoroughly so that the total volume of the washing medium (1 mM EDTA) was about twice that of the homogenate, and this was followed by centrifugation at 35,000 × *g* for 10 min. The above resuspension and washing procedures were repeated twice more. The final pellet was suspended in enough 1 mM EDTA to give a protein concentration of about 1 mg/ml. Protein concentration was determined by the method of Lowry *et al.* (16).

Crude microsomal fractions of vas deferens and nictitating membrane were prepared by homogenizing these tissues in 10 volumes of medium I with a Brinkman Polytron PT-10. The whole homogenate was centrifuged for 10 min at 1000 × *g*. The pellet (crude nuclear fraction) was suspended in medium I, and the previous step was repeated. The two supernatant fractions were combined and centrifuged for 10 min at 7710 × *g*. The supernatant was collected and centrifuged at 100,000 × *g* for 45 min in a Spinco ultracentrifuge. The final pellet was suspended in 1 mM EDTA to obtain the crude microsomal fraction.

The assay for specific binding of [^{3}H]ouabain to microsomal fractions was similar to that described for the binding of [^{125}I]-labeled nerve growth factor to sympathetic ganglion cell membranes (17). The binding assay used consisted of incubation of the microsomal suspension (0.7–1.5 mg of protein per milliliter) at 37° for 20 min in 1 ml of 0.05 M Tris-HCl buffer (pH 7.4) containing various concentrations of [^{3}H]ouabain, 4 mM $MgCl_2$, and 1 mM inorganic phosphate. After incubation, 3 ml of ice-cold 0.05 M Tris-HCl buffer were added to each tube, and the mixture was filtered and washed over glass fiber filter papers (Reeve Angel) as described previously (17). Corrections were made for nonspecific binding of [^{3}H]ouabain by assaying parallel incubations in which Mg^{2+} and inorganic phosphate were replaced by 0.2 M Na^+. Specific binding was obtained by subtracting from the total radioactivity the counts per minute in the presence of excess Na^+.

The filter papers were dried, and each paper was transferred to a counting vial containing 10 ml of Scintiverse (Fisher Scientific) and counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3380) at 30% efficiency as determined with internal standards.

[^{3}H]Ouabain (10 Ci/mmole) was obtained from New England Nuclear Corporation.

RESULTS

Specific [^{3}H]ouabain binding to the microsomal fractions obtained from various organs of cats is shown in Table 1. The highest specific [^{3}H]ouabain binding, found in the microsomal fraction obtained from salivary glands, was about 4 times the binding observed for whole kidney microsomes. This result is consistent with the (Na^+ + K^+)-ATPase activity of 40 μmoles of P_i per milligram of protein per hour found in microsomal suspensions of submaxillary gland (18), which is reported to be 4–5 times greater than the enzyme activity found in the guinea pig kidney preparation (19). The specific [^{3}H]ouabain binding to the microsomes obtained from heart and brain was similar. This is in agreement with an earlier observation of Erdmann and Schoner (20), who reported similar apparent affinities as well as number of binding sites for the glycoside in membrane preparations of beef heart and

TABLE 1

[^{3}H]Ouabain binding to microsomal fractions obtained from various organs of cats

The concentration of [^{3}H]ouabain was 0.16 μM, and its specific activity was 5700 cpm/pmole. Values are means ± standard errors of nine determinations.

Organ	[^{3}H]Ouabain binding						$T/C \times 100$
	Control animals (C)			6-Hydroxydopamine-treated animals (T)			
	4 mM Mg^{2+} + 1 mM P_i[a]	0.2 M Na^+	Specific binding	4 mM Mg^{2+} + 1 mM P_i[a]	0.2 M Na^+	Specific binding	
	cpm × 10^{-4}/mg protein						%
Salivary gland	294.0 ± 0.7	9.4 ± 0.7	284.6	50.4 ± 1.5	1.5 ± 0.08	48.9	17.2
Kidney	71.8 ± 1.6	1.6 ± 0.06	70.2	74.1 ± 1.7	1.8 ± 0.08	72.3	103
Heart	29.4 ± 1.7	2.8 ± 0.3	26.6	14.5 ± 0.2	1.3 ± 0.1	13.2	49.6
Brain	27.2 ± 0.2	1.8 ± 0.1	25.4	34.1 ± 1.2	1.6 ± 0.04	32.5	127
Spleen	8.3 ± 0.09	0.7 ± 0.001	7.6	8.5 ± 0.07	1.2 ± 0.02	7.3	96
Nictitating membrane	8.0 ± 0.01	2.5 ± 0.02	5.5	1.5 ± 0.02	0.6 ± 0.01	0.9	16.3
Vas deferens	5.2 ± 0.08	1.0 ± 0.07	4.2	0.9 ± 0.02	0.5 ± 0.005	0.4	9.5

[a] Inorganic phosphate.

brain. The specific [^{3}H]ouabain binding to spleen microsomes was about 30% of that of the brain microsomes. This is similar to the ratio of (Na^+ + K^+)-ATPase activity reported in cat brain white matter and spleen (21). Since the microsomal suspensions derived from nictitating membrane and vas deferens were not exposed to deoxycholate or sodium iodide, the specific [^{3}H]ouabain binding data in these two preparations were relatively low.

Administration of 6-hydroxydopamine to cats produced alterations in specific [^{3}H]ouabain binding to microsomal fractions derived from several organs (Table 1). Kidney (Na^+ + K^+)-ATPase is found predominantly in the renal tubules (22), and lipophobic 6-hydroxydopamine does not penetrate the blood-brain barrier (23). Therefore destruction of sympathetic nerve endings of the peripheral organs by the administration of 6-hydroxydopamine did not result in the reduction of specific [^{3}H]ouabain binding in kidney and brain. However, in most other tissues examined, there was a marked decrease in specific [^{3}H]ouabain binding following administration of 6-hydroxydopamine. Chemical sympathectomy did not modify specific [^{3}H]ouabain binding to microsomes obtained from spleen. This observation was surprising; we therefore measured specific [^{3}H]ouabain binding to microsomal preparations obtained from heart and spleen of rabbits and guinea pigs before and after 6-hydroxydopamine administration. The results were similar to those found in the heart and spleen of cats.[2] Another unexpected observation in Table 1 is the significant increase in specific [^{3}H]ouabain binding to the cat brain membrane preparation following administration of 6-hydroxydopamine. In order to ascertain whether this increase in specific [^{3}H]ouabain binding to brain microsomes was due to a change in affinity or number of binding sites, specific binding was measured in the presence of various concentrations of [^{3}H]ouabain (Table 2). There was an increase in specific [^{3}H]ouabain binding following 6-hydroxydopamine treatment at all four concentrations of [^{3}H]ouabain. A Scatchard plot of the data in Table 2 revealed that the apparent dissociation constants for ouabain of control and 6-hydroxydopamine-treated cat brain membrane preparations are 0.08 μM and 0.1 μM, respectively. These values are similar to the dissociation constant of about 0.18 μM found for ouabain at a high-affinity binding site of an ox brain (Na^+ + K^+)-ATPase preparation (24). On the other hand, the maximal number of binding sites for [^{3}H]ouabain increased from 155 to 260 pmoles/mg of protein following the administration of 6-hydroxydopamine. Thus intravenous injection of 6-hydroxydopamine, which does not readily cross the

[2] Unpublished observations.

TABLE 2

Specific [³H]ouabain binding to microsomes obtained from brains of control and 6-hydroxydopamine-treated cats

The procedures for the administration of 6-hydroxydopamine and the measurement of [³H]ouabain binding are described in the text. Values are means ± standard errors of six determinations.

[³H]Ouabain	Specific [³H]ouabain binding	
	Control animals	Treated animals
nM	*pmoles/mg protein*	
40	7.22 ± 0.01	13.27 ± 0.02
80	13.70 ± 0.01	16.08 ± 0.01
160	24.96 ± 0.02	33.52 ± 0.02
320	44.23 ± 0.03	56.95 ± 0.03

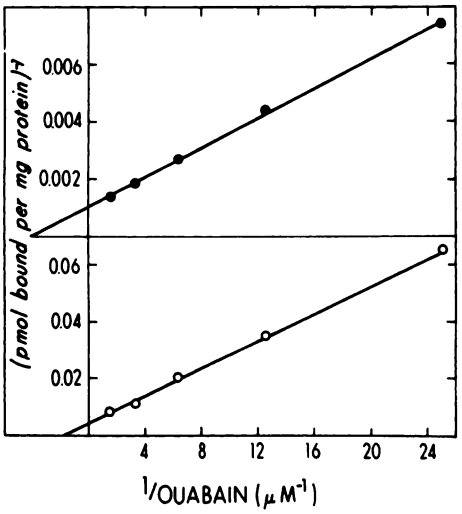

FIG. 1. *Double-reciprocal plots of specific [³H]ouabain binding to microsomal preparations derived from sympathetically innervated and denervated salivary glands*

Specific [³H]ouabain binding to microsomes obtained from salivary glands of control (●) and 6-hydroxydopamine-treated (○) cats was measured as described in the text. Each experimental point is the average of nine determinations of three separate measurements, which varied less than 10%.

blood-brain barrier in adult animals (23), produced a significant increase in the number of specific [³H]ouabain binding sites in cat brain microsomes without significant alterations in the apparent affinity for the cardiac glycosides.

Double-reciprocal plots of specific [³H]ouabain binding to salivary gland microsomes obtained from control and 6-hydroxydopamine-treated cats revealed significant alterations in maximal number of binding sites as well as an apparent affinity for ouabain following chemical sympathectomy (Fig. 1). Similar results were obtained when these data were analyzed by Scatchard plots (Table 3). Following chemical sympathectomy, the number of specific [³H]ouabain binding sites in salivary gland microsomes decreased by more than one-fifth of the original value, and the dissociation constant increased 2-fold (Table 3). Similar results were obtained with the membrane suspension obtained from cat vas deferens (Fig. 2 and Table 3).

TABLE 3

Distribution of specific [³H]ouabain binding in some sympathetically innervated organs of cat

The specific binding of various concentrations of [³H]ouabain to microsomal preparations from different organs of the cat was assayed as described in MATERIALS AND METHODS. The equilibrium dissociation constant (K_D) and maximal number of binding sites (B_{max}) were estimated from Scatchard plots. Values are averages of two separate determinations, which varied less than 10%.

Organ	Control animals		6-Hydroxydopamine-treated animals	
	K_D	B_{max}	K_D	B_{max}
	μM	*pmoles/mg*	*μM*	*pmoles/mg*
Salivary gland	0.22	1000.0	0.43	170.0
Heart	0.39	112.0	0.085	16.5
Nictitating membrane	0.88	30.0	0.087	1.35
Vas deferens	0.19	11.1	0.72	2.95

Results obtained with microsomal suspensions derived from cat heart and nictitating membrane were somewhat different. The maximal number of binding sites in control heart and nictitating membrane preparations was 112 and 30 pmoles/mg of protein, respectively. After 6-hydroxydopamine administration, the number of binding sites decreased to 16.5 and 1.35 pmoles/mg of protein, respectively (Table 3). Thus 85–95% of the total ($Na^+ + K^+$)-ATPase is located at the sympathetic nerve endings of the heart and nictitating membrane of cats. This is qualitatively similar to our observations with salivary glands and vas deferens. On the other

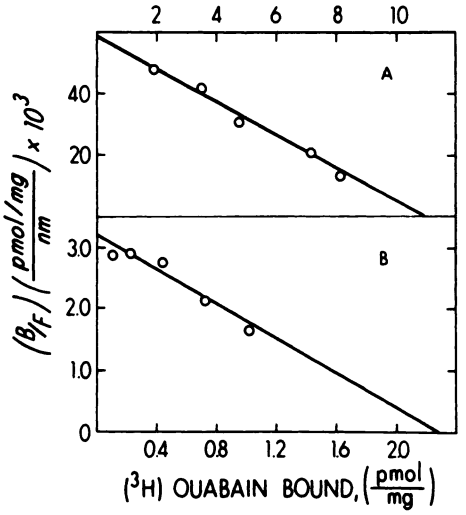

FIG. 2. *Scatchard plots of specific [^{3}H]ouabain binding to microsomal suspensions derived from vas deferens of control and 6-hydroxydopamine-treated cats*

The procedure for the measurement of specific [^{3}H]ouabain binding is described in the text. A. Scatchard plot obtained with microsomes derived from control vas deferens, which gave an apparent K_D of 0.19 μM and a specific binding density of 11.1 pmoles/mg of protein. B. Scatchard plot obtained with microsomes derived from vas deferens of 6-hydroxydopamine-treated cats, which gave an apparent K_D of a 0.72 μM; the maximal number of binding sites was found to be 2.95 pmoles/mg protein. Values are averages of six determinations. Abscissae for parts A and B are at the top and bottom of the figure, respectively.

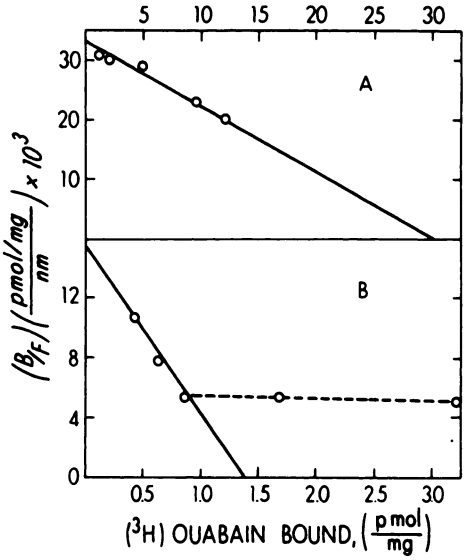

FIG. 3. *Scatchard plots of specific [^{3}H]ouabain binding to microsomal preparations derived from nictitating membrane of control and 6-hydroxydopamine-treated cats*

Specific [^{3}H]ouabain binding to microsomes obtained from nictitating membrane of control (A) and 6-hydroxydopamine-treated (B) cats was measured as described in the text. The apparent dissociation constants were 0.88 μM (control) and 0.087 μM (6-hydroxydopamine-treated), while the maximal number of binding sites was found to be 30.0 and 1.35 pmoles/mg of protein in nictitating membrane preparations from control and 6-hydroxydopamine-treated cats, respectively. Each experimental point is the average of six determinations. Abscissae for parts A and B are at the top and bottom of the figure, respectively.

hand, the apparent affinities of (Na^+ + K^+)-ATPase for ouabain located at the sympathetic nerve endings of the heart and nictitating membrane were less than the affinities of enzyme systems present in other parts of these organs (Table 3).

Scatchard plots for specific [^{3}H]ouabain binding to cat nictitating membrane and heart microsomal suspensions are shown in Figs. 3 and 4. These plots appeared to be linear for both tissue preparations derived from control animals, suggesting the presence of a single binding site for [^{3}H]ouabain in microsomal suspensions obtained from cat heart and nictitating membrane. The Scatchard plots for specific [^{3}H]-ouabain binding to heart and nictitating membrane from 6-hydroxydopamine-treated cats, however, were nonlinear (Figs. 3 and 4). Since the dashed lines shown in Figs. 3 and 4 are almost parallel to the x axis, it seems highly unlikely that these lines represent second binding sites. Furthermore, under conditions of high ligand or binding site concentrations, serious methodological problems may arise which can complicate proper analysis by Scatchard plots (1, 25). For example, with very high concentrations of labeled ligand, where only a very small fraction (e.g., about 2-5%) of the total ligand is bound, the errors in estimating small changes in the concentration of the bound ligand may be large and magnified on Scatchard representations. The points on the dashed lines in Figs. 3 and 4 were obtained with

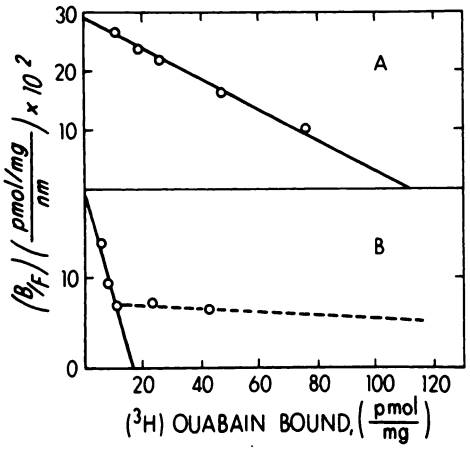

FIG. 4. *Scatchard analysis of specific [³H]ouabain binding to microsomal preparations derived from sympathetically innervated and denervated heart*

Specific [³H]ouabain binding to microsomes obtained from the hearts of control (A) and 6-hydroxydopamine-treated (B) cats was measured as described in MATERIALS AND METHODS. A. The Scatchard plot gave an apparent K_D of 0.39 μM and a specific binding density of 112 pmoles/mg of protein. B. The Scatchard plot gave an apparent K_D of 0.085 μM and a specific binding density of 16.5 pmoles/mg of protein.

higher concentrations of [³H]ouabain in tissue preparations obtained from 6-hydroxydopamine-treated animals. Under these conditions the fraction of bound [³H]ouabain was less than 0.1%. Therefore we believe that only a single binding site for [³H]ouabain is present in the microsomal fractions of cat heart and nictitating membrane.

DISCUSSION

Cardiac glycosides are specific inhibitors of $(Na^+ + K^+)$-ATPase (1). An impressive body of indirect evidence points to a link between the inhibition of $(Na^+ + K^+)$-ATPase and the positive inotropic effect of the cardiac glycosides (26, 27). In spite of such numerous published correlations between $(Na^+ + K^+)$-ATPase inhibition and the positive inotropic effects of ouabain, some new evidence has been obtained indicating that enzyme inhibition may not be responsible for the inotropic actions of cardiotonic steroids (6–9).

The results shown in Table 1 indicate that a major fraction of $(Na^+ + K^+)$-ATPase found in the microsomal fractions of several peripheral organs of the cat appears to be localized at the sympathetic nerve endings, provided that 6-hydroxydopamine has no effects on different tissues other than destruction of sympathetic nerve endings. Since this drug is selectively accumulated at the catecholaminergic nerve endings by a specific high-affinity uptake system (23), and its intravenous administration leads to no morphological alteration other than loss of sympathetic nerve endings (14), it is unlikely that 6-hydroxydopamine has nonspecific effects on peripheral organs. Furthermore, regeneration of sympathetic innervation to heart and salivary glands following chemical sympathectomy leads to increases in specific [³H]ouabain binding to microsomal fractions of these organs.[2]

In heart microsomal fractions, over 80% of the total $(Na^+ + K^+)$-ATPase molecules were found to be localized at the sympathetic nerve endings (Table 3). Since release of myocardial norepinephrine plays no role in the pharmacological actions of cardiac glycosides on the heart (28), it is unlikely that $(Na^+ + K^+)$-ATPase found at the sympathetic nerve ending of the heart would participate in the inotropic action of ouabain. Therefore 80% of the $(Na^+ + K^+)$-ATPase present in the heart microsomal fraction cannot be considered as "pharmacological receptors" for cardiotonic steroids. This predominant distribution of $(Na^+ + K^+)$-ATPase at the sympathetic nerve endings of heart muscle may help to explain the inability of Rhee and associates (8) to observe inhibition of canine cardiac $(Na^+ + K^+)$-ATPase, using a dose regimen for ouabain that would allow only positive inotropy and maintain it for a long period of time. The dissociation constant for specific [³H]ouabain binding to the microsomal preparation derived from control cat heart is about 5 times higher than the value obtained with cardiac membranes of 6-hydroxydopamine-treated animals (Table 3). Therefore it is likely that administration of low concentrations of ouabain would inhibit primarily the $(Na^+ + K^+)$-ATPase of the heart muscle cells rather than the enzyme sys-

tem localized at the sympathetic nerve endings.

The physiological significance of remarkable concentrations of (Na^+ + K^+)-ATPase at the sympathetic nerve endings of different sympathetically innervated peripheral organs remains to be established. Nevertheless, it should be pointed out that the neurochemical activity of norepinephrine at the sympathetic nerve endings appears to be terminated by its reuptake into the nerve ending from which it was released (29). This norepinephrine transport system appears to be linked in an ill-defined manner to the (Na^+ + K^+)-ATPase localized in the plasma membrane of the sympathetic nerve endings (29–31). Again, there is some evidence that the cardiac arrhythmia induced by cardiotonic steroids is due, at least in part, to alterations in the activity of the sympathetic innervation of the heart (32–36). Therefore it is possible that the inhibition by ouabain of (Na^+ + K^+)-ATPase at the sympathetic nerve endings could cause inhibition of norepinephrine reuptake by these nerve terminals, and this may lead to alterations in the neuronal regulation of cardiac muscle. Thus the existence of high concentrations of (Na^+ + K^+)-ATPase with low affinity for ouabain at the sympathetic nerve endings may provide a biochemical basis for ouabain-induced cardiac arrhythmia.

The norepinephrine content and specific [^{3}H]ouabain binding in innervated and sympathetic nerve terminal denervated organs are shown in Table 4. The values for norepinephrine content were obtained from the references cited, and denervation of various organs was achieved by either chemical or surgical methods. The data for [^{3}H]ouabain binding are derived from present results (Table 1), where peripheral organs were denervated by 6-hydroxydopamine. Despite these differences, there is a remarkable correspondence between the percentage decrease in concentration of norepinephrine and specific [^{3}H]ouabain binding following sympathetic denervation in various tissues except kidney and spleen. The reason for differences in the results between kidney and spleen and other organs is not known. However, since the spleen and kidney are secretory organs, it is possible that (Na^+ + K^+)-ATPase may be involved in their secretory function.

In conclusion, a high percentage of total (Na^+ + K^+)-ATPase appears to be localized at the sympathetic nerve endings of many peripheral organs of the cat. The data presented here are consistent with the hypothesis that (Na^+ + K^+)-ATPase localized on the heart muscle cell may be, in part, the "pharmacological receptors" of cardiac glycosides and that inhibition of

TABLE 4

Specific [^{3}H]ouabain binding and norepinephrine content in innervated and denervated organs of cats and other species

The specific [^{3}H]ouabain binding was measured in the presence of 0.16 μM [^{3}H]ouabain as described in the text and Table 1. Unless stated otherwise, all results were obtained from cat tissues.

Organ	Norepinephrine content			Specific [^{3}H]ouabain binding		
	Innervated	Denervated	Decrease	Innervated	Denervated	Decrease
	μg/g		%	*pmoles/mg protein*		%
Salivary gland	1.4[a]	0.19[a]	86	364.0	49.0	86
Kidney	0.34[b]	0.08[b]	76	73.0	78.0	0
Heart	1.0,[a] 2.0[c]	–, 0.05[c]	97	25.6	11.0	57
Brain	0.22[a]	–		25.0	32.0	0
Spleen	1.4,[a] 4.28[c]	–, 0.135[c]	97	6.9	7.3	0
Nictitating membrane	9.31[c]	0.788[c]	92	5.0	0.6	88
Vas deferens	4.4,[d] 8.8[e]	1.66[e]	81	4.7	0.4	91

[a] From von Euler (37).

[b] From von Euler (37), derived from sheep kidney.

[c] From Thoenen and Tranzer (14).

[d] From Sjostrand (38).

[e] From Thoenen and Tranzer (14), derived from rat vas deferens.

$(Na^+ + K^+)$-ATPase on the sympathetic nerve endings may be indirectly involved in the development of cardiac arrhythmias by decreasing the reuptake of norepinephrine at the presynaptic sites.

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