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Mercury Binding Site on Na^+/K^+-ATPase: A Cysteine in the First Transmembrane Segment

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SUMMARY

Mercury is an element of great pharmacological and toxicological importance. It reacts with sulfhydryl groups on proteins to form mercaptides. Mercuric mercury (Hg^{2+}), a form that shows primarily epithelial toxicity, can inhibit Na^+/K^+-ATPase at low concentration, but its molecular target site on the protein is not known. To investigate the interaction of Hg^{2+} with Na^+/K^+-ATPase, we studied the inhibition of Na^+/K^+ pump activity by inorganic mercury ($HgCl_2$) in *Xenopus laevis* oocytes expressing wild-type and mutant forms of Na^+/K^+-ATPase. Na^+/K^+ pump potassium-activated current was inhibited with first-order kinetics ($K_{on} = 7*10^3$ $M^{-1}\cdot sec^{-1}$) and an estimated K_d of ≤170 nM. To study the hypothesis that the cysteine (C113) of the first transmembrane segment of the α subunit participates in a Hg^{2+} binding site, we investigated the inhibition of Na^+/K^+ pump activity produced by a 1-min exposure to 5 μM $HgCl_2$. Wild-type and C113S and C113Y mutant Na^+/K^+ pumps were inhibited by 43 ± 7%, 12 ± 2%, and 5 ± 3%, respectively. Because C113 is a component of the cardiac steroid binding site, we studied the interaction of mercury with strophanthidin by exposing oocytes for 2 min to 5 μM $HgCl_2$ in the presence or absence of 50 μM strophanthidin. Strophanthidin reduced the inhibition by mercury from 68 ± 5% to 30 ± 7%. Based on the position of C113 in the first transmembrane segment, these results suggest that Hg^{2+} binding to C113 from the extracellular side is one of the mechanisms by which mercury inhibits Na^+/K^+-ATPase.

Na^+/K^+-ATPase (EC 3.6.1.37) is an ubiquitous membrane protein that is responsible for the transport of Na^+ and K^+ across the plasma membrane against their electrochemical gradient (1). The Na^+ and K^+ gradients established by Na^+/K^+-ATPase are essential for regulation of cell volume, membrane potential, and secondary active transport. The minimal functional structure of the pump consists of an α and a β subunit (2). The cardiac steroid compounds (e.g., ouabain, strophanthidin, digoxin) are specific inhibitors of Na^+/K^+-ATPase. These compounds bind to the extracellular part of the α subunit (1, 3). Recently, many residues and segments that affect the ouabain affinity to the pump have been identified (4–6), but the structure of the ouabain binding site remains unclear.

Mercury exists in several chemical forms (i.e., metallic, organic, or inorganic) that display a large range of toxic effects. The toxicity of mercuric mercury, Hg^{2+}, mostly concerns epithelial cells; although acute ingestion of $HgCl_2$ causes corrosive ulceration, bleeding diarrhea, necrosis of the gastrointestinal tract mucosa, and acute renal tubular necrosis, chronic exposure of $HgCl_2$ results mainly in kidney damage. Among other proteins present in the membrane of epithelial cells, mercuric mercury is known to inhibit Na^+/K^+-ATPase.

Mercury is a sulfhydryl reagent, and its main biological mechanism of action is to bind to exposed cysteine residues on proteins (7). Earlier experiments (see review in Ref. 8) have shown that in purified Na^+/K^+-ATPase, many sulfhydryl groups can be titrated by mercurials. The number of accessible sites was estimated to be 24 sites/α2β2 complex. Titration of slightly more than half of these sites led to a state with considerable modification of the enzymatic properties; Na^+/K^+-ATPase activity was abolished, but some partial reactions, such as Na-ATPase or K-NPPase activity, were preserved. Titration of additional sites abolished all ATPase activity. Later *in vitro* experiments on purified Na^+/K^+-ATPase reconstituted into liposomes showed that mercuric mercury inhibited Na^+/K^+-ATPase (9, 10), and this effect was proposed to occur primarily as a result of action on a cytosolic site (11).

Recent work with the *Xenopus laevis* (12) or sheep (6) Na^+/K^+ pump has shown that a cysteine residue in the first transmembrane segment of the α subunit of the *X. laevis* Na^+/K^+ pump plays an important role in the interaction between Na^+/K^+ pump and cardiac glycosides (12). Substitution of this cysteine, C113 (according to the *X. laevis* α1 sequence numbering; Refs. 12 and 13), with tyrosine (C113Y)

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ABBREVIATIONS: I_{pump}, K-activated Na^+/K^+ pump current; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

or phenylalanine (C113F) confers ouabain resistance (K_i >1000-fold larger than wild-type), but substitution by serine (C113S) does not change ouabain affinity. In addition, labeling experiments using the cysteine-reactive cardiac steroid derivative *N*-hydroxysuccinimidyl digoxigenin-3-methylcarbonyl-*E*-aminocaproate demonstrated covalent labeling in wild-type Na^+/K^+ pump but not in C113 mutants (14). These results imply that this cysteine, although located inside the membrane according to the present topological model, is accessible from the extracellular side.

To more precisely define the molecular site of action of Hg^{2+} on Na^+/K^+-ATPase, we studied the effect of $HgCl_2$ on the functions of wild-type and C113 mutant *X. laevis* Na^+/K^+ pumps expressed in *X. laevis* oocytes and the interaction between mercury and cardiac steroids.

Materials and Methods

Expression of wild-type and site mutant Na^+/K^+ pumps in *X. laevis* oocytes. Stage V–VI *X. laevis* oocytes were obtained and prepared as described previously (15). Seven nanograms of Na^+/K^+-ATPase cRNA encoding wild-type or mutants α1 *X. laevis* subunit was coinjected with 1 ng of β subunit cRNA in a volume of 50 nl (13). In experiments using *Bufo* Na^+/K^+-ATPase, 7 ng of α subunit cRNA and 4 ng of β1 subunit cRNA were injected (16). Three to 5 days later, the oocytes were loaded with Na^+ by a 2-hr exposure to a K^+-free solution before measurement (15).

Electrophysiological measurements. The oocytes were studied by the two-electrode voltage-clamp technique with a TEV-200 Voltage Clamp (Dagan, Minneapolis, MN). The fluid volume in the chamber was ~120 μl, and the fluid exchange rate was 4 ml/min, resulting in a fluid exchange time of a few seconds. The current signal was filtered at 20 Hz and recorded on a Gould chart recorder (model 220, Gould, Cleveland, OH). The intracellular potential was held at −50 mV. For all experiments, the oocytes were kept in the control solution until the base-line current was stable, and I_{pump}, defined as the current activated by 10 mM K^+, was measured. This value reflects the maximal activity of the Na^+/K^+ pump and is an index of the number of the pump units on the oocyte plasma membrane (17).

Drugs and solutions. All of the drugs and chemicals, including ouabain, strophanthidin, and $HgCl_2$, were purchased from Sigma Chemical (St. Louis, MO). The control solution for electrophysiological measurements contained 92.4 mM Na^+, 0.82 mM Mg^{2+}, 5 mM Ba^{2+}, 0.41 mM Ca^{2+}, 10 mM tetraethylammonium, 22.46 mM Cl^-, 2.4 mM HCO_3^-, 80 mM gluconate, and 10 mM HEPES. The different concentrations of Hg^{2+} were obtained by adding an appropriate quantity of a 10 mM $HgCl_2$ stock solution to the control solution. Strophanthidin was added from a stock solution of 50 mM in dimethylsulfoxide. All experiments were performed at room temperature.

Data are presented as mean ± standard error. Statistical significance of the difference between mean values was evaluated by the Student's *t* test for unpaired data.

Results

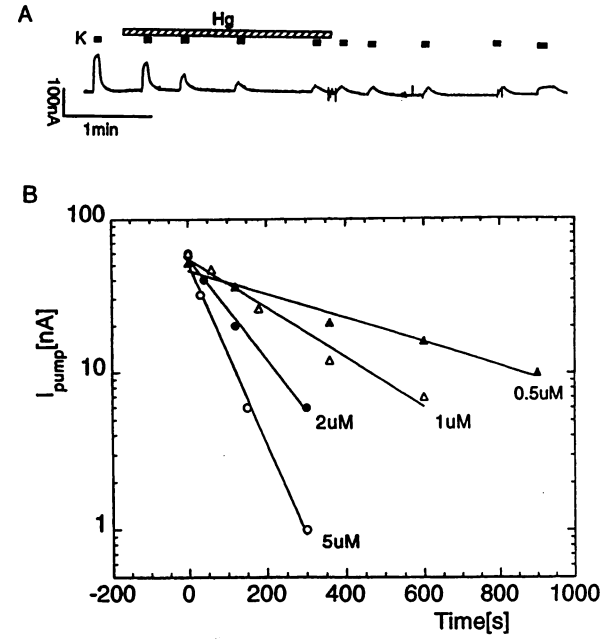
Expression of the Na^+/K^+ pump in oocytes. The endogenous Na^+/K^+-ATPase of the *X. laevis* oocytes, measured in noninjected oocytes, usually gives an I_{pump} of 40–100 nA (15, 18). In these experiments, I_{pump} was 58 ± 1 nA (24 measurements) in noninjected oocytes. Oocytes expressing *X. laevis* wild-type or C113S mutant α and β Na^+/K^+-ATPase produced an ~4-fold higher current: 238 ± 14 nA (12 measurements) and 239 ± 34 nA (5 measurements), respectively. These activities represent the sum of the endogenous and exogenous Na^+/K^+ pump populations. Because the C113Y mutant is resistant to ouabain (K_i = 250 μM; Ref. 12), we took advantage of this fact and incubated the oocytes for 2 hr in a solution containing a low concentration of ouabain (0.2 μM) to inhibit the endogenous Na^+/K^+ pump. In this case, I_{pump} (80 ± 6 nA; five measurements) essentially reflected the activity of the C113Y mutant Na^+/K^+ pump.

Kinetics of Na^+/K^+ pump inhibition by mercuric mercury. The kinetics of Na^+/K^+ pump inhibition by Hg^{2+} were first studied on the endogenous Na^+/K^+ pump of noninjected oocytes. I_{pump} was measured repeatedly before and during exposure to various concentrations of Hg^{2+} (Fig. 1A). After a 10-min exposure to Hg^{2+} or a stable >90% inhibition, mercury was removed and the recovery was assessed by repeated measurements of I_{pump}. Fitting a single exponential function to the I_{pump} values versus time yielded the time constant of the onset of inhibition [τ (in sec)] for each Hg^{2+} concentration. We tested the Hg^{2+} concentrations of 0.5–5 μM, and τ ranged from 242 sec for 0.5 μM to 35 sec for 5 μM (Fig. 1B). Within a time frame of 10 min, the recovery from inhibition was <5%, indicating that the time constant of dissociation was much slower that the time constant of the association at a micromolar concentration of Hg^{2+}. This indicates that the association rate constant can be estimated by $K_{on} = 1/(\tau*[Hg^{2+}])$, and we obtained a mean value for K_{on} of $6.8 \pm 0.4*10^3$ $M^{-1}\cdot sec^{-1}$ (24 measurements). Because of the slow dissociation kinetics, it was not possible to obtain a direct measurement of the affinity in our system. However, from a lower limit of the dissociation rate constant (K_{off} = <0.1 min^{-1}) and the association rate constant ($K_{on} = 6.8*10^3$ $M^{-1}\cdot sec^{-1}$), we could obtain an upper limit for the affinity constant: K_d = <0.24 μM. The K_d of Hg^{2+} can also be estimated from the plot of the onset rate of inhibition ($k = 1/\tau$) versus mercury concentration (Fig. 1C); the slope of this plot corresponds to K_{on}, and the intercept yields K_{off}. From these two values, for $K_d = K_{off}/K_{on}$ we obtained an estimate of 0.170 μM, a value similar to one previously reported (19).

To determine the effect of Hg^{2+} on different conformations of the Na^+/K^+ pump, we measured the inhibition kinetics of 5 μM Hg^{2+} in the presence or absence of 10 mM K^+ (Fig. 2). In the presence of 10 mM K^+, τ = 40 ± 2 sec (nine measurements), which was slightly higher than that in the absence of K^+ (35 ± 3 sec; seven measurements), but the difference was not significant ($p > 0.10$).

We also measured the inhibition by 5 μM Hg^{2+} of the I_{pump} in oocytes expressing the *Bufo* Na^+/K^+-ATPase after inhibition of the endogenous Na^+/K^+ pump with 0.2 μM ouabain. The association time constant (τ) was 50.9 ± 7.7 sec (five measurements), a value similar to that measured in oocytes expressing the *X. laevis* Na^+/K^+ pump.

Effect of C113 substitution. To examine the possibility that C113 was implicated in the effect of Hg^{2+}, we studied $HgCl_2$ inhibition of wild-type and C113 mutant Na^+/K^+ pumps. We examined the inhibition of the Na^+/K^+ pump current produced by a 1-min exposure to 5 μM $HgCl_2$ (Fig. 3). In oocytes expressing wild-type Na^+/K^+ pump, $HgCl_2$ produced a 56 ± 5% inhibition (seven measurements). In the C113Y group (oocytes expressing only the C113Y Na^+/K^+ mutant pump; the endogenous pump had been inhibited by exposure to a low dose of ouabain), there was no significant inhibition: 5 ± 3%; seven measurements. In the C113S group, the inhibition was 35 ± 1% (seven measurements), a



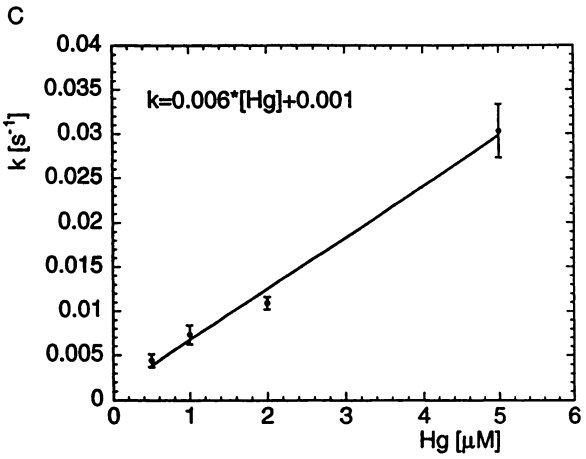


Fig. 1. Kinetics of Hg^{2+} inhibition of the Na^+/K^+ pump current. A, Current tracing of an oocyte voltage-clamped at −50 mV showing the inhibition of I_{pump} (outward current activated by 10 mM K^+, ■) by 1 μM Hg^{2+} (▨). After maximal inhibition was observed, the oocyte was kept in the control solution for 5 min to allow assessment of recovery of the Na^+/K^+ pump activity. B, Exponential decrease in I_{pump} with different concentrations of Hg^{2+} as a function of time. *Straight lines*, best-fitting exponential function $I_{pump} = I_{pump}0*\exp(-t/\tau)$, where $I_{pump}0$ is the value of I_{pump} before Hg^{2+} exposure. C, Plot of the rate of inhibition ($k = 1/\tau$; in sec^{-1}) versus Hg^{2+} concentration. The slope of the regression line yields an estimate of the association rate constant, K_{on}, and the intercept yields an estimate of the dissociation rate constant, K_{off}, according to first-order binding kinetics: $1/\tau = K_{on}*[Hg^{2+}] + K_{off}$.

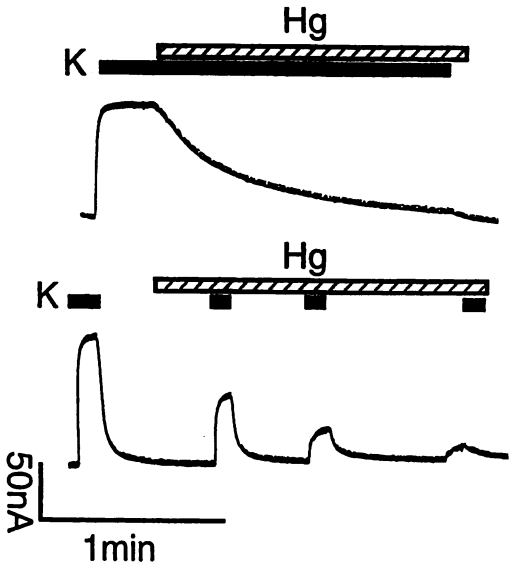


Fig. 2. Inhibition of Na^+/K^+ pump by Hg^{2+} in the presence or absence of external K^+. Original recordings show the time course of inhibition of K^+-activated current by 5 μM Hg^{2+} (▨) when 10 mM K^+ (■) was continuously present (*top*) or added intermittently (*bottom*) to measure Na^+/K^+ pump activity.

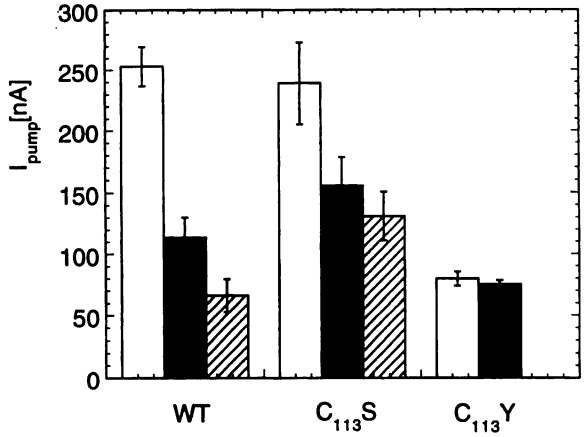


Fig. 3. Inhibition of wild-type and C113 mutant forms of Na^+/K^+-ATPase by Hg^{2+}. Mean I_{pump} measured before (□) and after (■) a 1-min exposure to 5 μM Hg^{2+} in wild-type (*WT*; seven measurements) and C113S (seven measurements) and C113Y (seven measurements) mutants. ▨, Effect of a second 1-min exposure to 5 μM Hg^{2+} for the wild-type and C113S groups. The oocytes of the C113Y group had been previously exposed to 0.2 μM ouabain to inhibit the endogenous *X. laevis* Na^+/K^+ pump. In each case, I_{pump} was measured twice before Hg^{2+} application to be sure there was no time-dependent change in I_{pump} within the time frame of our experiments.

value significantly lower ($p < 0.005$) than that observed in the wild-type group. The partial inhibition observed in the C113S group could be accounted for, at least in part, by the presence of the endogenous wild-type Na^+/K^+ pump. We attempted to estimate the real inhibition of the C113S mutant by subtracting the expected activity of the endogenous component [i.e., the mean I_{pump} measured in noninjected oocytes before (58 nA) and after (25 nA) exposure to $HgCl_2$]. This calculation resulted in an estimated inhibition of 27 ± 2% (seven measurements) for the C113S mutant. We also performed a second exposure of 1 min to 5 μM $HgCl_2$. After the first exposure to Hg^{2+}, we expected the fraction of C113S pump in the total pump population to increase because of selective inhibition of the wild-type endogenous Na^+/K^+ pump. The second exposure indeed resulted in lesser inhibition (12 ± 2%; seven measurements) in the C113S group ($p < 0.001$ compared with the first exposure to 5 μM Hg^{2+}), whereas in the wild-type group, the inhibition was 43 ± 7% (seven measurements) after the second exposure, a value similar to that measured during the first exposure (see Fig. 3).

Effect of strophanthidin on Hg^{2+} inhibition. To examine the relationship between cardiac steroid binding and

inhibition by $HgCl_2$, we studied the effect of preexposure to strophanthidin on the inhibitory effect of $HgCl_2$ in oocytes injected with the wild-type *X. laevis* α and β subunit cRNAs. We used strophanthidin because of its relatively fast dissociation rate constant (12). We first confirmed that strophanthidin produced a reversible inhibition of the *X. laevis* Na^+/K^+ pump. No K-activated current could be detected in the presence of 50 μM strophanthidin, but I_{pump} recovered to 79 ± 2% of its initial value after a 10-min rinse period. We then exposed a group of oocytes to 5 μM $HgCl_2$ for 2 min (Fig. 4A) and a second group to 50 μM strophanthidin for 20 sec and then to 5 μM $HgCl_2$ plus 50 μM strophanthidin for 2 min (Fig. 4B). The oocytes were allowed to recover for 10 min in the control solution, and I_{pump} was measured again (Fig. 4C). The presence of strophanthidin reduced the inhibition due to a 2-min exposure to 5 μM Hg^{2+} from 68 ± 5% (five measurements) to 30 ± 7% (five measurements) ($p < 0.005$).

Discussion

Our results demonstrate that extracellular exposure to Hg^{2+} in the micromolar range inhibits the Na^+/K^+ pump activity. This inhibition was irreversible within the time course of our experiments. The onset of the Hg^{2+} inhibition of the Na^+/K^+ pump current was well described by first-order binding kinetics, with K_{on} = ~6800 $M^{-1} \cdot sec^{-1}$. These results are compatible with the hypothesis that mercury acts on a site that is directly accessible from the external solution.

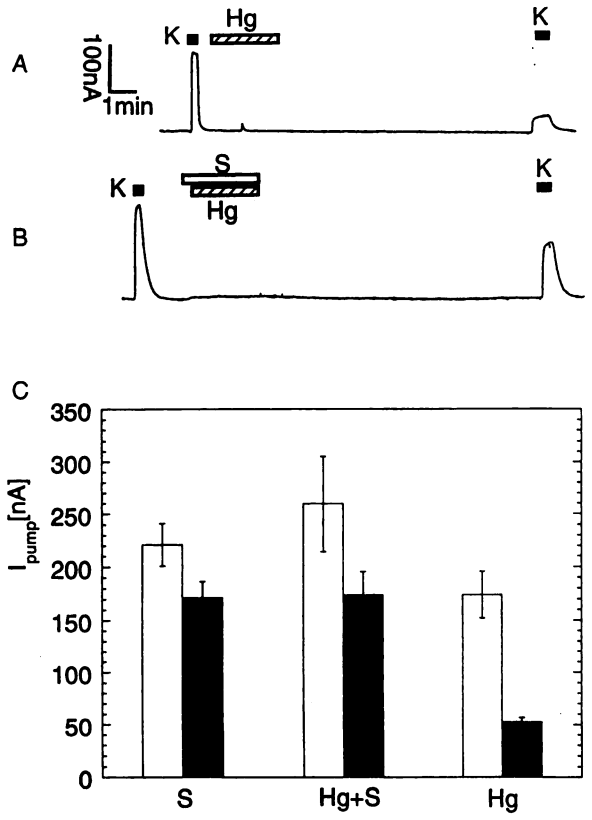


Fig. 4. Effect of strophanthidin on Na^+/K^+ pump inhibition by Hg^{2+}. A, Original current recordings show the effect of a 2-min exposure to 5 μM Hg^{2+} (▨) on I_{pump} measured after a 10-min recovery period. B, Effect of a similar exposure to Hg^{2+} in the presence of 50 μM strophanthidin (□). Strophanthidin was first applied alone for 20 sec; then, Hg^{2+} was added. ■, Addition of 10 mM K^+. C, Mean Na^+/K^+ pump current observed after exposure to 50 μM strophanthidin and a 10-min recovery time showing an 80% recovery of activity (*S*). After a similar exposure to strophanthidin followed by a 2-min exposure to 5 μM Hg^{2+} and strophanthidin (*Hg + S*) (in the presence of strophanthidin), recovery occurred to ~70% of initial activity. When the oocytes were exposed to 5 μM Hg^{2+} alone, in the absence of strophanthidin (*Hg*), the current recovered by only ~30%.

Hg^{2+} readily reacts with exposed cysteines on proteins. Among the total of 23 cysteines on the α subunit, 16 are in the cytoplasmic domain, and none are in the extracellular loops. There is one cysteine in each of the seven transmembrane domains: H1, H2, H4, H6, H8, H9, and H10. We knew that the cysteine in the first transmembrane segment (C113 in *X. laevis* α1) is accessible from the extracellular side of the membrane because it can be labeled by the membrane-impermeant digoxigenin derivative *N*-hydroxysuccinimidyl digoxigenin-3-methylcarbonyl-*E*-aminocaproate (14). We wanted to determine whether C113 was also accessible to Hg^{2+} and whether binding to C113 was a mechanism of Na^+/K^+ pump inhibition by mercury.

Our results show that two mutants in which C113 was replaced by either tyrosine or serine showed a decreased sensitivity to the effect of Hg^{2+}. These observations may be explained in two ways: either C113 is the binding site for Hg^{2+}, or C113 mutants are less sensitive to mercury because the mutation induces a conformational change that decreases the accessibility of another cysteine. The C113Y mutation induces a high resistance to ouabain, which may indicate an important change in the conformation of the protein. However, the C113S mutant shows no detectable functional alteration compared with the wild-type Na^+/K^+ pump (12). In addition, the inhibition kinetics of Hg^{2+} are not significantly altered by the presence or the absence of external K^+. This indicates that the two main conformations of the Na^+/K^+ pump, E1 and E2, are more or less equally sensitive to Hg^{2+}. Taken together, these observations support the conclusion that the lower sensitivity to Hg^{2+} inhibition of the C113 mutants is due to the removal of the site of direct interaction between Hg^{2+} and the α subunit rather than to a conformational change induced by the mutation. Therefore, these results strongly suggest that C113 is one of the mercury reactive sites on the Na^+/K^+ pump. It certainly is not the only one; other experiments have shown that several sulfhydryl group can be titrated by mercurial compounds in the isolated enzyme (8) and that inhibition by $HgCl_2$ can occur by interaction with the intracellular part of the enzyme (11). The rather large difference in the inhibition rate between wild-type and C113 mutants shows that C113 is part of the mercury binding site that seems to be most readily accessible from the external side of the membrane. Other mercury binding sites may have an even higher affinity than the C113 site but may not have been detected in our experiments because of the sidedness and the short duration of exposure to $HgCl_2$.

Using purified Na^+/K^+-ATPase reconstituted into liposomes, Anner and Moosmayer (11) observed that $HgCl_2$ inhibited Na^+/K^+-ATPase primarily by interaction with the cytoplasmic side of the protein. There are large differences in the technical approaches, which may account for this apparent divergence. One of the differences is the very large difference in surface-to-volume ratio between oocytes and liposomes; because of the large volume and comparatively small surface area of the *X. laevis* oocyte, it is probable that during

the short exposure time, the intracellular concentration of $HgCl_2$ did not increase to biologically relevant concentrations. In addition, the protein-rich oocyte cytoplasm might offer many mercury acceptor sites other than those present on Na^+/K^+-ATPase. In contrast, the concentration of $HgCl_2$ in the extremely small intravesicular volume of the liposomes might have rapidly approached that in the external solution.

As shown in Table 1, we confirm the results of Anner *et al.* (10) with regard to the relationship between sensitivities to ouabain and Hg^{2+}. The sensitivity to Hg^{2+} was not dependent on the sensitivity to ouabain but rather on the presence of a cysteine in position 113 (C111 for *Bufo* α1 Na^+/K^+-ATPase; Ref. 16).

Experiments with strophanthidin showed that occupancy of the cardiac steroid binding site decreases the sensitivity to Hg^{2+}. Thus, C113 is less accessible when the cardiac steroid binding site is occupied. Because it is known that cardiac steroids such as strophanthidin act on the pump from the extracellular side, the protective effect of strophanthidin on the Hg^{2+} inhibition also supports an action of Hg^{2+} from the extracellular side of the membrane. A simple explanation of these results could be that strophanthidin blocks the access pathway of Hg^{2+} ions to C113, which (on the basis of topological models) is located inside the membrane. Another explanation could be that strophanthidin stabilizes a conformation in which C113 is not accessible from the external solution. This explanation is unlikely because strophanthidin stabilizes the E2 conformation, which seems to be at least as sensitive as the E1 conformation, according to the results obtained with and without 10 mM K^+ in the external solution.

Mercurial compounds have also been used as diuretics, and it is known that, in general, organomercury compounds undergo cleavage of the carbon-mercury bond and release ionic inorganic mercury (20), so the actual effector is likely to be Hg^{2+}. The action of mercurial diuretics on renal function includes inhibition of the reabsorption of water, sodium, and chloride and depression of the secretion of potassium (20). The high affinity inhibition of Na^+/K^+-ATPase by Hg^{2+} would be compatible with the hypothesis that the altered Na/K transport in kidney by mercurial diuretics may be due to inhibition of Na^+/K^+-ATPase. However, results of experiments that compared Na^+/K^+-ATPase inhibition by diuretic and nondiuretic mercurials indicate that inhibition of Na^+/K^+-ATPase probably has no direct role in the inhibition of tubular sodium transport by mercurial diuretics (21).

TABLE 1

Relationship between ouabain-resistant phenotype, presence of a cysteine in position 113, and sensitivity to Hg^{2+}

	Ouabain-sensitive forms	Ouabain-resistant forms
Cysteine in position 113	*Xenopus* wild-type	*Bufo* wild-type
K_d (ouabain) (μM)	<0.1[c]	50[d]
τ (sec)[a]	35.0 ± 3.5 (7)	50.9 ± 7.7 (5)
No Cysteine in position 113	*Xenopus* C113S	*Xenopus* C113Y
K_d (ouabain) (μM)	<0.1[c]	250[c]
τ (sec)[b]	529 ± 91 (7)	>1000

[a] τ values were measured directly by exponential fitting to the current decrease during exposure to 5 μM Hg^{2+} in *Xenopus* and *Bufo* wild-type groups.
[b] For the C113S and C113Y mutants, τ was estimated from the inhibition produced by a 2-min exposure to 5 μM Hg^{2+}.
[c] Ref. 12.
[d] Ref. 16.

Acknowledgments

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References

1. Lingrel, J. B., and T. Kuntzweiler. Na^+,K^+-ATPase. *J. Biol. Chem.* **269:** 19659-19662 (1994).
2. Ackermann, U., and K. Geering. Mutual dependence of Na, K-ATPase α- and β-subunits for correct posttranslational processing and intracellular transport. *FEBS Lett.* **269:**105-108 (1990).
3. Horisberger, J.-D., V. Lemas, J.-P. Kraehenbühl, and B. C. Rossier. Structure-function relationship of Na, K-ATPase. *Annu. Rev. Physiol.* **53:**565-584 (1991).
4. Kirley, T. L., and M. Peng. Identification of cysteine residues in lamb kidney (Na, K)-ATPase essential for ouabain binding. *J. Biol. Chem.* **266:** 19953-19957 (1991).
5. Price, E. M., and J. B. Lingrel. Structure-function relationships in the Na, K-ATPase *alpha* subunit: site-directed mutagenesis of glutamine-111 to arginine and asparagine-122 to aspartic acid generates a ouabain-resistant enzyme. *Biochemistry* **27:**8400-8408 (1988).
6. Schultheis, P. J., and J. B. Lingrel. Substitution of transmembrane residues with hydrogen-bonding potential in the α subunit of Na, K-ATPase reveals alterations in ouabain sensitivity. *Biochemistry* **32:**544-550 (1993).
7. Lau, S., and B. Sarkar. Inorganic mercury(II)-binding components in normal human blood serum. *J. Toxicol. Environ. Health* **5:**907-916 (1979).
8. Askari, A., W. Huang, and G. R. Henderson. Na, K-ATPase: functional and structural modifications induced by mercurials, in Na, K-ATPase, in *Structure and Kinetics* (J. C. Skou and J. G. Norby, eds.). Academic Press, New York, 205–215 (1979).
9. Anner, B. M., M. Moosmayer, and E. Imesch. Chelation of mercury by ouabain-sensitive and ouabain-resistant renal Na, K-ATPase. *Biochem. Biophys. Res. Commun.* **167:**1115-1121 (1990).
10. Anner, B. M., M. Moosmayer, and E. Imesch. Mercury blocks Na-K-ATPase by a ligand-dependent and reversible mechanism. *Am. J. Physiol.* **262:**F830–F836 (1992).
11. Anner, B. M., and M. Moosmayer. Mercury inhibits Na-K-ATPase primarily at the cytoplasmic side. *Am. J. Physiol.* **262:**F843–F848 (1992).
12. Canessa, C. M., J.-D. Horisberger, D. Louvard, and B. C. Rossier. Mutation of a cysteine in the first transmembrane segment of Na-K-ATPase α subunit confers ouabain resistance. *EMBO J.* **11:**1681-1687 (1992).
13. Verrey, F., P. Kairouz, E. Schaerer, P. Fuentes, K. Geering, B. C. Rossier, and J.-P. Kraehenbühl. Primary sequence of *Xenopus laevis* Na^+-K^+-ATPase and its localization in A6 kidney cells. *Am. J. Physiol.* **256:**F1034–F1043 (1989).
14. Antolovic, R., W. Schoner, K. Geering, B. C. Rossier, and J.-D. Horisberger. Labeling of a cysteine in the cardiotonic glycoside binding site by the steroid derivative HDMA. *FEBS Lett.* **368:**169-172 (1995).
15. Jaisser, F., P. Jaunin, K. Geering, B. C. Rossier, and J.-D. Horisberger. Modulation of the Na, K-pump function by β subunit isoforms. *J. Gen. Physiol.* **103:**605-623 (1994).
16. Jaisser, F., J.-D. Horisberger, and B. C. Rossier. Primary sequence and functional expression of a novel ouabain-resistant Na, K-ATPase. *J. Biol. Chem.* **267:**16895-16903 (1992).
17. Jaunin, P., J.-D. Horisberger, K. Richter, P. J. Good, B. C. Rossier, and K. Geering. Processing, intracellular transport and functional expression of endogenous and exogenous α-β3 Na, K-ATPase complexes in *Xenopus* oocytes. *J. Biol. Chem.* **267:**577-585 (1992).
18. Horisberger, J.-D., P. Jaunin, P. J. Good, B. C. Rossier, and K. Geering. Coexpression of α1 with putative β3 subunits results in functional Na-K-pumps in *Xenopus* oocyte. *Proc. Natl. Acad. Sci. USA* **88:**8397-8400 (1991).
19. Imesch, E., M. Moosmayer, and B. M. Anner. Mercury weakens membrane anchoring of Na-K-ATPase. *Am. J. Physiol.* **262:**F837–F842 (1992).
20. Goyer, R. A. Toxic effects of metals, in *Casarett and Doull's Toxicology: The Basic Science of Poisons* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.). Pergamon Press, New York, 646–651 (1991).
21. Nechay, B. R. Relationship between inhibition of renal $Na^+ + K^+$-ATPase and natriuresis. *Ann. N. Y. Acad. Sci.* **242:**501-518 (1974).

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