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Sulfhydryl-Reactive Heavy Metals Increase Cell Membrane K^+ and Ca^{2+} Transport in Renal Proximal Tubule

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Summary. The cellular mechanisms by which nephrotoxic heavy metals injure the proximal tubule are incompletely defined. We used extracellular electrodes to measure the early effects of heavy metals and other sulfhydryl reagents on net K^+ and Ca^{2+} transport and respiration (QO_2) of proximal tubule suspensions. Hg^{2+}, Cu^{2+}, and Au^{3+} (10^{-4} M) each caused a rapid net K^+ efflux and a delayed inhibition of QO_2. The Hg^{2+}-induced net K^+ release represented passive K^+ transport and was not inhibited by barium, tetraethylammonium, or furosemide. Both Hg^{2+} and Ag^+ promoted a net Ca^{2+} uptake that was nearly coincident with the onset of the net K^+ efflux. A delayed inhibition of ouabain-sensitive QO_2 and nystatin-stimulated QO_2, indicative of Na^+, K^+-ATPase inhibition, was observed after 30 sec of exposure to Hg^{2+}. More prolonged treatment (2 min) of the tubules with Hg^{2+} resulted in a 40% reduction in the CCCP-uncoupled QO_2, indicating delayed injury to the mitochondria. The net K^+ efflux was mimicked by the sulfhydryl reagents pCMBS and N-ethylmaleimide (10^{-4} M) and prevented by dithiothreitol (DTT) or reduced glutathione (GSH) (10^{-4} M). In addition, both DTT and GSH immediately reversed the Ag^+-induced net Ca^{2+} uptake. Thus, sulfhydryl-reactive heavy metals cause rapid, dramatic changes in the membrane ionic permeability of the proximal tubule before disrupting Na^+, K^+-ATPase activity or mitochondrial function. These alterations appear to be the result of an interaction of the metal ions with sulfhydryl groups of cell membrane proteins responsible for the modulation of cation permeability.

Key Words mercury · Ca^{2+} transport · K^+ transport · sulfhydryl groups · heavy metals · rabbit

Introduction

Sulfhydryl (SH)-reactive heavy metals and reagents are known to alter the permeability of many biological membranes. Specific effects of these compounds on cation transport have been demonstrated in a variety of cell types (Passow, Rothstein & Clarkson, 1961; Knauf & Rothstein, 1971; Klyce & Marshall, 1982; Van Driessche, 1987; Lauf, 1988), and have been thought to contribute to their cytotoxicity (Passow et al., 1961; Klyce & Marshall, 1982). These agents are particularly toxic to the kidney, and have long been used experimentally to produce acute renal failure. Despite numerous investigations, however, the initial cellular mechanisms leading to renal tubule cell injury remain poorly understood. Previous studies of heavy metal-induced nephropathy have identified the proximal tubule as the principal site of injury (Gritzka & Trump, 1968; Zalme et al., 1976; Reugg et al., 1987), and have suggested mitochrondrial dysfunction to be the primary mechanism of cell damage (Weinberg, Harding & Humes, 1982). However, the contribution of initial cell membrane lesions to the evolution of proximal tubule injury from heavy metals remains unknown.

Recently, we reported that Ag^+, a nephrotoxic heavy metal, dramatically increased the K^+ and Na^+ permeabilities of the proximal tubule in vitro at concentrations that did not disrupt Na^+, K^+-ATPase or mitochondrial function (Kone, Kaleta & Gullans, 1988). These permeability disturbances, manifested as a rapid net K^+ efflux and an increase in ouabain-sensitive oxygen consumption (QO_2), were rapidly reversed by the thiol reagents dithiothreitol (DTT) and reduced glutathione (GSH). These results suggested that the changes in K^+ and Na^+ transport were mediated by a reversible interaction of Ag^+ with SH groups of cell membrane proteins responsible for the modulation of cation permeability. The present study was designed to characterize the early cytotoxic effects of other heavy metals, including the classic nephrotoxin mercury (Hg^{2+}), on the proximal tubule in vitro, and to examine in further detail the effects of SH-reactive compounds on the ion transport properties of this nephron segment.

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Table 1. Experimental solutions

	I Control	II HCO_3^- free	III Cl^- free
NaCl	115	115	0
$NaHCO_3$	25	0	25
KCl	5	5	0
Na_2HPO_4	1.6	1.6	1.6
NaH_2PO_4	0.4	0.4	0.4
$CaCl_2$	1.2	1.2	0
$MgSO_4$	1.0	1.0	1.0
Na HEPES	0	32	0
Na gluconate	0	0	115
K gluconate	0	0	5
Ca gluconate	0	0	6.7[a]
D-glucose	5	5	5
Na lactate	4	4	4
L-alanine	1	1	1
O_2	95%	100%	95%
CO_2	5%	0%	5%
pH	7.40	7.40	7.40

[a] Additional Ca^{2+} was used to maintain a constant ionized Ca^{2+} concentration.

To address specifically these issues, we used extracellular electrodes to probe the acute, in vitro effects of several heavy metals and SH reagents on the major ion transport and metabolic functions of the proximal tubule. Net K^+ fluxes were measured as an index of passive K^+ transport, Na^+, K^+-ATPase activity, and cell membrane integrity, whereas measurements of oxygen consumption (QO_2) provided an index of Na^+ transport and oxidative metabolism. Since Ag^+ has been shown to alter the Ca^{2+} permeability of the cell membrane (Van Driessche, 1987) and organellar membranes (Abramson et al., 1983), and since altered cell Ca^{2+} homeostasis is thought to be a mechanism of toxic cell injury (Humes & Weinberg, 1986; Smith et al., 1987), we also used a high-resolution extracellular Ca^{2+} electrode to measure net Ca^{2+} fluxes in response to Ag^+ and Hg^{2+}. These continuous, parallel measurements provided a direct assessment of multiple, essential cell functions and permitted the temporal distinction of cytotoxic responses. The results indicated that specific heavy metals promote rapid fluxes of K^+ and Ca^{2+} through passive permeability pathways in the proximal tubule and that these changes precede mitochondrial injury. These responses appear to be mediated, in part, by a reaction of these agents with SH groups of cell membrane proteins, and likely represent initial mechanisms of proximal tubule injury common to SH-reactive heavy metals.

Materials and Methods

Preparation of Proximal Tubules

Suspensions enriched in proximal tubule fragments were prepared from female New Zealand White rabbits by in situ collagenase perfusion as previously described (Kone et al., 1988). The final three washes were performed in the appropriate experimental buffer solution and the tubules were suspended at a concentration of 4–8 mg protein/ml (~2–4% cytocrit). The control buffer was a bicarbonate Ringer's solution (solution I, Table 1). Buffers nominally free of either Cl^- or HCO_3^- were prepared by substituting gluconate$^-$ for Cl^-, (solution II, Table 1) or by substituting N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer for HCO_3^- (solution III, Table 1). For experiments using barium chloride, magnesium chloride was substituted for magnesium sulfate in equimolar concentrations to prevent the precipitation of barium sulfate. Prior to ion transport or QO_2 measurements, 2.5-ml aliquots of tubules suspended in the appropriate experimental buffer were placed in capped polycarbonate flasks under an atmosphere of 95% O_2/5% CO_2 or 100% O_2, as appropriate, and incubated at 37°C for 30 min. This preincubation allowed the intracellular contents of ions and ATP to achieve steady-state levels.

Measurement of Net K^+ Fluxes

Net K^+ fluxes were monitored with an extracellular, solid state K^+ electrode (WPI Model POT-1, World Precision Instruments, New Haven, CT) and an ultrawick glass reference electrode (MERE-1, WPI) filled with 1 M *n*-methyl-D-glucamine chloride, pH 8.0. The slope of the K^+ electrode was determined on each experimental day and in each of the experimental buffers: it ranged from 54–59 mV/decade K^+ concentration with a "linear" response between $10^{-4.5}$ and 10^{-2} M K^+. The selectivity of K^+:Na^+ was 10^4:1, and the response time, including mixing, was 1–2 sec. Both electrodes were sealed into a thermoregulated and magnetically stirred, 2-ml glass chamber and connected to a high-impedance electrometer (Model VF-2, WPI). The voltage difference was fed into an 8-pole, lowpass Bessel filter (Model 902LPF, Frequency Devices, Haverhill, MA) with a cutoff frequency of 1 Hz, amplified 10×, and converted to a digital signal at 2 Hz with a 12-bit A-D converter (Model #DT2801, Data Translation, Marlboro, MA). DT Notebook (Data Translation) software was used for data acquisition and display, and the voltage output was converted to K^+ concentration with a computer program. Initial rates of net K^+ flux were determined from the slope of the linear phase of the tracing with a resolution of 16–27 μM K^+, using a computer program written for this purpose. An increase or decrease in the medium K^+ concentration was interpreted as the net efflux or influx, respectively, of K^+ from the tubules. Given the low cytocrits used, changes in cell volume were estimated to alter the ion flux measurements by <4% (Avison et al., 1987).

To begin an experiment, 2-ml aliquots of the preincubated tubule suspension were placed in the chamber, continuously stirred, and bubbled with 95% O_2/5% CO_2 or 100% O_2 (as indicated in Table 1) at 37°C. At the end of each experiment, digitonin (20 μg/mg tubular protein) was added to the suspension to permeabilize selectively the cell membrane and allow for equilibration of cytosolic and extracellular K^+; cytosolic K^+ content

could then be estimated from the difference of the basal and the maximum, postdigitonin K^+ concentrations.

Measurement of Net Ca^{2+} Fluxes

Net Ca^{2+} fluxes were measured with an extracellular Ca^{2+}-sensitive electrode (WPI model CAL1) and an ultrawick glass reference electrode in a manner similar to that described for K^+. The electrode was calibrated, using buffered Ca^{2+} solutions (CALBUF, WPI) with concentrations of free Ca^{2+} ranging from 10^{-8} to 10^{-1} M. The slope of the electrode determined in this manner was 26–29 mV/decade Ca^{2+} concentration and "linear" from 10^{-7} to 10^{-1} M Ca^{2+}. The selectivity coefficients were Na^+ ($10^{-6.1}$), K^+ ($10^{-6.2}$), and Mg^{2+} ($10^{-5.1}$). Since Ca^{2+} interacts with other ions, such as phosphate and bicarbonate, in the Ringer's solution, measurements of cellular Ca^{2+} fluxes were based on electrode calibrations performed with the Ringer's solution. A nominally Ca^{2+}-free Ringer's solution was prepared by omitting $CaCl_2$ from the bicarbonate Ringer's solution described above. This solution was placed in the chamber at 37°C and bubbled with 95% O_2/5% CO_2 (pH = 7.40), and the response of the electrode to increments in $CaCl_2$ (0.1 to 5 mM) was recorded. This calibration procedure indicated that, in the bicarbonate Ringer's solution over the physiologically relevant range of extracellular Ca^{2+} concentrations, the electrode displayed a "linear" response to Ca^{2+} concentration with a slope of 25 ± 1 mV/decade (n = 5). The typical response time of the electrode (including mixing) was 4–6 sec. The experimental chamber and electronics for converting the electrode output to a digital tracing were identical to that used for the measurement of K^+ fluxes, except that a DC amplifier (WPI model FC-23B) was interposed between the Bessel filter and the A-D converter. This modification increased the final amplification to 120×. In addition, the computer program was improved to allow signal averaging. Four to six data points, acquired at 2 Hz, were averaged and converted to the final digital tracing. Using these modifications, changes in the extracellular medium of as little as 1–2 μM Ca^{2+} could be resolved. To our knowledge, this degree of resolution for an extracellular Ca^{2+} electrode in physiological studies has not been previously described. Initial rates of net Ca^{2+} flux were also measured during the initial linear phase of the recording.

Measurement of Respiration (QO_2)

The O_2 tension of the extracellular medium was monitored polarographically using an O_2 electrode connected to an oxymeter (Yellow Springs Instruments, Yellow Springs, OH). The electrode was inserted into a closed 1.8-ml glass chamber (Gilson, Middleton, WI) that was maintained at 37°C by a circulating water bath. The O_2 tension was plotted as a function of time on a chart recorder (Kipp and Zonen, Holland); the slope of the tracing represented the QO_2. Changes in QO_2 were measured as the steady-state changes in the slope following an experimental maneuver. The response time of the electrode, measured by the addition of sodium hydrosulfite to previously oxygenated, well-stirred buffer solution, was 1–2 sec. Ouabain was used to distinguish cellular respiration supporting Na^+ transport from that related to other ATP-consuming processes. The cationophore nystatin was used to assess ADP-coupled, Na^+, K^+-ATPase-mediated QO_2. The nystatin-stimulated QO_2 is equivalent to the mitochondrial state 3 respiratory state (Harris et al., 1982). Mitochondrial respiratory capacity was probed by the addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation. For experiments involving nystatin or CCCP, sodium butyrate (1 mM) was added to stimulate mitochondrial oxidative metabolism to maximal ADP-coupled and -uncoupled rates (Harris et al., 1981). Using this additional substrate, mitochondrial dysfunction may be detected with greater sensitivity.

Chemicals

Analytical grade reagents obtained from standard commercial sources were used. Concentrated stock solutions of the heavy metal salts, ouabain (protected from light), *p*-chloromercuribenzene sulfonate (*p*CMBS), N-ethylmaleimide (NEM), DTT and GSH were prepared on the day of experiment in the appropriate Ringer's solution. Furosemide, nystatin (10 mg/10 μl) and digitonin (10 mg/10 μl) were prepared daily in dimethylsulfoxide. CCCP was prepared in methanol. A stock solution of sodium butyrate (0.5 M, pH 7.40) was stored frozen until used. Experimental additions of the stock solutions were made in μl quantities that never exceeded 0.2% of the suspension volume. Aside from voltage offsets, neither the reagents nor the solvents affected electrode performance, and the solvents did not measurably affect cell function.

Data Analysis

Net K^+ and Ca^{2+} fluxes and QO_2 were normalized to tubular protein as determined by the Lowry method (Lowry et al., 1951), using bovine serum albumin as the standard. Data are expressed as the means ± SEM and were analyzed by the paired or unpaired Student's t test, as appropriate. A value of $P < 0.05$ was taken to represent a statistically significant difference between group means. Lines drawn in the Eadie-Hofstee plots were determined by least-squares linear regression analysis.

Results

Validation of Methods

Numerous studies using this (or a similar) tubule preparation have established that measurements of net K^+ fluxes and QO_2 with extracellular electrodes represent almost exclusively proximal tubule functions (Harris et al., 1981; Soltoff & Mandel, 1986; Gullans et al., 1988). Net K^+ fluxes have been used as an index of cell membrane integrity, passive K^+ transport and Na^+, K^+-ATPase activity (Soltoff & Mandel, 1986; Kone et al., 1988). Measurement of QO_2 provides a measurement of Na^+ transport (Harris et al., 1981), mitochondrial integrity and cellular energetics.

The extracellular Ca^{2+} electrode, however, has not previously been used to study net Ca^{2+} transport in renal epithelia. Therefore, we used three

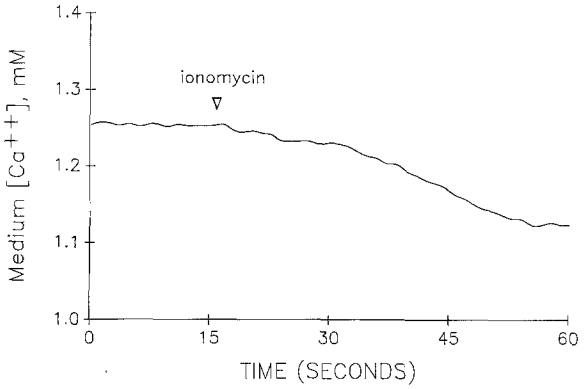

Fig. 1. Digital output of the extracellular Ca^{2+} electrode from a representative experiment (n = 3) showing the rapid net uptake of Ca^{2+} by the tubules in response to ionomycin (5 μM)

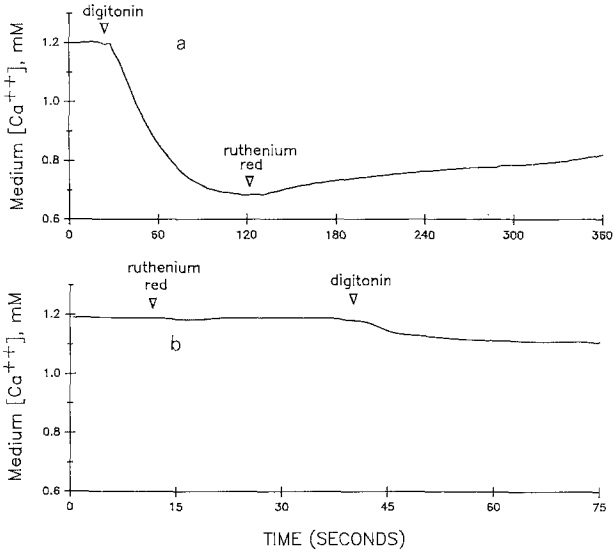

Fig. 2. Representative experiments (n = 4) demonstrating the effects of digitonin and ruthenium red on net Ca^{2+} transport by proximal tubule suspensions. (*a*) Net Ca^{2+} influx resulting from permeabilization of the plasma membrane with digitonin. The subsequent addition of ruthenium red caused a gradual net release of Ca^{2+}. (*b*) Inhibition of digitonin-induced net Ca^{2+} uptake by ruthenium red. Note that addition of ruthenium red to the intact tubules caused no net flux of Ca^{2+}

experimental perturbations to demonstrate that changes in medium Ca^{2+} concentration detected by the Ca^{2+} electrode represented net, transmembrane fluxes of Ca^{2+}. To measure the response to an increase in Ca^{2+} permeability of the plasma membrane, the Ca^{2+} ionophore ionomycin (5 μM) was added to the tubule suspensions (Fig. 1). This maneuver resulted in a rapid decrease in medium Ca^{2+} concentration, representing entry of Ca^{2+} down its concentration gradient into the cell and avid uptake by subcellular organelles, primarily the mitochondria (Murphy & Mandel, 1982). Similarly, acute addition of digitonin (20 μg/mg protein) to permeabilize selectively the plasma membrane also resulted in a rapid net Ca^{2+} influx (Fig. 2*a*). As expected, the subsequent addition of ruthenium red (16 nmol/mg protein), an inhibitor of mitochondrial Ca^{2+} uptake (Ash & Bygrave, 1975), to the permeabilized tubules resulted in a net release of Ca^{2+} into the medium (Fig. 2*a*). A similar response was observed when mitochondrial oxidative phosphorylation was poisoned with CCCP (10^{-6} M, *not shown*). If free mitochondria, possibly contaminating the tubule preparation, contributed significantly to the net Ca^{2+} transport of the tubule suspension, a similar increase in medium Ca^{2+} concentration would be expected upon addition of ruthenium red to untreated (i.e., nonpermeabilized) suspensions. Instead, no change in medium Ca^{2+} concentration was observed when ruthenium red was acutely added (Fig. 2*b*). However the digitonin-induced net Ca^{2+} uptake was dramatically attenuated by ruthenium red, presumably because mitochondrial Ca^{2+} uptake was inhibited (Fig. 2*b*). Taken together, these results indicate that changes in plasma membrane Ca^{2+} permeability can be readily detected from changes in medium Ca^{2+} concentration by the electrode: a decrease in medium Ca^{2+} concentration represents the net influx of Ca^{2+} across the plasma membranes of functionally intact tubules.

Effects of Heavy Metals on K^+ Transport and QO_2

To determine which of the heavy metals (in addition to Ag^+) affected proximal tubule function, we studied the effects of several heavy metal salts on net K^+ transport and QO_2. Of the compounds tested, only the salts of Hg^{2+}, Cu^{2+} and Au^{3+} significantly changed net K^+ transport or QO_2 (Table 2). Each of these metal ions, like Ag^+, caused a net release of K^+ from the tubules, although at slower initial rates. However, unlike Ag^+ which stimulated QO_2, these ions significantly inhibited QO_2. In contrast, equimolar concentrations (10^{-4} M) of the salts of cobalt, lead, manganese, platinum, cadmium, and iron altered neither net K^+ transport nor QO_2. Thus, based on their in vitro effects on these parameters, the heavy metals can be separated into three categories: those that cause no acute cellular injury (Co^{3+}, Mn^{2+}, Pt^{4+}, Pb^{2+}, Cd^{2+}, and Fe^{3+}), that which increases cell membrane passive K^+ transport and stimulates QO_2 (Ag^+), and those that increase cellular K^+ transport and inhibit QO_2 (Hg^{2+}, Cu^{2+} and Au^{3+}). To characterize this latter group of

Table 2. Effects of heavy metals on proximal tubule net K^+ transport and QO_2

Metal salt	Net K^+ efflux (nmol K^+/min/mg)	QO_2 (% of basal)
$AgNO_3$	178 ± 20	150 ± 3
$HgCl_2$	98 ± 4	51 ± 5
$CuSO_4$	80 ± 7	86 ± 3
$AuCl_3$	55 ± 7	90 ± 3

Aqueous solutions of various heavy metal salts were added to the tubule suspensions to achieve a final concentration of 10^{-4} M, and net K^+ transport or QO_2 was monitored. The measurements of net K^+ efflux represent initial rates. QO_2 is presented as the % of the basal QO_2 before addition of the metals. Each value represents the mean ± SEM, $n = 6$. Data for $AgNO_3$ are taken from Kone et al., 1988.

metals, we studied in further detail the effects of $HgCl_2$ on proximal tubule function.

Effects of $HgCl_2$

Dose and Time Dependence

Under steady-state conditions, the addition of $HgCl_2$ to the tubules resulted in a rapid and dose-dependent net K^+ release and net Ca^{2+} uptake, followed by an inhibition of QO_2 (Fig. 3). The K^+ efflux gradually slowed and plateaued after approximately 2 min (*not shown*). Comparison of the dose-response curves (Fig. 4) for the initial rates of net K^+ efflux and net Ca^{2+} influx to the inhibition of QO_2 induced by $HgCl_2$ indicated different thresholds and sensitivities of these processes to $HgCl_2$. The threshold concentration for changes in net K^+ transport and QO_2 was 10 μM $HgCl_2$, whereas the net Ca^{2+} influx was observed only at concentrations of $HgCl_2 \geq 30$ μM. Concentrations of $HgCl_2$ above 300 μM caused a significant Ca^{2+} electrode artifact, precluding accurate quantitation of initial rates and kinetic analysis. However, Eadie-Hofstee plots of the K^+ flux and QO_2 data were constructed and yielded single linear regression lines ($r \geq 0.95$). Using this kinetic analysis, the V_{max} and K_i for the initial rate of K^+ efflux were 171 nmol/min/mg protein and 75 μM, respectively, whereas the V_{max} and K_i for QO_2 were 84% and 180 μM, respectively. Hence, the K_i for QO_2 inhibition was more than twofold greater than that for K^+ efflux. Based on these dose-response relationships, K^+ transport appeared to be more vulnerable than QO_2 or Ca^{2+} transport to the effects of $HgCl_2$.

Given these differences in dose dependence, detailed time-course experiments were performed to distinguish further the effects of $HgCl_2$ on net K^+ and Ca^{2+} transport and QO_2. In these experiments, the effects of 10 μM $MgCl_2$ on net K^+ fluxes, net Ca^{2+} fluxes or QO_2 were measured. As seen in the composite graph of representative experiments (Fig. 3), a net K^+ efflux (4 ± 1 sec after addition) and net Ca^{2+} influx (6 ± 2 sec after addition) were observed well before the inhibition of QO_2 (16 ± 2 sec after addition, $P < 0.05$, $n = 6$). At high concentrations of Hg^{2+} (≥10 μM), a delayed net release of Ca^{2+} followed the initial net Ca^{2+} uptake. Thus, the response to $HgCl_2$ (>30 μM) is characterized by an early net efflux of K^+ and net influx of Ca^{2+}, followed by an inhibition of respiration. We next studied these two phases of cell injury in greater detail.

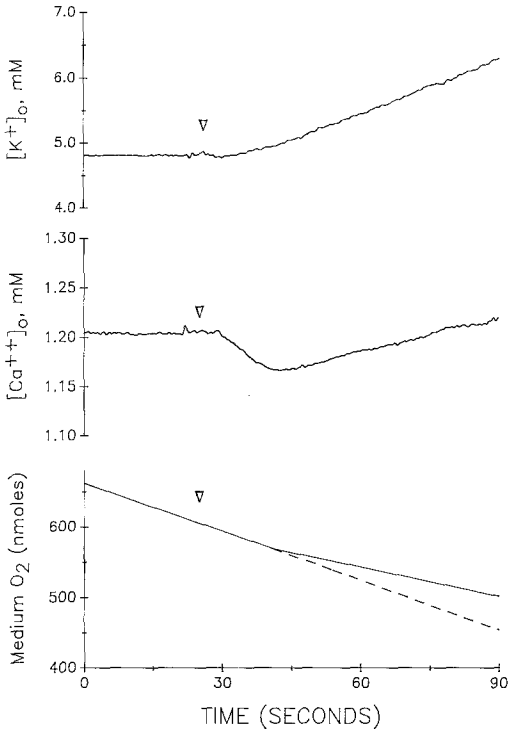

Fig. 3. Effects of 10^{-4} M $HgCl_2$ on net K^+ transport (upper tracing), net Ca^{2+} transport (middle tracing), and QO_2 (bottom tracing) of proximal tubule suspensions. The plots for the K^+ and Ca^{2+} fluxes are actual tracings from representative experiments ($n = 6$). The QO_2 plot was redrawn for illustrative purposes from the steady-state slopes of a representative ($n = 6$) chart recording and accurately reflects the time of onset of QO_2 inhibition. The final steady-state slope was achieved within 6–8 sec of the onset of QO_2 inhibition. Note that spontaneous QO_2 was unchanged during the period of initial net K^+ release and net Ca^{2+} uptake. The delayed net Ca^{2+} release was roughly coincident with the onset of QO_2 inhibition

Early Effects of $HgCl_2$

Since it occurred during a period of unchanged QO_2, the initial period of net K^+ efflux appeared to

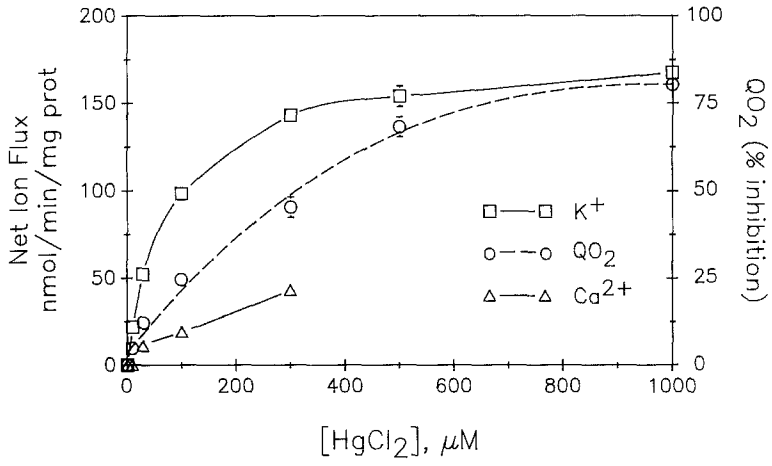

Fig. 4. Dose-response curves of $HgCl_2$-induced net K^+ efflux, net Ca^{2+} influx and inhibition of QO_2. Each datum represents the mean ± SEM, $n = 6$. When error bars are not evident, they were less than the data symbol. The best fit curve for the QO_2 data was determined by least-squares regression analysis

Table 3. Effects of $HgCl_2$ and/or ouabain on net K^+ transport

	Net K^+ efflux nmol K^+/min/mg protein
$HgCl_2$	98 ± 4
Ouabain	141 ± 5
$HgCl_2$ + ouabain	200 ± 15[a]

The initial rate of net K^+ efflux was measured following the acute addition of $HgCl_2$ (0.1 mM), ouabain (0.1 mM) or $HgCl_2$ and ouabain simultaneously. Each value represents the mean ± SEM of six observations.

[a] $P < 0.02$ *vs.* ouabain alone.

represent an increase in passive K^+ transport, rather than an inhibition of the Na^+, K^+-ATPase (which would have coincidentally decreased QO_2). To confirm that Na^+, K^+-ATPase turnover was unaffected during the initial phase of cell injury, we measured the time-course response of nystatin-stimulated QO_2 to 10 μM $HgCl_2$. As with basal QO_2, the nystatin-stimulated QO_2 was initially unaffected by $HgCl_2$ addition, but decreased after 19 ± 2 sec ($n = 5$) of exposure to the metal. These results indicated that neither the Na^+, K^+-ATPase nor the mitochondria were inhibited during the initial period of net K^+ efflux. As further proof that passive K^+ transport was increased by $HgCl_2$, we measured the initial rate of net K^+ efflux promoted by $HgCl_2$ in the presence and absence of 10 μM ouabain [a maximal inhibitory concentration for this tubule preparation (Brady, Kone & Gullans, 1989)]. Since active uptake of K^+ by the Na^+, K^+-ATPase is inhibited by ouabain, the resulting net K^+ efflux is a measurement of the total passive K^+ leak of the plasma membrane (Soltoff & Mandel, 1986). As seen in Table 3, the net K^+ efflux induced by complete inhibition of the Na^+, K^+-ATPase was 141 nmol/min/mg protein. Simultaneous addition of ouabain and $HgCl_2$ (10 μM) promoted a net K^+ efflux of 200 nmol/min/mg protein, a value 42% greater than that observed with ouabain alone. Thus, $HgCl_2$ alone caused an initial net K^+ efflux without inhibiting Na^+, K^+-ATPase activity (as evidenced by the initial period of unchanged basal and nystatin-stimulated QO_2), and the initial rate of this net K^+ efflux was additive to that promoted by ouabain. These data indicate that $HgCl_2$ accelerated the rate of K^+ efflux through passive K^+ permeability pathways.

We next considered whether this increased passive K^+ transport could be secondary to a change in the membrane permeability to other ions such as Na^+, HCO_3^-, Cl^- or Ca^{2+}. Since the initial net K^+ release occurred in the absence of any significant increase in Na^+ entry (as evidenced by the initial period of unchanged QO_2), a change in Na^+ permeability did not appear to be involved. Anion substitution experiments (Table 4) indicated HCO_3^- transport was not involved in the increase in K^+ transport since removal of HCO_3^- did not alter the initial rate of K^+ efflux. In comparison, replacement of Cl^- with gluconate$^-$, a maneuver designed to deplete both intracellular (Cassola, Mollenhauer & Fromter, 1983; Lipman, Harris & Lechene, 1987) and extracellular Cl^-, resulted in an ~50% decrease in the initial rate of net K^+ release, implicating Cl^- permeability as a primary and/or secondary determinant of K^+ efflux. Moreover, the net K^+ efflux promoted by $HgCl_2$ did not appear to be the result of the net Ca^{2+} influx (i.e., Ca^{2+}-activated K^+ channels), since changes in K^+ transport could be observed at a concentration of $HgCl_2$ (10 μM) that did not measurably alter Ca^{2+} transport (Fig. 4) and since ionomycin, in concentrations that promoted a net Ca^{2+} influx (1–10 μM), failed to cause any measurable net K^+ flux (*not shown*).

To distinguish whether known K^+ permeability

pathways were directly affected by the $HgCl_2$, we tested the ability of agents known to block passive K^+ permeability pathways in the rabbit proximal tubule to prevent the initial $HgCl_2$-induced K^+ release. As shown in Table 5, Ba^{2+} and tetraethylammonium (TEA), agents known to block K^+ channels, depolarize the cell membrane potential (Gogelein & Greger, 1984; Kawahara, Hunter & Giebisch, 1987), and (in the case of Ba^{2+}) promote a net K^+ uptake (Soltoff & Mandel, 1986), had absolutely no effect on the initial rate of net K^+ efflux induced by $HgCl_2$. Furthermore, neither furosemide (1 mM), an inhibitor of KCl cotransport in the proximal tubule (Eveloff & Warnock, 1987; Avison et al., 1988), nor tolbutamide, an inhibitor of ATP-sensitive K^+ channels in cardiac myocytes (Misler, 1987) and pancreatic β cells (Gillis et al., 1987), slowed the rate of K^+ release induced by $HgCl_2$. Thus $HgCl_2$, like $AgNO_3$, rendered the major passive K^+ permeability pathways of the proximal tubule insensitive to known inhibitors suggesting a direct action of these metals on K^+ permeability pathways.

Unfortunately, the Ca^{2+} transport inhibitors verapamil, diltiazem and nifidipene produced significant Ca^{2+} electrode artifacts, preventing a similar transport inhibitor study of the net Ca^{2+} influx induced by $HgCl_2$. However, the net Ca^{2+} influx produced by Hg^{2+} was similar to that caused by ionomycin, suggesting that it, too, was the result of an increase in cell membrane permeability to Ca^{2+}. Since it *preceded* the reduction in QO_2, the net Ca^{2+} influx was presumably not caused by inhibition of the Na^+, K^+-ATPase or Na^+/Ca^{2+} exchange. As further support for this hypothesis, ouabain (10 μM) alone produced no net Ca^{2+} flux (*not shown*).

Late Effects of $HgCl_2$

To distinguish whether the delayed inhibition of QO_2 by $HgCl_2$ was the result of an effect on Na^+ transport-dependent respiration, ouabain-sensitive and -insensitive QO_2 were measured 30 sec after addition of $HgCl_2$ (10 μM) to the tubules (Fig. 5). Under basal conditions, QO_2 was 20.4 ± 0.9 nmol O_2/min/mg for the vehicle-treated tubules (control) and 20.1 ± 0.6 nmol O_2/min/mg for the tubules subsequently treated with $HgCl_2$ (NS). At 30 sec, $HgCl_2$ decreased ouabain-sensitive QO_2 by ~40% (from 9.0 ± 0.7 to 5.6 ± 0.8 nmol O_2/min/mg), but did not alter ouabain-insensitive QO_2 (11.0 ± 0.7 *vs.* 11.7 ± 0.4 nmol O_2/min/mg, respectively). These data indicated that $HgCl_2$ caused a delayed inhibition of Na^+ transport-dependent ATP utilization.

To distinguish whether the delayed inhibition of ouabain-sensitive QO_2 was the result of a direct effect of $HgCl_2$ to reduce either Na^+ entry or Na^+, K^+-ATPase turnover, nystatin-stimulated QO_2 was measured after 30-sec exposure to $HgCl_2$. Additionally, since mitochondrial injury could account for the reduced ouabain-sensitive QO_2, CCCP-uncoupled QO_2 was measured in control tubules and in tubules treated for 30 sec with $HgCl_2$. CCCP stimulates QO_2 to maximal rates, independent of ADP or Na^+, K^+-ATPase activity, and thus, provides an index of the respiratory capacity of the mitochondria. As seen in Fig. 6, nystatin-stimulated QO_2 was 50% less in the $HgCl_2$-treated tubules compared to controls, whereas CCCP-uncoupled QO_2 was not significantly different between the two groups. These results indicated that, after 30 sec of treatment, $HgCl_2$ inhibited the Na^+, K^+-ATPase, but not Na^+ entry or mitochrondrial function. The inhibition of Na^+, K^+-ATPase turnover by $HgCl_2$ was substantiated by the observation that ouabain (10 μM) did not accelerate the rate of K^+ release from tubules pretreated (30 sec) with 10 μM $HgCl_2$ (*not shown*). If residual Na^+, K^+-ATPase activity remained after treatment with $HgCl_2$, ouabain would

Table 4. Anion dependence of the $HgCl_2$-induced K^+ efflux

	Net K^+ efflux (nmol K^+/min/mg)
Control	98 ± 4
HCO_3^- free	108 ± 6
Cl^- free	52 ± 6[a]

Initial rates of net K^+ efflux were measured following the addition of 100 μM $HgCl_2$ to tubules suspended in control (solution I), HCO_3^--free (solution II), or Cl^--free (solution III) buffer. Each datum represents the mean ± SEM, $n = 6$.
[a] $P < 0.05$ *vs.* control.

Table 5. Effects of K^+ transport inhibitors on $HgCl_2$-induced net K^+ efflux

Inhibitor	Net K^+ efflux (nmol/min/mg protein)
$HgCl_2$ ($n = 6$)	98 ± 4
+ $BaCl_2$ (5 mM, $n = 5$)	91 ± 6
+ TEA (1 mM, $n = 4$)	102 ± 8
+ Furosemide (1 mM, $n = 4$)	100 ± 5
+ Tolbutamide (1 mM, $n = 4$)	92 ± 7

The initial rate of $HgCl_2$-induced net K^+ efflux was measured in the presence of various K^+ transport inhibitors. $HgCl_2$ (0.1 mM) was added simultaneously with $BaCl_2$ or 30–60 sec after TEA, furosemide or tolbutamide. In comparison to $HgCl_2$ alone, none of the inhibitors significantly altered the initial rate of $HgCl_2$-induced net K^+ efflux rate ($P \geq 0.20$).

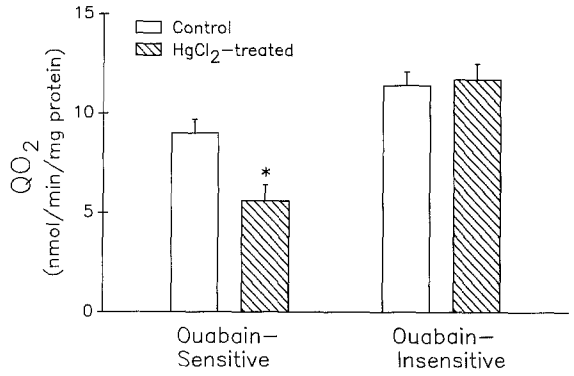

Fig. 5. Inhibition of basal, ouabain-sensitive, and ouabain-insensitive QO_2 by 10 μM $HgCl_2$. QO_2 was measured 30 sec after addition of $HgCl_2$ to the tubule suspensions. Ouabain was used at a concentration of 10 μM. Each bar represents the mean ± SEM, $n = 6$. * denotes $P < 0.01$

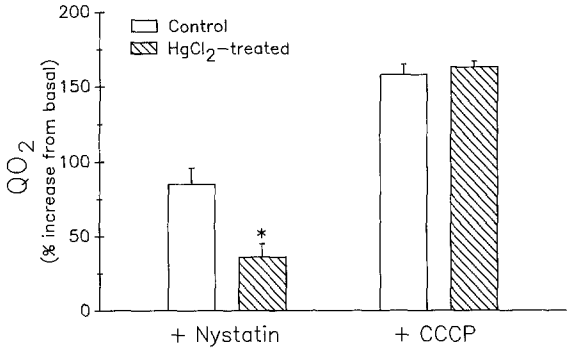

Fig. 6. Effects of $HgCl_2$ on nystatin-stimulated and CCCP-uncoupled QO_2. Nystatin or CCCP was added 30 sec after treatment of the tubules with 10 μM $HgCl_2$. $n = 5$, * $P < 0.05$.

have augmented the net efflux of K^+ through unopposed permeability pathways. More prolonged exposure (2 min) of the tubules to $HgCl_2$ resulted in a ~40% reduction in the CCCP-uncoupled QO_2, from 73 ± 5 nmol/min/mg to 43 ± 2 nmol/min/mg ($n = 7$, $P < 0.001$), indicating delayed disruption of mitochondrial function.

Effects of SH Reagents

Although Ag^+ and Hg^{2+} are known to complex with a variety of biological macromolecules, they react preferentially with protein SH groups (Gurd & Wilcox, 1956). To ascertain whether plasma membrane SH groups were primarily responsible for the Hg^{2+}-induced cell dysfunction, the acute effects of other, purportedly specific SH reagents were tested to see if they could mimic the effects of Ag^+ or Hg^{2+}

Table 6. Effects of sulfhydryl reagents on proximal tubule function

Reagent	Net K^+ efflux (nmol K^+/min/mg)	QO_2 (% of control)	Net Ca^{2+} influx (nmol Ca^{2+}/min/mg)
*p*CMBS	51 ± 11	119 ± 3	0
NEM	21 ± 4	79 ± 4	0

The SH reagents were added to achieve a final concentration of 10^{-4} M, and the response of the tubules was measured with extracellular K^+, O_2 or Ca^{2+}-sensitive electrodes. Each value represents the mean ± SEM, $n = 4$. No measurable net Ca^{2+} flux was produced by either agent.

on K^+ and Ca^{2+} transport, and QO_2 (Table 6). The organic mercurial agent, *p*CMBS (10^{-4} M) promoted an initial net K^+ efflux followed by an increase in QO_2. NEM (10^{-4} M), a membrane-permeant reagent that alkylates SH groups, also caused a net K^+ release, but later inhibited basal QO_2. At 10^{-3} NEM, the inhibition of QO_2 was even more profound (80 ± 2%, $n = 4$). Neither reagent, in concentrations of up to 10^{-3} M, measurably altered net Ca^{2+} transport. Thus, in comparison to Ag^+ and Hg^{2+}, the SH reagents produced similar, although slower rates of net K^+ loss, but no changes in net Ca^{2+} transport. In addition, *p*CMBS (like Ag^+) stimulated QO_2, whereas NEM (like Hg^{2+}) inhibited QO_2.

Since the effects of Ag^+ on K^+ and Na^+ transport previously reported (Kone et al., 1988) were similar to those caused by *p*CMBS but different from those observed with $HgCl_2$, we were interested to know whether Ag^+ also affected net Ca^{2+} transport in the proximal tubule. As seen in Fig. 7, addition of 10^{-4} M $AgNO_3$ caused an early, rapid net Ca^{2+} influx. We previously found that this concentration of $AgNO_3$ produced no deleterious effects on mitochondrial function. In comparison to the Ag^+-induced increases in net K^+ release and QO_2 we previously reported (Kone et al., 1988), the time of onset for the net Ca^{2+} influx was roughly coincident with the net K^+ efflux, and preceded the increase in ouabain-sensitive QO_2.

Effects of DTT and GSH

Given the effects of the SH-reactive metals and reagents on ion transport and QO_2, the acute, protective effects of DTT and GSH were studied in our preparation. These reagents maintain SH groups in their reduced form under oxidizing conditions (Cleland, 1964). Under basal conditions, neither DTT (1

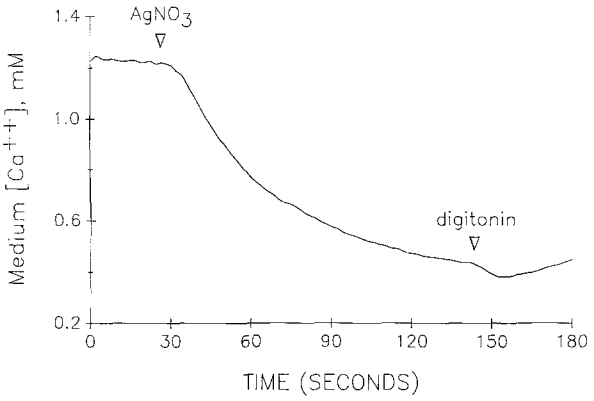

Fig. 7. $AgNO_3$-induced net Ca^{2+} uptake by suspensions of proximal tubules. $AgNO_3$ was added to achieve a final concentration of 10^{-4} M. The subsequent addition of digitonin caused only a slight, further net uptake of Ca^{2+}. This result is representative of three other experiments

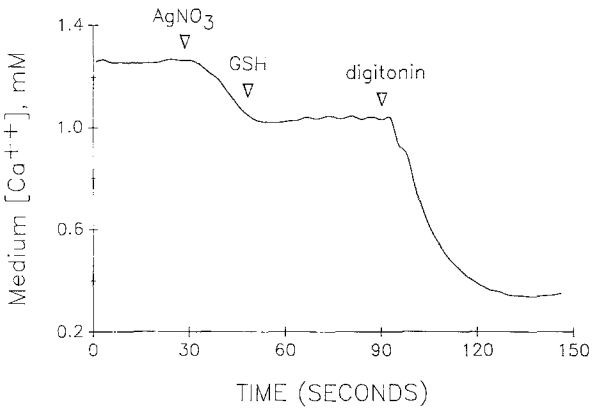

Fig. 8. Ca^{2+} electrode tracing demonstrating the effects of GSH (10^{-4} M) on the $AgNO_3$-induced net Ca^{2+} uptake of proximal tubules. Under steady-state conditions, $AgNO_3$ (10^{-4} M) was added to the tubule suspensions. During the ensuing period of net Ca^{2+} uptake, GSH was added and immediately prevented further Ca^{2+} influx. Similar results were obtained when DTT (10^{-4} M) was substituted for GSH. The subsequent addition of digitonin caused a significant net Ca^{2+} uptake, suggesting that cell membrane Ca^{2+} permeability had been restored to its basal condition by GSH (*cf.* Fig. 2*a*), and that the Ca^{2+} buffering capacity of the mitochondria remained intact

mM) nor GSH (1 mM) alone altered net K^+ or Ca^{2+} transport or QO_2 in the tubule suspensions. Addition of either reagent (1 mM) to the suspension immediately before treatment with either $HgCl_2$, *p*CMBS, or NEM, prevented the changes in ion transport and QO_2 described above (*not shown*). However, neither DTT nor GSH reversed the net K^+ efflux or net Ca^{2+} influx induced by $HgCl_2$. When either reagent was given after $HgCl_2$, during the initial phases of cation transport, no change in the rate of net K^+ efflux or net Ca^{2+} influx was observed. In contrast, the Ag^+-induced net Ca^{2+} influx, like the changes in K^+ transport and QO_2 caused by this metal ion (Kone et al., 1988), were immediately reversed by 10^{-4} M GSH (Fig. 8).

Discussion

In response to a nephrotoxin, the proximal tubule must defend its intracellular ionic milieu by maintaining normal ionic permeabilities of the cell membrane, Na^+, K^+-ATPase activity, and mitochondrial function. The mechanisms by which nephrotoxins disrupt these coordinated processes are incompletely defined. In this study, we demonstrated that Hg^{2+}, Cu^{2+} and Au^{3+} each caused a rapid net K^+ efflux and a delayed inhibition of respiration in suspensions of renal proximal tubules. A detailed analysis of the effects of Hg^{2+}, the most nephrotoxic of these compounds, demonstrated a complex sequence of cellular responses characterized by a nearly coincident Cl^--dependent net efflux of K^+ and net influx of Ca^{2+} (responses also produced by Ag^+), followed by an inhibition of ouabain-sensitive QO_2 and nystatin-stimulated QO_2. These results suggest that Hg^{2+} (in concentrations commonly used in experimental models of renal tubular injury) causes an early increase in membrane ionic permeability followed by an inhibition of the Na^+, K^+-ATPase, responses that clearly precede mitochondrial injury. Given the ability of the SH reagents *p*CMBS and NEM to mimic the early K^+ release, and the ability of GSH and DTT to reverse the Ag^+-induced net Ca^{2+} influx, an interaction of Hg^{2+} and Ag^+ with cell membrane thiol groups appears to contribute to the altered ionic permeabilities of the cell membrane.

The common response of the tubules to Hg^{2+}, Cu^{2+}, Au^{3+}, *p*CMBS, and NEM was the net release of cellular K^+. Such a response could represent an increase in passive K^+ efflux across the cell membrane as occurs following treatment with Ag^+, and/or an inhibition of Na^+, K^+-ATPase activity. In the case of Hg^{2+}, the initial phase of net K^+ efflux was not related to an inhibition of Na^+, K^+-ATPase activity since both basal and nystatin-stimulated QO_2 were unchanged. Furthermore, $HgCl_2$ augmented by approximately 50% the total K^+ leak flux caused by ouabain alone, indicating that passive K^+ efflux was increased. Whether the increase in K^+ efflux was a primary increase in K^+ permeability or was secondary to an increase in the permeability of the

membrane to other ions (which could indirectly augment K^+ exit by depolarizing the membrane or activating K^+ transport) was not directly ascertained. However, several lines of evidence suggest that Hg^{2+} directly increased K^+ permeability: (*i*) the K^+ efflux preceded any changes in Na^+ entry and was unaffected by removal of HCO_3^-, suggesting conductive pathways for these ions were not involved; (*ii*) low concentrations (10 μM) of $HgCl_2$ promoted a net K^+ efflux without causing a net Ca^{2+} influx, and an ionomycin-induced increase in Ca^{2+} permeability failed to elicit a net K^+ release, thus dissociating the K^+ efflux from the net Ca^{2+} influx; (*iii*) Ba^{2+} and TEA, agents known to block K^+ channels in the proximal tubule, failed to elicit a response in the presence of $HgCl_2$, suggesting that these pathways were rendered insensitive to these inhibitors. In support of these observations, a recent electrophysiological study of cultured renal epithelial cells (MDCK cells) revealed that $HgCl_2$ caused an initial membrane hyperpolarization by irreversibly increasing the K^+ conductance of the plasma membrane (Jungwirth, Paulmichl & Lang, 1989). Furthermore, like us, these investigators found that in the presence of $HgCl_2$, Ba^{2+} was unable to block K^+ conductance. The decrease in K^+ efflux with replacement of Cl^- by gluconate$^-$, observed in the present study, suggests that Cl^- was a limiting factor in the K^+ efflux. Whether this effect is a primary action of the heavy metals on Cl^- permeability or KCl cotransport, or a secondary effect whereby K^+ efflux was limited by the low native Cl^- permeability of the cells (Bello-Reuss, 1982) remains to be elucidated. The ion dependence of the remaining, Cl^--independent K^+ efflux is not yet known. However, the net Ca^{2+} influx associated with higher concentrations of $HgCl_2$ could account for a portion of the charge balance; alternatively an influx of H^+ (i.e., directly or indirectly coupled K^+/H^+ exchange, as described in some other cell types) could charge balance the residual component of net K^+ efflux. Nonetheless, heavy metals appear to increase selectively the ionic permeability of the plasma membrane of the proximal tubule.

Similar alterations in cation transport have been observed in other epithelia treated with these heavy metals and SH reagents. Ag^+ and Cu^{2+} increased the cation conductance of rabbit corneal epithelium, an effect mimicked by NEM (Klyce & Marshall, 1982), and frog skin (Ferreira, 1970; Curran, 1972). Ag^+ has also been shown to increase the cation conductance of toad bladder (Walser, 1970) and rat ileum (Clarkson & O'Toole, 1964). Similarly, *p*CMBS has been shown to increase the cation conductance of total urinary bladder (Spooner & Edelman, 1976) and to stimulate short-circuit current (and by inference Na^+ transport) across frog skin (Benos, Mandel & Simon, 1980).

In addition to the Hg^{2+}-induced increase in cell membrane K^+ permeability, we also observed a delayed inhibition of both ouabain-sensitive and nystatin-stimulated QO_2. These results indicate an inhibition of the Na^+, K^+-ATPase, an effect that further contributed to the loss of cell K^+ and likely accounted, at least in part, for the acceleration of the K^+ efflux (Fig. 3) occurring after approximately 20 sec of treatment. Such direct inhibition of Na^+, K^+-ATPase activity by similar concentrations of $HgCl_2$ has been observed in whole kidney homogenates (Rifkin, 1965). In addition, a progressive increase in membrane K^+ permeability caused by $HgCl_2$ could have caused this increased rate of K^+ loss.

Several of our results also point to significant mitochondrial injury following prolonged treatment of the tubules with $HgCl_2$. Hg^{2+} maximally inhibited basal QO_2 by 80%, and significantly attenuated the maximal rates of ADP-coupled (nystatin-stimulated) and -uncoupled (CCCP-stimulated) QO_2. These findings are in agreement with Weinberg et al. (1983) who demonstrated that $HgCl_2$ produced severe dysfunction of mitochondria isolated from renal cortex. The fact that the changes in QO_2 observed in the present study were delayed in time and produced only by more prolonged exposures to higher concentrations of $HgCl_2$ (when compared to the early increase in passive K^+ transport), suggests that, unlike membrane transport pathways, injury to the mitochondria is not the primary, but rather a secondary mechanism of proximal tubule injury by this metal. Furthermore, the vulnerability of this nephron segment to injury by these metals may relate in part to the relatively high density of K^+ permeability pathways, as reflected by the high rates and magnitudes of K^+ loss in response to these metals.

Nearly coincident with the onset of net K^+ release, a rapid net Ca^{2+} uptake was observed in tubules treated with Hg^{2+} or Ag^+. Since the electrochemical gradient across the plasma membrane favors Ca^{2+} entry, this response represents either an increase in Ca^{2+} permeation through passive permeability pathways or an inhibition of Ca^{2+} extrusion mechanisms (i.e., Na^+/Ca^{2+} exchange, Ca^{2+}-ATPase) of the cell. Given the rate and magnitude of the net Ca^{2+} influx induced by these metal ions, it seems unlikely that inhibition of Ca^{2+} extrusion pathways was solely responsible for the increase in cell membrane permeability to Ca^{2+}. Indeed, Van Driessche (1987) reported that Ag^+ and Hg^{2+} activate Ca^{2+} channels in the apical membrane of toad urinary bladder. Similar interactions of Ag^+ with Ca^{2+} channels of human platelets (Adunyah &

Dean, 1986) and the sarcoplasmic reticulum of skeletal mucle (Abramson et al., 1983) have also been noted. In addition, Hg^{2+} has been found to increase the cytosolic Ca^{2+} concentration of cultured renal tubular cells via an apparent increase in Ca^{2+} entry (Smith et al., 1987).

Perhaps most striking was the ability of GSH and DTT to prevent an immediately reverse the Ag^+-mediated net Ca^{2+} influx. These thiol reagents also rapidly reversed the net K^+ efflux produced by Ag^+ in this tubule preparation (Kone et al., 1988). These results suggest an effect of Ag^+ to interact reversibly with SH groups of cell membrane proteins responsible for the modulation of Ca^{2+} and K^+ permeabilities. Ag^+ is known to react preferentially with SH groups to form hemi-silver sulfides (Gurd & Wilcox, 1956). Hg^{2+}, Cu^{2+}, and Au^{3+} are also extremely SH reactive. Presumably, alterations of the critical SH group(s) of K^+ transporter(s), whether by oxidation (Ag^+, Hg^{2+}, Cu^{2+}) or alkylation (NEM), led to the K^+ efflux we observed. A similar thiol-dependent mechanism of KCl cotransport has been well characterized in the sheep erythrocyte (Lauf, 1988). The fact that the net Ca^{2+} uptake produced by Ag^+ and Hg^{2+} in the present study was not mimicked by NEM and *p*CMBS may reflect the inaccessibility of these organic SH reagents to the critical SH-bearing ligands of the Ca^{2+} transport proteins. Van Driessche (1987) observed NEM and *p*CMBS to be similarly ineffective in mimicking the Ag^+-induced activation of Ca^{2+} channels of toad urinary bladder. He postulated that the site of Ag^+ action may be on the cytosolic face of the channel proteins, and thus inaccessible to the organic SH reagents. Similar reasoning may apply to our results.

Several lines of evidence indicate that the various heavy metals alter proximal tubule function in vitro by different mechanisms. Of the 10 metals tested, only four (Ag^+, Hg^{2+}, Cu^{2+}, and Au^{3+}) altered net K^+ transport or QO_2. Of these four metal ions, Ag^+ and Hg^{2+} promoted far more rapid rates of net K^+ efflux, but produced opposite effects (stimulation and inhibition, respectively) on QO_2. Moreover, GSH and DTT rapidly reversed the net K^+ release (Kone et al., 1988) and net Ca^{2+} uptake caused by Ag^+, but not by Hg^{2+}. Furthermore, neither NEM nor *p*CMBS mimicked all the actions of either Ag^+ or Hg^{2+}, suggeting different mechanisms of actions for all of these SH-reactive agents. These variable cellular responses likely reflect subtle physical chemical differences among the metal ions, and suggest that the common outcome following exposure to heavy metals, namely proximal tubule injury, may be produced by different cellular mechanisms.

In conclusion, we have demonstrated rapid changes in K^+ transport and respiration after exposure of renal proximal tubules to Hg^{2+}, Cu^{2+} and Au^{3+}. The response to Hg^{2+} was characterized by initial increases in cell membrane K^+ and Ca^{2+} transport, as also occurred with Ag^+, followed by inhibition of Na^+, K^+-ATPase activity and impairment of mitochondrial function. These results suggest that disruption of the ionic permeability of the cell membrane, particularly K^+ permeability, appears to represent the initial and most sensitive mechanism of proximal tubule injury common to SH-reactive heavy metals. Given the limitations of tubule suspensions, the precise location of the altered transport pathways within the plasma membrane remains to be determined. However, since K^+ conductive pathways (Biagi, Sohtell, & Giebisch, 1981), KCl cotransport (Eveloff & Warnock, 1987; Sasaki et al., 1988), and the Na^+, K^+-ATPase reside primarily in the basolateral membrane of the proximal tubule, it seems likely that this membrane is most affected by the metals. In addition, we demonstrated an effect of *p*CMBS and NEM to mimic the K^+ release produced by the heavy metals, and an effect of GSH and DTT to reverse rapidly the Ag^+-induced net Ca^{2+} uptake. These results extend our previous observations of the effects of Ag^+ on the proximal tubule, and suggest an important role for cell membrane thiols in the modulation of proximal tubule K^+ and Ca^{2+} permeabilities.

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