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Cadmium and transport of ions and substances across cell membranes and epithelia

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Abstract Toxic metals such as cadmium (Cd^{2+}) pose serious risks to human health. However, even though the importance of Cd^{2+} as environmental health hazards is now widely appreciated, the specific mechanisms by which it produces its adverse effects have yet to be fully elucidated. Cd^{2+} is known to enter cells, it binds and interacts with a multitude of molecules, it may indirectly induce oxidative stress and interfere with gene expression and repair of DNA. It also interacts with transport across cell membranes and epithelia and may therefore disturb the cell's homeostasis and function. Interaction with epithelial transport, especially in the kidney and the liver, may have serious consequences in general health. A lot of research still needs to be done to understand the exact way in which Cd^{2+} interferes with these transport phenomena. It is not always clear whether Cd^{2+} has primary or secondary effects on cell membrane transport. In the present review we try to summarize the work that has been done up to now and to critically discuss the relevance of the experimental work in vitro with respect to the in vivo situation.

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Abbreviations

BLMV	Basolateral membrane vesicles
BBMV	Brushborder membrane vesicles
sc	Subcutaneous
iv	Intravenous
ip	Intra-peritoneal
TEA	Tetraethylammonium
PAH	p-aminohippurate
FL-MTX	Fluorescein, methotrexate, fluorescent model substrate for Mrp2
i/o	Inside/out
CdAc2	Cadmium acetate
ECaC	Epithelial Ca^{2+}channel
NCX	Na+/Ca^{2+} exchanger
Pi	Inorganic phosphate

Introduction

More than 100 years ago, Seiffert (1897) observed emphysema and proteinuria in cases of "lead poisoning" among workers in a zinc smelter (Nordberg 2009). It is to be suspected that this was due to Cd^{2+} exposure. Cd^{2+} most probably was present in that environment and the symptoms fit with chronic Cd^{2+}

poisoning as we know it from later studies. This observation suggests glomerular defects or impairment of kidney tubular reabsorption. That Cd^{2+} has a toxic effect on cellular transport mechanisms in the kidney may also be inferred from the development by intoxicated patients of symptoms resembling the Fanconi syndrome (Fanconi 1936; Bergeron et al. 1976). The syndrome is characterized by tubular proteinuria with variable expression of amino acid, glucose, phosphate (P_i), calcium (Ca^{2+}), uric acid and potassium (K^+) tubular leaks. Examples of effects on the functioning of the kidney in humans and animals are given in Table 1. In 1948 (Friberg 1948) presented the first report on the characteristic combination of proteinuria and emphysema in chronic Cd^{2+} poisoning occurring among cadmium workers. In 1950 the author found evidence of decreased renal efficiency in workers exposed to cadmium oxide (Friberg 1950). In another early study of urinary amino acid excretion by workers exposed to heavy metals Clarkson and Kench pointed to the frequency of abnormal aminoaciduria appearing among workers absorbing Cd^{2+} (Clarkson and Kench 1956).

Since then a vast literature has been produced on Cd^{2+} exposure and health. But the details of the mechanisms by which Cd^{2+} affects membrane transport molecules or processes have not always been really clarified.

In this review we want to focus on membrane transport mechanisms and Cd^{2+} exposure in vivo and in vitro and try to find out whether the data may contribute to a better understanding of the toxicity of Cd^{2+} in living organisms. The impact of Cd^{2+} on the integrity of an epithelium is included as this is important in maintaining the vectorial transport function of such a tissue. In each section we start from what is known about renal epithelia, which have been most extensively studied in this respect, extending the discussion to other tissues, if information is available. A summary of the literature on membrane transport processes and Cd^{2+} discussed in this review can be found in Table 1.

Table 1 Overview of effects of Cd^{2+} on renal reabsorptive function

Cd^{2+} effect	Tissue/animal	Cd^{2+} administration route in vivo	Reference
Proteinuria	Workers in Zn smelter	Occupational exposure	Seiffert (1897)
Proteinuria & emphysema	Cd workers	Chronic Cd poisoning	Friberg (1948)
Aminoaciduria	Workers exposed to CdO dust	Occupational exposure, [Cd^{2+}] in urine 0.1–3 μM	Clarkson and Kench (1956)
Polyuria, hyposthenuria, urinary loss of protein, glucose, urea, Ca^{2+}, P_i Cl^-, K^+	Intoxicated rats	Sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight for 16 days	Kim et al. (1988)
Increased excretion of 15 different amino acids, from neutral, acidic and basic families	Male Sprague–Dawley rats	Sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight for 2 weeks	Kim et al. (1990)
Polyuria, glucosuria, proteinuria, and phosphaturia	Exposed adult male Sprague–Dawley rats	$CdCl_2$ sc at a dose of 2 mg Cd^{2+}/kg body weight/day for 2 weeks	Ahn and Park (1995)
Polyuria, hypercalciuria, hyperphosphaturia and loss of Na^+, K^+, Cl^-	Rat kidney	~5 μM Cd^{2+} via intravenous infusion	Barbier et al. (2004)
No effects during the injection period, increased excretion fraction during the 15 days recovery period of Cl^-, P_i, Na^+, K^+, Ca^{2+} and Mg^{2+}	Rat	Daily ip injections of 0.5 mg of $CdCl_2$/kg for 5 days	Jacquillet et al. (2006)
Protection by Zn^{2+} from Cd^{2+} effect on renal reabsorption of Cl^-, P_i, Na^+, K^+, Ca^{2+} and Mg^{2+}	Rat	Idem, with co-injection with 0.5 mg $ZnCl_2$/kg	Jacquillet et al. (2006)

Cadmium and salt excretion in rat kidney

The group of Poujeol et al. performed an elaborate study with clearance, microinjection and micropuncture experiments in rat kidney (Barbier et al. 2004). The authors exposed the animals to Cd^{2+} via intravenous (iv) infusion. The plasma free Cd^{2+} concentration obtained was $\sim$3 μM. This reduced the reabsorption of several ions. The plasma concentration of Na^+, K^+, Mg^{2+}, Ca^{2+} and PO_4^{2-} decreased significantly and the excretion fraction (EF) of all these ions increased during the 30–120 min of exposure. Such an acute perfusion with Cd^{2+} caused polyuria, hypercalciuria, hyperphosphaturia and loss of all these ions without modification of GFR. The applied dose seemed to have been massive and produced an acute Fanconi-like syndrome in these animals (Table 1).

In another paper from this group, exploring kidney Cd^{2+} intoxication and the possible protection by Zn^{2+}, clearance measurements were performed after daily intra-peritoneal (ip) injections of 0.5 mg of $CdCl_2$/kg for 5 days (Jacquillet et al. 2006). The authors found no effects during the injection period but observed increased excretion fractions of Cl^-, P_i, Na^+, K^+, Ca^{2+} and Mg^{2+} during the 15 days recovery period together with polyuria and diminished GFR. Co-injection with 0.5 mg $ZnCl_2$/kg protected the animals from this insult. The study gives us a detailed picture of ion and water excretion after a Cd^{2+} load: Cd^{2+} clearly has an effect on fluid and ion transport in the rat kidney after the cells had accumulated the Cd^{2+}. But the exact molecular mechanisms cannot be derived from this work.

In an attempt to elucidate the underlying mechanisms, $^{65}Zn^{2+}$ was microinjected with or without 50 μM Cd^{2+}, a rather high concentration, at different sites along the nephron (Barbier et al. 2004). The authors claim that there is an "important inhibition of $^{65}Zn^{2+}$ transport along the terminal segments of the nephron". No statistical analysis of the data (Table 6 in their paper) was given however, and the amount of $^{65}Zn^{2+}$ recovered in the urine, i.e. not reabsorbed, after the microinjections at different sites along the nephron seemed higher in all experiments. Therefore from the data (mean and SE) we calculated a one-tailed *t*-value and found that the Cd^{2+} effect was significant in all conditions ($P \leq 0.05$ or less), except for the late proximal microinjection. So, Cd^{2+} does seem to inhibit the $^{65}Zn^{2+}$ reabsorption in different segments of the nephron. Presenting the data in a different way, i.e. as the fraction of injected $^{65}Zn^{2+}$ reabsorbed, at the more proximal micro-injection sites this corresponded to a reabsorption of only 86 to 88% in the presence of Cd^{2+} when compared to control. When microinjecting at the early distal site only 14% of the injected $^{65}Zn^{2+}$ was reabsorbed in control conditions and almost none in cadmium, which seems to correspond to a total inhibition of $^{65}Zn^{2+}$ absorption in the downstream segment. If however, as stated in the Methods, 3 nl was injected at each site within 20–90s, the early distal tubule must have been flooded with the injected fluid, whereas in the early proximal tubule with most of the snGFR still present, the solution (and the Cd^{2+}) must have been diluted more or less. Therefore, the inhibition in the early proximal tubule may even have been more efficient than what appears from the results and the one in the distal tubule may have been overestimated. So in our opinion, Cd^{2+} does affect $^{65}Zn^{2+}$ reabsorption in all segments, but possibly more in the distal segment.

Barbier et al. (2004) also focussed on Ca^{2+} and PO_4^{2+}. After 30 min of infusion with $\sim$3 μM Cd^{2+} the plasma concentration of Ca^{2+} and PO_4^{2-} decreased significantly and the EF of these ions increased within 30 min. This was maintained for up to 120 min of exposure. As mentioned above, the acute infusion with Cd^{2+} caused polyuria, hypercalciuria, hyperphosphaturia without modification of GFR. Very little of the filtered Cd^{2+} was excreted by the kidney. Microinjections of $^{109}Cd^{2+}$ at different sites along the nephron showed that Cd^{2+} was primarily taken up by the proximal tubule. By microinjecting $^{45}Ca^{2+}$ together with (or without) 20 μM Cd^{2+} into the early proximal, late proximal, early distal or late distal tubule of the Wistar rat tubule and measuring the $^{45}Ca^{2+}$ recovered in the urine, the authors could show that Cd^{2+} significantly reduced Ca^{2+} reabsorption in different parts of the nephron. The effect in the proximal tubule may be partly explained by the diminished fluid uptake, reducing the solvent drag effect and paracellular Ca^{2+} reabsorption. ECaC channels may also be involved: like Cd^{2+}, gadolinium and lanthanum inhibit Ca^{2+} currents across ECaC channels. These metals had effects on Ca^{2+} reabsorption similar to those of Cd^{2+}, nifedipine, a dihydropyridine Ca^{2+}channel blocker, did not (Barbier et al. 2004).

In a recent study of Poujeol's group, in an attempt to unravel the mechanisms and membrane transport

phenomena involved in the onset of apoptosis, L'Hoste et al. (L'Hoste et al. 2009) suggest a role for the CFTR channel in Cd^{2+} induced apoptosis in mouse proximal tubule cells. Proximal tubule cell cultures from wild type *cftr*$^{+/+}$ and *cftr*$^{-/-}$ mice were used. In the presence of 5 μM Cd^{2+}—within minutes—a CFTR-like Cl^- conductance was activated, apparently via activation of ERK1/2. The open CFTR channel allows for Cd^{2+} bound to GSH to be extruded from the cell (for more details on CFTR channels and Cd^{2+}, see Thévenod, this issue, 2010). Cd^{2+} also induces reactive oxygen species (ROS) and the depletion of GSH in turn leads to an increase in the level of ROS, which activates caspase 3, apoptosis and cell shrinkage via KCl loss through activation of the proximal tubule cell TASK2 K^+channel (Barriere et al. 2003)) and VSOR Cl^- channels.

It is clear from these elaborate studies that Cd^{2+} may interfere with several transport processes along the nephron.

Cadmium and the Na^+K^+-ATPase

A key membrane transporter in cell homeostasis and often the prime mover in active transepithelial transport is the Na^+K^+-ATPase. It is an integral enzyme that couples the hydrolysis of ATP to the vectorial transport of Na^+ and K^+ across the plasma membrane, maintaining electrochemical gradients for Na^+ and K^+. The Na^+ electrochemical gradient is the primary energy source for the secondary and tertiary active transport of various inorganic ions and small organic molecules (Reuss et al. 1996). The K^+ electrochemical gradient creates an inside negative membrane potential which provides an extra driving force for electrogenic transporters. Damage to this enzyme will therefore have a major impact on basic cellular functions and may lead indirectly to dysfunction of other Na^+-dependent transporters, even if those transporters remain undamaged themselves (Reuss et al. 1996).

The papers discussed below concern renal tissue, cells or cell fractions exposed to Cd^{2+} either in vivo (Table 2) or in vitro (Table 3) or other tissues (Table 4). Along the way we each time refer to the relevant table, where details of the experimental conditions can be found. Earlier studies had already shown that exposure to Cd^{2+} inhibited Na^+K^+-ATPase activity in the renal cortex of dogs (Nechay and Saunders 1977), rats (Kim et al. 1988, Table 2) and rabbits (Kinne-Saffran et al. 1993, Table 3). In

Table 2 Effects of Cd^{2+} on membrane transport systems: renal Na^+K^+-ATPase activity in vivo

Cd^{2+} effect	Tissue/animal	Cd^{2+} administration route	Reference
Loss of basolateral invaginations in the cortical proximal tubules and decreased immunostaining of associated Na^+K^+-ATPase	Male Wistar rats	Sc 2 mg Cd^{2+}(as $CdCl_2$)/kg body weight/day for 2 weeks	Sabolic et al. (2006)
After 6–12 h loss of basolateral invaginations in the cortical proximal tubules, no decreased immunostaining of associated Na+K+ATPase	Male Wistar rats	Acute Cd^{2+} nephrotoxicity, single sc injection of 0.4 mg Cd^{2+} (as Cd MT)/kg body weight	Sabolic et al. (2006)
60% reduction in activity, extensive loss of the basal plasma membrane infoldings	Rat kidney cortex homogenate of exposed animals	Repetitive ip administration of Cd^{2+}, cortical Cd^{2+} 200 ppm wet weight	Gonick (2008)
Inverse relationship between Cd^{2+} content and Na^+K^+-ATPase activity	Rat kidney microsomes of exposed animals	Repetitive ip administration of Cd^{2+}, cortical Cd^{2+} 200 ppm wet weight	Gonick (2008)
Downregulation after 4 weeks, recovery within 30 weeks, downregulation after 1 year	Mouse kidney cortex of exposed animals	100 mg Cd^{2+}/l (as $CdCl_2$) in the drinking water for up to 1 year	Jolling (2008)
Amount of protein was doubled after 1 week, still slightly increased after 3 weeks	Rat renal cortex of intoxicated animals, immunoblots	2 mg Cd^{2+}/kg/day sc for 3 weeks	Ahn et al. (2005)
Significantly inhibited	Renal cortical slices of intoxicated rats	Sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight for 16 days	Kim et al. (1988)

Table 3 Effects of Cd^{2+} on membrane transport systems: renal Na^+K^+-ATPase activity in vitro

Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$	Reference
Inhibition I_{50} 19 μM	Lyophilised membranes of rabbit kidney outer medulla at 37°	10 min 1 μM to 1 mM $CdCl_2$ concentrations	Kinne-Saffran et al. (1993)
$CdCl_2$ induces ROS that causes degradation of Na^+/K^+-ATPase via proteasomal and endo-/lysosomal proteolytic pathways	Immortalized cells (WKPT-0293 Cl.2) of the S1 segment of the proximal tubule of normotensive Wistar-Kyoto rats (RPTC)	5 μM $CdCl_2$ up to 72 h	Thévenod and Friedmann (1999)
Decrease in Na^+K^+-ATPase activity to 68.1% and 49.8%, in 2.5 and 5 μM $CdAc_2$, respectively, correlated with production of ROS and mitochondrial dysfunction	Primary cultures of rat proximal tubular cells on solid support, first passage	2.5 and 5 μM $CdAc_2$ in serum free medium for 12 h	Wang et al. (2009)
Reduction of transepithelial resistance (basolateral exposure), but no effect on Na^+K^+-ATPase activity,	Primary culture of human proximal tubule cells on Millicell HA filters in serum free medium, passage 5–7	0.5, 1.0 and 3.0 μg/ml (=4.5, 9 and 27 μM) of $CdCl_2$ apical and basolateral exposure for 4 days	Hazen-Martin et al. (1993)
Delayed effect on basolateral Na^+-K^+-ATPase detected via electrophysiological measurements	in vitro luminally perfused isolated S2 segments of renal proximal tubules of female Japanese White rabbits	Luminal Cd^{2+} as CdMT, 1–10 min, 8.9 μM; $CdCl_2$ or basolateral CdMT are less potent	Tsuruoka et al. (2000)

Table 4 Effects of Cd^{2+} on membrane transport systems: Na^+K^+-ATPase activity, other tissues

Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
Inhibition IC_{50} 2.6 μM	Tilapia intestine i/o BLMV	$CdCl_2$ nM to μM concentrations		Schoenmakers et al. (1992)
Decrease of enzyme activity after 1 week, recovery after 1 month	Hepatic microsomes from exposed adult male Charles-Foster rats		Single ip injection of 0.84 mg Cd^{2+} (as $CdAc_2$)/kg body weight	Modi et al. (2008)
Inhibition EC_{50} 50 μM, protection by sulfhydryl agents	Rat brain microsomes	Treatment with Cd^{2+} in vitro		Chetty et al. (1992)
Inhibition I_{50} 13 μM	Lyophilised membranes of shark rectal gland at 15°	30 min 1 μM to 1 mM $CdCl_2$ concentrations		Kinne-Saffran et al. (1993)
Maximal inhibition (80%) at 5 μM (branchial) and 50 μM (intestinal)	Crude homogenates of intestinal & branchial tissue of European eel	1 h 0.5–50 μM $CdCl_2$		Lionetto et al. (1998)

addition, Cd^{2+} exposure also reduced Na^+K^+-ATPase activity in a variety of tissues such as *Tilapia* intestine (Schoenmakers et al. 1992, Table 4), membranes of shark rectal gland, outer medulla in rabbits (Kinne-Saffran et al. 1993, Tables 3, 4) and intestinal and branchial tissue of the European eel (Lionetto et al. 1998, Table 4). In a short term exposure study on luminally perfused rabbit S2 proximal tubules, Tsuruoka et al. could mimick the depolarizing effect of a basolateral application of ouabain with the luminal application of 8.9 μM cadmium metallothionein (CdMt) (Tsuruoka et al. 2000, Table 3). The CdMt has been taken up by the cells and exerts an effect via the cytoplasm. The group of Thévenod studied the causal mechanisms behind these observations in renal cells in culture (Thévenod 2003). Although Cd^{2+} is not a Fenton metal and cannot directly generate ROS, free Cd^{2+} generates ROS in the cytosol of proximal tubule cells in the presence of metalloproteins containing Fenton metals (e.g. Fe^{2+}), as mentioned in the review by Moulis (Moulis 2010) (this issue). This suggests that

Cd^{2+}-mediated production of ROS takes place as a consequence of Cd^{2+}-induced displacement of redox active metals and subsequent damage to critical organelles or is due to a decrease of endogenous radical scavengers. Thévenod et al. exposed immortalized cells (WKPT-0293 Cl.2) of the S1 proximal tubule segment of normotensive Wistar-Kyoto rats (RPTC) to 5 μM $CdCl_2$ (Thévenod and Friedmann 1999 (Table 3), Thévenod 2003). Cd^{2+} increased the production of reactive oxygen intermediates, decreasing the stability of the α1-subunit of the Na^+K^+-ATPase, which is subsequently degraded by both endo-lysosomal proteases and the ubiquitine-proteasome complex. Gonick suggested an alternative, or possibly complementary explanation for the decreased activity, next to the oxidative stress-related theory of the group of Thévenod, i.e. a direct effect of intracellular Cd^{2+} on the Na^+K^+-ATPase which was enriched in the microsomal fraction (Reviewed in Gonick 2008, Table 2). Rats developed the Fanconi syndrome after 3 weeks of repetitive ip administration of Cd^{2+}, accompanied with a 60% drop in Na^+K^+-ATPase activity of the renal cortex homogenate. The authors studied the distribution of Cd^{2+} by fractionation of the kidney homogenate after having exposed the animals and found an inverse relationship between the Cd^{2+} content and the Na^+K^+-ATPase activity of the microsomes. They hypothesize that, after saturation of the kidney cortex with Cd^{2+} mostly complexed to Mt and after exhaustion of the organism's capacity to synthesize new Mt, the excess Cd^{2+} in the kidney spills over from the saturated protein-binding sites to the Cd^{2+} sensitive enzymes, including the Na^+K^+-ATPase, found in soluble cytoplasm as well as in microsomal and mitochondrial fractions. The authors concluded overall that the effect of Cd^{2+} exposure on Na^+K^+-ATPase activity might contribute to the clinical manifestation of Cd^{2+}-induced nephropathy (Thévenod and Friedmann 1999 (Table 3); Thévenod 2003; Gonick 2008 (Table 2)). A recent study on the effect of cadmium acetate, $CdAc_2$ (2.5 and 5 μM) on primary cultures of rat proximal tubular cells showed a significant production of ROS in a dose-dependent manner. At the same time, the Na^+K^+-ATPase activity was significantly decreased also in a dose-dependent manner (Wang et al. 2009, Table 3), providing a confirmation of the findings of Thévenod and Friedman (Thévenod and Friedmann 1999, Table 3). In contrast, Hazen-Martin et al. observed no effect on the activity of Na^+K^+-ATPase in cell cultures of human proximal tubule cells after 4 days of exposure to 0.5, 1.0 and 3.0 μg/ml (4.5, 9 and 27 μM) of $CdCl_2$ (Hazen-Martin et al. 1993, Table 3). Sabolic et al. exposed male Wistar rats to a subchronic nephrotoxicity by injecting them subcutaneously (sc) with 2 mg Cd^{2+}/kg body weight/day for 2 weeks or to an acute Cd^{2+} nephrotoxicity by injecting a single dose of 0.4 mg CdMt/kg body weight (Sabolic et al. 2006, Table 2). Both experimental treatments caused a loss of basolateral invaginations in the cortical proximal tubules, which was accompanied by a decreased immunostaining for Na^+K^+-ATPase. The loss of basolateral invaginations appeared to be independent of clathrin, indicating that the decreased activity of the enzyme was not due to clathrin-dependent endocytosis. Although oxidative stress levels were not measured, the observed effects were attributed to an enhanced cellular production of ROS, as described by Thévenod and Friedmann (1999) (Table 3).

Ahn et al. on the other hand reported an increase of the Na^+K^+-ATPase alpha subunit in the rat kidney cortex after 1 and 3 weeks of daily sc injections of 2 mg Cd^{2+}/kg body weight/day (Ahn et al. 2005, Table 2). Preliminary results in mice, exposed to 100 mg Cd^{2+}/l (as $CdCl_2$) in the drinking water for up to 1 year, show that the relative gene expression of the Na^+K^+-ATPase in the kidney cortex was downregulated after 4 weeks, recovered the following weeks (up to 30 weeks). But after 1 year the expression was reduced again and so was the amount of protein as observed in a Western blot (Jolling 2008, Table 2). Interestingly, a biphasic response of enzymes in defense against ROS, was found in parallel with this change in expression of the Na^+K^+-ATPase (and of the SGLT cotransporters, see section on Cd^{2+} and glucose transport) (Thijssen et al. 2007a).

A few studies have been done on other tissues than the kidney. Modi et al. induced Cd^{2+} nephropathy in vivo by treating adult male Charles-Foster rats with a single ip injection of 2 mg of $CdAc_2$/kg body weight, i.e. 0.84 mg Cd^{2+}/kg body weight (Modi et al. 2008, Table 4). One group of animals was killed 1 week and the other group 1 month after treatment, after which hepatic microsomes were isolated. The activity of the Na^+K^+-ATPase decreased in the 1-week Cd^{2+}-treated animals, whereas it recovered in the 1-month Cd^{2+} -treated rats. A possible explanation for the

reduced activity after 1 week might be that Cd^{2+} interfered with the phosphorylation state of the ATPase, whereas after 1 month, the Cd^{2+} level in the system became too low to have an effect (Modi et al. 2008, Table 4). It should be noted that oxidative stress levels were not measured in this study. Reduced activity of the Na^+K^+-ATPase by Cd^{2+} in microsomes in vitro, isolated from rat brain tissue, has also been reported, although the EC_{50} for Cd^{2+} was rather high (50 μM) and may not be relevant for the in vivo situation. Thiol reagents protected against the Cd^{2+} inhibition (Chetty et al. 1992, Table 4).

Summarizing, exposure to cadmium has a detrimental effect on Na^+K^+-ATPase activity. From some studies, it seems that after an initial decline in the activity of the ATPase, intoxicated animals may show a defensive pattern. The cells try to cope with oxidative stress and upregulate the Na^+K^+-ATPase, while if doses are too high and/or time of exposure becomes too long the toxic effect overrules.

Cadmium and ion transport across membranes and epithelia

First of all it needs to be mentioned, that in research on ion channels and ionotropic receptors, heavy metals are often used primarily as a tool to unravel the properties of the channels as such, e.g. selectivity, activation, inactivation. These properties may be altered by binding of the metals to charged amino acids, sugar groups, thiol groups and/or by screening of the negative surface charges of the membrane by the metals. The authors often (but not always e.g. (Thévenod and Jones 1992)) use rather high Cd^{2+} concentrations in the millimolar or high micromolar range. These are not necessarily relevant to the Cd^{2+} concentrations found in an exposed or a fortiori in the general population or even in animals exposed experimentally. Such effects of heavy metals on ion channels in excitable membranes were reviewed in 1994 by Kiss and Osipenko (Kiss and Osipenko 1994). Briefly, the authors reviewed effects on voltage-activated ion channels, Ca^{2+} activated currents, ionic pumps and agonist-operated channels. They also discussed conductances activated by the toxic metals as such.

In this review we want to focus on the interference of Cd^{2+} with cell membrane transport processes in relationship with toxic effects observed in organisms.

Calcium transport

A survey of the papers discussed on Cd^{2+} effects on Ca^{2+} pathways is given in Table 5.

The epithelial Ca^{2+} channel (ECaC) was found to be blocked by Cd^{2+} (IC_{50} 2.5 μM, (Vennekens et al. 2001)). The ECaC channel is one of the steps involved in transcellular Ca^{2+} reabsorption in the kidney.

As discussed above (see section "Cadmium and salt excretion in rat kidney") the Ca^{2+} reabsorption is hampered by Cd^{2+} in the rat kidney at different sites along the nephron. ECaC channels may be involved. The effect in the proximal tubule may be partly explained by the diminished fluid uptake, reducing the solvent drag effect and paracellular Ca^{2+} reabsorption (Barbier et al. 2004). Leffler et al. made a single sc injection of CdMt (0.4 mg Cd^{2+}/kg body weight rat) and isolated basolateral (BLMV) and luminal membrane vesicles (BBMV) at different moments during the following period of 24 h (Leffler et al. 2000). The results suggested an impaired Ca^{2+} uptake across the luminal membrane and an even larger inhibition of Ca^{2+} extrusion across the basolateral membrane. The net result would be a decreased Ca^{2+} reabsorption with an increased Ca^{2+} content in the kidney cortex, as observed by the authors. Wang et al. treated primary cultures of rat proximal tubule cells with 2.5 and 5 μM $CdAc_2$ for 12 h and found a reduction in the Ca^{2+}-ATPase activity to 65 and 58% respectively (Wang et al. 2009).

Verbost et al. used the gill of the rainbow trout as a model for a tight epithelium transporting Ca^{2+} (Verbost et al. 1987a). The gills possess apical Ca^{2+} channels and a basolateral Ca^{2+}-ATPase as in kidney and intestinal epithelium. The authors measured unidirectional $^{45}Ca^{2+}$ fluxes and found that the influx was reduced after 16 h pre-incubation of the gills with 0.1 μM $Cd(NO_3)_2$. Cd^{2+} needed to enter the cells first. In a further study they found that the ATP-dependent Ca^{2+} transport in BLMV from the gills was inhibited by nanomolar concentrations of Cd^{2+} (Verbost et al. 1988). This may be a direct effect on the Ca^{2+} pump. A similar low nanomolar Cd^{2+} effect on the Ca^{2+}-ATPase was found in red blood cells ($K_i = 2$ nM Cd^{2+}) and in intestinal epithelium (Verbost et al. 1987b, 1988, 1989). Schoenmakers reports a reduction in ATP driven

Table 5 Effects of Cd^{2+} on membrane transport systems: Ca^{2+} pathways

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
ECaC	Block by Cd^{2+} of whole cell Ca^{2+} current, IC50 2.5 μM	Rabbit ECaC expressed in HEK 293 cells	0–100 μM Cd^{2+} in bath solution		Vennekens et al. (2001)
Ca^{2+}reabsorption, possibly ECaC channels	Inhibition of reabsorption, possibly at several levels along the nephron	Rat kidney		Luminal micro-injection of $^{45}Ca^{2+}$ with 20 μM Cd^{2+} at several sites along the nephron	Barbier et al. (2004)
Unidirectional $^{45}Ca^{2+}$ uptake	Impaired Ca^{2+} uptake across the luminal membrane, inhibition of Ca^{2+} extrusion across the basolateral membrane, overall effect Ca^{2+} accumulation in the kidney cortex	Rat kidney cortex, BLMV and BBMV 4, 12, 24 h after injection		Single sc injection of cadmium-metallothionein, CdMt, 0.4 mg Cd^{2+}/kg body weight	Leffler et al. (2000)
Ca^{2+}-ATPase activity	Decrease in the Ca^{2+}-ATPase activity to 65.2 and 58.4% of the control value in 2.5 and 5 μM $CdAc_2$, respectively, correlated with production of ROS and mitochondrial dysfunction	Primary cultures of rat proximal tubular cells on solid support, first passage	2.5 and 5 μM $CdAc_2$ in serum free medium for 12 h		Wang et al. (2009)
Unidirectional $^{45}Ca^{2+}$ fluxes	Analysis of effects of preincubation with Cd^{2+} suggests inhibition of Ca^{2+} uptake, possibly via blocking of Ca^{2+} pump	Gill of the rainbow trout		Saline perfused head preparation, 16 h pre-incubation of the gills with 0.1 μM $Cd(NO_3)_2$	Verbost et al. (1987a)
Ca^{2+}-ATPase	Nanomolar concentrations of Cd^{2+} inhibit ATP dependent ^{45}Ca uptake (IC of competitive unhibition in intestinal vesicles: 1.6 nM)	Permeabilized red blood cells, basolateral membrane vesicles from intestinal epithelium and of kidney cortex of rat	Nanomolar concentrations Cd^{2+}		Verbost et al. (1987b)
ATP-dependent Ca^{2+} transport	Possibly direct inhibitory effect of Cd^{2+} on Ca^{2+}-ATPase, I_{50} value of 3.0 nM, no effect on V_{max}, increase in $K_{0.5}$ for Ca^{2+}	BLMV from gill of the rainbow trout	Nanomolar concentrations		Verbost et al. (1988)
Ca^{2+}-ATPase	Cd^{2+} competitively inhibits ATP-dependent transmembrane $^{45}Ca^{2+}$ transport, K_i 2 nM	Human erythrocytes	Nanomolar concentrations $Cd(N0_3)_2$		Verbost et al. (1989)
ATP driven Ca^{2+} uptake	Inhibition IC_{50} 8.2 pM	*Tilapia* intestine i/o BLMV	$CdCl_2$ nM to μM concentrations		Schoenmakers et al. (1992)

Table 5 continued

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
Na^+/Ca^{2+} exchange	Inhibition IC_{50} 73 nM	*Tilapia* intestine i/o BLMV	$CdCl_2$ nM to µM concentrations		Schoenmakers et al. (1992)
ECaC and NCX mRNA levels	No effect on expression of the transporters, except NCX mRNA after 1 week	Rainbow trout		Cd^{2+} exposure (3 µg/l waterborne Cd^{2+}, 500 mg/kg dietary Cd^{2+}) for up to 4 weeks	Galvez et al. (2007)
Ca^{2+}channel	50% block of whole cell Ca^{2+} current in 300 nM Cd^{2+}	Neuron isolated from caudal paravertebral sympathetic ganglia in frog	0–3 µM $CdCl_2$ in bath solution		Thévenod and Jones (1992)
Ca^{2+} uptake at 0.5 mM Ca^{2+}	Inhibition of Ca^{2+} uptake, K_i of Cd^{2+} 36 µM	Cell suspension of the rainbow trout head kidney	0–100 µM Cd^{2+} (mostly as nitrate)		Gagnon et al. (2007)
Ca^{2+} uptake	Cd^{2+} concentration dependent inhibition (possibly via Cd^{2+}/thiol interaction), increase in K_m of Ca^{2+}, no change in V_{max}	WRL-68 cells (fetal hepatic cell line)	Range between 1 and 100 µM, 30 min		Souza et al. (1996)

Ca^{2+} uptake into i/o vesicles of fish intestinal basolateral membrane vesicles with an IC_{50} for Cd^{2+} as low as 8 pM (Schoenmakers et al. 1992). From these studies it seems that Cd^{2+} may interfere with the Ca^{2+}-ATPase at very low doses, similar to those found in vivo.

As far as other Ca^{2+} transporters in the fish gill are concerned Galvez et al. found little or no interference of waterborne or dietary Cd^{2+} (3 µg/l waterborne Cd^{2+}, 500 mg/kg dietary Cd^{2+}) with the expression of other Ca^{2+} transporters (ECaC and Na^+-Ca^{2+} exchanger (NCX) mRNA levels) in the gill, except NCX mRNA after 7 days and no interaction of Ca^{2+} dietary supplementation on Cd^{2+} effects (Galvez et al. 2007).

Cd^{2+} block of Ca^{2+} channels (Hille 2001) may also affect the function of excitable cells where Ca^{2+} channels play a role in electrophysiological phenomena (Thévenod and Jones 1992) or cells with Ca^{2+} channels involved in signal transduction. For instance, Cd^{2+} is an endocrine disruptor in fish and has been shown to have a direct effect on fish head kidney cells (Lacroix and Hontela 2004). These cells are steroidogenic and secrete cortisol when needed. Ca^{2+} plays a role in the cellular signaling. Gagnon et al. studied the interaction of Cd^{2+} with Ca^{2+} uptake in head kidney cells of the rainbow trout and found a concentration-dependent reciprocal inhibition between Ca^{2+} and Cd^{2+}: Ca^{2+} uptake at 0.5 mM was inhibited by Cd^{2+} with a Ki of 36 µM (Gagnon et al. 2007).

In liver cells, Cd^{2+} concentrations in the range between 1 and 100 µM were tested and have an inhibitory effect on Ca^{2+} uptake by a human fetal hepatic cell line (WRL-68 cells). The channel or transporter could only be speculated on, but Cd^{2+}/thiol interaction seemed to play a role (Souza et al. 1996).

Finally, Cd^{2+} may use Ca^{2+} channels to enter cells (for more details on Cd^{2+} uptake and interference with Ca^{2+} channels, see review by Thévenod, this issue (2010)).

Zinc transport

A survey of the papers discussed on Cd^{2+} effects on Zn^{2+} pathways is given in Table 6.

The studies by Poujeol's group (see section "Cadmium and salt excretion by the kidney" above) suggested an interference of Cd^{2+} with Zn^{2+} reabsorption in rat kidney. Kaur et al. investigated Zn^{2+} transport in rat renal BLMVs and its inhibition by

Table 6 Effects of Cd^{2+} on membrane transport systems: Zn^{2+} pathways

Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
Inhibition of reabsorption, possibly at several levels along the nephron (no statistics)	Rat kidney		Luminal micro-injection of $^{65}Zn^{2+}$ with 50 μM Cd^{2+} at several sites along the nephron	Barbier et al. (2004)
Competitive (K_i Cd^{2+} 13 μM) and non-competitive (K_i Cd^{2+} 72 μM) inhibition of 0.5 min $^{65}Zn^{2+}$ uptake	Isolated proximal tubule cells from rabbit kidney	15 and 50 μM $CdCl_2$		Gachot and Poujeol (1992)
Competitive inhibition of Zn^{2+} uptake by Cd^{2+} with a K_i of 3.9 mM	BLMVs isolated from normal rat kidney	2 mM Cd^{2+} up to 60 min		Kaur et al. (2006)
Reduction of Zn^{2+} reabsorption, possibly decrease in the number of Zn^{2+} transporters	BLMVs isolated from kidneys of Cd^{2+} exposed rats		Sc injections of $CdCl_2$ dose of 0.6 mg/kg/day during 12 weeks (5 days/week)	Kaur et al. (2006)

Cd^{2+} in vivo and in vitro (Kaur et al. 2006). The authors first measured Zn^{2+} uptake by BLMVs isolated from normal rat kidney and found that the uptake was competitively inhibited in the presence of 2 mM Cd^{2+} with a K_i of 3.9 mM. This seems to be a very high concentration however compared to Cd^{2+} concentrations found in vivo. Rats that received subcutaneous (sc) injections of 0.6 mg $CdCl_2$/kg/day for 5 days/week during 12 weeks, developed renal failure with proximal tubular degeneration and a significantly high urinary excretion of Cd^{2+}, Zn^{2+} and proteins. Zn^{2+} uptake in BLMVs isolated from kidneys of Cd^{2+} exposed rats was significantly reduced, possibly due to a decrease in the number of Zn^{2+} transporters. In 1992, Gachot and Poujeol observed an inhibition of Zn^{2+} uptake in isolated proximal tubule cells from rabbit kidney in the presence of 15 and 50 μM $CdCl_2$ (Gachot and Poujeol 1992).

Zn^{2+} transporters have been identified by now (see Moulis, this issue 2010; Himeno et al. 2009) and interaction with Cd^{2+} and other metals have been described (reviewed by Himeno et al. 2009; Moulis 2010).

Chloride transport

A survey of the papers discussed on Cd^{2+} effects on Cl^- pathways is given in Table 7.

A6 cells, derived from the kidney of Xenopus laevis, are commonly used as a model to explore the cell biology of distal nephron epithelial cells. The distal nephron is highly regulated and is involved in NaCl reabsorption and dilution of the tubular luminal fluid (Boron and Boulpaep 2009). Interfering with its function may disturb the salt and water homeostasis in the body. Faurskov and Bjerregaard (1997, 2000, 2002) studied A6 cells in an Ussing chamber and found that the transepithelial resistance (TER) of the cell layer started to decrease after 40 min when exposed to $CdCl_2$ either from the apical (EC_{50} of 174 μM Cd^{2+}) or the basolateral side (EC_{50} of 148 μM Cd^{2+}). When applying Cd^{2+} (>100 μM, EC_{50} 563 μM) to the basolateral side for less than 40 min, before the drop in TER occurred, a significant transient increase in short circuit current was observed. It could be mimicked by applying thapsigargin, a blocker of the endoplasmic Ca^{2+} pump or by inducing an increase in intracellular Ca^{2+} with the Ca^{2+} ionophore A23187. The increase in short circuit current was abolished in Cl^--free media or by blockers of Ca^{2+} activated Cl^- channels (apical side) and of Na/Cl cotransport (basolaterally), but not by amiloride (apical side), a Na^+ channel blocker. A rise in intracellular Ca^{2+} and a fall in Cl^- after applying Cd^{2+} could be demonstrated with the appropriate fluorescent dyes. The Cd^{2+} effect could be mimicked (and abolished) by applying the closely related metals Zn^{2+} or Ni^{2+}. The authors hypothesize that Cd^{2+} at the basolateral side causes intracellular Ca^{2+} mobilization that activates chloride channels leading to chloride secretion in A6 cells (Faurskov and Bjerregaard 1997, 2000, 2002). It must be noted

Table 7 Effects of Cd^{2+} on membrane transport systems: Cl^- and K^+ pathways

Transport system	Cd^{2+} effect	Tissue/animal	[Cd^{2+}] in vitro	Cd^{2+} administration route in vivo	Reference
Cl^- channels and Cl^- current	40 min or less of $CdCl_2$ mobilizes intracellular Ca^{2+} and activates Cl^- current, Cd^{2+} threshold 100 μM	Confluent A6 cells grown on Costar filters	1 mM $CdCl_2$ in serum free medium in Ussing chamber, basolateral side		Faurskov and Bjerregaard (1997)
Cl^- channels and Cl^- current	Cd^{2+} mobilizes intracellular Ca^{2+}, which activates Cl^- current, half stimulation at 386 μM Cd^{2+}	Confluent A6 cells grown on Costar filters	1 mM $CdCl_2$ in serum free medium in Ussing chamber, basolateral side		Faurskov and Bjerregaard (2000, 2002)
Cl^- conductance/ CFTR channel	Activation of CFTR in minutes, loss of GSH	Proximal tubule cell cultures from wild type *cftr*$^{+/+}$ and *cftr*$^{-/-}$ mice	5 and 10 μM Cd^{2+} within minutes		L'Hoste et al. (2009)
TASK-2 K^+channel and VSOR Cl-channels	Cd^{2+} induces increase in ROS followed by activation of K^+ and Cl^- channels	Proximal tubule cell cultures from wild type *cftr*$^{+/+}$ and *cftr*$^{-/-}$ mice	5 and 10 μM Cd^{2+} up to 60 min		L'Hoste et al. (2009)
K^+ conductance	Increase possibly via intracellular Ca^{2+} release, half maximal effect of Cd^{2+} at ~0.2 μM	Subconfluent MDCK cells on solid support	(sub)micromolar concentrations of Cd^{2+} 1 min		Jungwirth et al. (1990)
K^+ conductance	Cd^{2+} caused an increase in K^+ conductance from the basolateral side	Proximal tubule cells in frog kidney in situ		Aortic (luminal) and peritubular (basolateral) perfusion with 1 or 3 μM $CdCl_2$	Nesovic-Ostojic et al. (2008)

that the Cd^{2+} concentrations used in these studies (10 μM–1 mM) are rather high.

Potassium transport

A survey of the papers discussed on Cd^{2+} effects on K^+ pathways is given in Table 7.

K^+ channels exist in many different forms and have diverse functions. The K^+ conductance is important in stabilizing membranes of excitable cells, or in creating a negative cell potential in transporting epithelia (see above). Apical K^+ channels also play a role in K^+ reabsorption or secretion in the kidney (Boron and Boulpaep 2009). Barbier et al. (2004), in clearance experiments in rats (see section "Cadmium and salt excretion in rat kidney"), suggested that blocking of the Renal Outer Medullary Potassium channel (ROMK) in the thick ascending limb by luminal Cd^{2+} may have prevented the K^+ recycling in the apical membrane and explain the urinary K^+ loss and increased EF they observe: part of the K^+ reabsorption occurs transcellularly in the TAL. It makes use of a luminal NKCC2 transporter (Na/K/Cl cotransporter) and a basolateral K^+ channel. The apical ROMK channel provides a mechanism for recycling enough K^+ from the cell back to the lumen so that luminal K^+ does not fall so low as to jeopardize Na/K/Cl cotransport (Boron and Boulpaep 2009).

Jungwirth et al. on the other hand used a kidney derived cell line (Madin Darbey canine kidney or MDCK cells), grown on solid support to incompletely confluent cell layers and found that (sub)micromolar concentrations of Cd^{2+} lead to a rapid, sustained and reversible hyperpolarization of the cell membrane, paralleled by an increase in the K^+selectivity and a

decrease in the membrane resistance (Jungwirth et al. 1990). Thus, Cd^{2+} increased the K^+conductance of the cell membrane in these cells. The half maximal effect is elicited at 0.2 μM Cd^{2+}. The results suggested that also in these cells Cd^{2+} induces an increase in intracellular Ca^{2+}, as reported by Faurskov and Bjerregaard in A6 cells (Faurskov and Bjerregaard 2002). This in turn activates the K^+ conductance, although the Cd^{2+} concentrations applied by Jungwirth et al. that induced a raise in intracellular Ca^{2+} were much lower than in the study of Faurskov and Bjerregaard mentioned above. Nesovic-Ostojic et al. measured the basolateral membrane potential of the proximal tubule cells in frog kidney in situ. The authors applied Cd^{2+} concentrations (1 or 3 μM Cd^{2+}), similar to those used by Jungwirth et al. (1990), at either the basolateral or the luminal side of the tubule via the aortic and peritubular perfusion. Cd^{2+} caused a comparable increase in K^+ conductance when applied from the basolateral side (Nesovic-Ostojic et al. 2008).

Interference of Cd^{2+} with the membrane potential may have an impact on the transepithelial electrogenic transport and deregulate normal transport rates.

Cadmium, calcium, phosphate and bone

In this section we consider studies that try to find evidence for the hypothesis that Cd^{2+} impairs Ca^{2+} and P_i reabsorption in the kidney in the first place and therefore causes bone loss in the second place, i.e. in an indirect way, versus studies that suggest that the effect of Cd might be a direct one on bone.

Urinary Ca^{2+} and P_i loss after exposure to Cd^{2+} has been described many times in humans and in experimental models. Also known is that Cd^{2+} exposure causes loss of bone. Well known for instance is the Itai-itai disease (Japanese for Ouch-Ouch disease), a bone disease with fractures and severe pain that occurred after World War II in Japan. The population was exposed to Cd^{2+} by the consumption of contaminated rice. Decalcification and fractures occurred as well as compression fractures of the spine (Nordberg 2009). The hypothesis arose that Cd^{2+} may impair the Ca^{2+} tubular reabsorption in the kidney, causing loss of plasma Ca^{2+}, which in turn induced Ca^{2+} extraction from bone. In a study on Japanese women exposed to Cd^{2+} contaminated rice Horiguchi et al. made a detailed statistical analysis of kidney function and bone loss and hypothesized that kidney impairment precedes bone loss and that impaired tubular reabsorption and renal loss of Ca^{2+} induced PTH release and extraction of Ca^{2+} from bone (Horiguchi et al. 2005) .

Normally about 99.5% of filtered Ca^{2+} is reabsorbed by the nephron (65% in the proximal tubule, 25% in the thick ascending limb (TAL), 8% in the distal convoluted tubule (DCT) and 1.5% in the collecting duct. The Ca^{2+} transport mechanisms that could be involved are apical Ca^{2+} channels (all segments), solvent drag (proximal tubule), the basolateral Ca^{2+} ATPase and the $3Na^+/Ca^{2+}$ exchanger (all segments) (Boron and Boulpaep 2009).

To assess whether Cd^{2+} interferes directly with Ca^{2+} reabsorption in the kidney, detailed studies on the Ca^{2+} transport mechanisms at the level of the kidney tubules, cells and cell membranes are needed. Studies by Barbier et al. (2004) and Leffler et al. (2000) were discussed earlier. Briefly, Ca^{2+} reabsorption, possibly via the ECaC, is reduced by micromolar Cd^{2+} in the rat kidney at different sites along the nephron. (Barbier et al. 2004). A blockage of the epithelial Ca^{2+} channel (ECaC) by Cd^{2+} (IC_{50} 2.5 μM) has also been mentioned earlier (Vennekens et al. 2001). Studies on rat kidney BBMV and BLMV of intoxicated animals showed that basolateral Ca^{2+} extrusion may be inhibited even more (Leffler et al. 2000).

A key element in the overall homeostasis of P_i is the handling by the kidney. A survey of the papers discussed on Cd^{2+} effects on P_i pathways is given in Table 8.

Of all P_i filtered, 80% is reabsorbed in the proximal tubule, 10% in the distal tubule and 10% is excreted (Boron and Boulpaep 2009). Proximal tubule reabsorption of P_i involves secondary active P_i transport mechanisms. Key players in this process are Na–P_i cotransporters which have been identified in the brush border membrane (BBM) of the proximal tubule (reviewed by Murer et al. 2000; Forster et al. 2006). Regulators are parathyroid hormone (PTH) and vitamin D. PTH induces phosphaturia by inhibiting brush border Na–P_i cotransport activity (by downregulation of the transporter), vitamin D is thought to increase tubular P_i reabsorption.

A direct acute inhibitory effect of Cd^{2+} on P_i transport has been shown in *Xenopus laevis* oocytes expressing the type IIa Na–P_i cotransporter (Wagner

Table 8 Effects of Cd^{2+} on membrane transport systems: P_i pathways

Transport system	Cd^{2+} effect	Tissue/animal	[Cd^{2+}] in vitro	Cd^{2+} administration route in vivo	Reference
Type IIa Na–P_i cotransporter	Cd^{2+} reduces P_i current with K_i of 0.32 mM, no change of K_m for P_i	Expressed in *Xenopus levis* oocytes	30 μM up to 1 mM, immediate effects on P_i current		Wagner et al. (1996)
Type II Na–Pi cotransporter	Reduction in expression of the protein	Renal cortical BBMV of exposed rats		2 mg Cd^{2+}/kg body weight, s.c. for 14 days	Herak-Kramberger et al. (1996)
Na^+-dependent P_i uptake	Free Cd^{2+} directly reduces P_i transport	Renal cortical BBMV of non-exposed rats	25–200 μM $CdCl_2$ 60 min		Ahn and Park (1995)
Na^+-dependent P_i uptake	Reduced P_i uptake in exposed animals	Renal cortical BBMV of exposed rats		$CdCl_2$ sc at a dose of 2 mg Cd^{2+}/kg body weight/day for 2 weeks	Ahn and Park (1995)
Na^+-dependent P_i uptake	Reduction of V_{max} (Cd^{2+} seems to act from inside the vesicle)	Renal cortical BBMVs of not pre-exposed rabbits	$CdCl_2$ (50 μM) 60 min 37°C		Park et al. (1997)
Na^+-dependent P_i uptake	CdMT had no effect	Renal cortical BBMVs of not pre-exposed rabbits	CdMT (0–100 μM) 60 min 37°C		Park et al. (1997)
Na^+-dependent P_i uptake	Dose dependent reduction in uptake, proportional with Cd^{2+} uptake, suggesting direct interaction with free Cd^{2+}	Rat renal cortical BBMV	Preincubation in 50, 100, 200 μM $CdCl_2$ 60 min		Ahn et al. (1999)

et al. 1996). Cd^{2+} reduced V_{max} (Cd^{2+} concentrations used were 30 μM up to 1 mM) (Wagner et al. 1996).

Proximal tubular brush border membrane Na–Pi cotransport is sensitive to intoxication by heavy metals. In vivo intoxication of rats with cadmium (2 mg Cd^{2+}/kg body weight, s.c. for 14 days) reduced brush border membrane Na–P_i cotransport rate accompanied by a loss of the type IIa Na–P_i cotransporter protein (Herak-Kramberger et al. 1996). Ahn and Park injected adult male Sprague–Dawley rats sc with $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight/day for 2 weeks. This induced marked polyuria, glucosuria, proteinuria, and phosphaturia, characteristic of chronic Cd^{2+} intoxication. The Cd^{2+} content of renal cortical BBMV from these rats was drastically increased and the Na^+-dependent P_i uptake was significantly attenuated. Brush border membrane vesicles from non-exposed animals directly exposed to free Cd^{2+} (25–200 μM) gave similar results (Ahn and Park 1995).

In a further study, the authors used rabbit renal cortical BBMVs of animals, not pre-exposed to Cd^{2+}. The BBMVs were exposed to $CdCl_2$ (50 μM) or CdMt (0–100 μM) in vitro for 60 min at 37°C, and P_i uptake was measured at 25°C. Na^+-dependent P_i uptake was decreased in the presence of $CdCl_2$, CdMt had no effect. Preincubation by $CdCl_2$ was necessary (30 min or more) and—after preincubation—extravesicular EDTA did not abolish the Cd^{2+} effect. So Cd^{2+} seems to act from inside the vesicle. Two Na^+ and 1 P_i were involved. Cd^{2+} decreased V_{max}, but had no effect on the stoechiometry or the equilibrium value (Park et al. 1997).

From these results it seems that Cd^{2+} intoxication, at the doses used in the in vitro studies at least, may directly impair the Na^+-phosphate cotransport system in the proximal tubular brush border membrane, which may lead to phosphaturia in the intact animal. Once again however, the question arises whether the Cd^{2+} concentrations used in vitro compare to those present in vivo in the intoxicated animal. Furthermore

recent developments suggest that kidney damage does not necessarily precede bone loss. For the inhabitants of the highly polluted Kakehashi river basin in Japan for instance Hayashi et al. found a correlation between Cd^{2+} exposure and renal damage, i.e. increased loss of urinary Ca^{2+}, P_i and beta2-microglobulin, but this did not greatly differ between groups with or without bone loss (Hayashi et al. 2003). Furthermore Schutte et al. actually measured PTH and showed that bone loss and urinary Ca^{2+} loss in humans went hand in hand with a decrease in PTH rather than an increase (Schutte et al. 2008). So it seems that the urinary Ca^{2+} loss may be due—at least in part—to a direct effect of Cd^{2+} on bone, causing an increased serum Ca^{2+} and filtration in the kidney and therefore loss of Ca^{2+} via the kidney and not the other way around.

Additionally, direct effects on bone have been described, reviewed by Bhattacharyya (2009). Cd^{2+} interferes with the pathways of osteclasts (at 10–500 nM Cd^{2+}) and osteoblasts (at 0.1–20 μM Cd^{2+}), the overall effect being that the balance between bone formation and breakdown is disturbed (Bhattacharyya 2009). This occurs at low Cd^{2+} exposures and at low Cd^{2+} blood concentrations in humans or experimental animals and may be independent of kidney effects in experimental animals and humans (see also Bhattacharyya et al. 1988; Honda et al. 2003; Schutte et al. 2008)

It is noteworthy that the nanomolar concentrations that seem to affect bone in vitro are close to those found in vivo. Again, this shows the importance of studying Cd^{2+} effects in vitro at relevant doses before making any conclusions concerning the in vivo situation.

Cadmium effects on membrane transport of glucose and amino acids

The glucose level in the plasma is highly regulated and glucose, as well as amino acids, is recovered from the renal ultrafiltrate. Under normal conditions glucose and amino acids are completely reabsorbed in the proximal tubule via Na^+-dependent transport processes (Boron and Boulpaep 2009). After Cd^{2+} intoxication, as mentioned earlier, glucosuria and aminoaciduria are commonly found clinical symptoms in experimental animals and humans. The effects of exposure to Cd^{2+} on Na^+-dependent glucose and amino acid transport have been studied in various experimental models. Experimental details of the papers discussed in this section can be followed in Table 9.

In BBMVs from the renal cortex of male Sprague–Dawley rats that were exposed daily to sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight during 2 weeks, the Na^+-dependent transport system for glucose and for the amino acid L-glutamate was seriously impaired compared to BBMVs from control rats receiving saline injections. Furthermore, Na^+-independent transport of glucose, the permeability of the BBM for Na^+, and glucose transport in BLMVs were not influenced by Cd^{2+} intoxication, suggesting that only the Na^+-dependent glucose transport in the BBMVs was affected. Kinetic analysis of the Na^+-dependent glucose transport in the BBMVs indicated that the reduced transport was due to a decrease in V_{max} and not to an effect on K_m, implying a Cd^{2+}-induced decrease of the number of transporters without a change in substrate affinity of the carrier (Lee et al. 1990). The authors also report a reduction in the Na^+-dependent L-glutamate uptake by BBMVs of intoxicated rats. Similar results were reported for Na^+-dependent glucose and L-glutamate transport from experiments with BBMVs isolated from the kidney cortex of male New Zealand white rabbits exposed to daily sc injections of $CdCl_2$ at a dose of 3 mg Cd^{2+}/kg body weight during 2 weeks (Lee et al. 1991). Furthermore, in primary cell cultures of mouse renal cortical cells, isolated from male C57bl6 mice, exposure to Cd^{2+} in doses ranging from 2.5 to 7.5 μM resulted in a dose-dependent decrease of the V_{max} of Na^+-glucose cotransport, without affecting the affinity of the transporters (Blumenthal et al. 1990). Kinne et al. demonstrated that the inhibition of L-glutamate transport in cortical BBMV isolated from male New Zealand white rabbits, preincubated for 30 min with 1 μM to 1 mM $CdCl_2$, could be attributed to a marked decrease in the V_{max} of the transport system (half maximal inhibition at 50 μM $CdCl_2$), whereas the affinity for glutamate seemed to increase if it changed at all (Kinne et al. 1995). Using phlorizin, a competitive inhibitor of Na^+-dependent glucose transport, Kim and Park demonstrated that the Cd^{2+}-induced decrease in V_{max} in renal cortical BBMV of rabbits may be accounted for by a decrease in the number of active Na^+-glucose cotransport units

Table 9 Effects of Cd^{2+} on membrane transport systems: transport of glucose and amino acids

Transport system	Cd^{2+} effect	Tissue/animal	[Cd^{2+}] in vitro	Cd^{2+} administration route in vivo	Reference
Na^+-dependent D-glucose uptake	Reduction in V_{max}, not K_m	Isolated renal proximal BBMV from $CdCl_2$ intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Lee et al. (1990)
Na^+-dependent L-glutamate uptake	Reduction in Na^+ dependent uptake	Isolated renal proximal BBMV from $CdCl_2$ intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Lee et al. (1990)
Na^+-dependent D-glucose and L-alanine transport	Seriously impaired (decrease in V_{max} and no effect on K_m)	Renal cortical BBMVs isolated from exposed male New Zealand white rabbits		3 mg Cd^{2+} as $CdCl_2$/kg/day for 2 weeks	Lee et al. (1991)
Na^+-dependent D-glucose and L-alanine transport	Similar results on direct exposure to free Cd^{2+}	Renal cortical BBMVs isolated from non-exposed male New Zealand white rabbits	in vitro exposure to Cd^{2+}		Lee et al. (1991)
Na+-dependent glucose uptake	Decrease of V_{max}, no effect on K_m	Primary culture of mouse kidney cortical tubule cells on solid support	0–6 μM Cd^{2+} preincubation for 24 h		Blumenthal et al. (1990)
Na^+-dependent L-glutamate transport	Marked decrease in the V_{max}, little effect on K_m, half maximal inhibition at 50 μM $CdCl_2$, CdMT no effect	Rabbit renal cortical BBMV	Preincubation with 1 μM to 1 mM $CdCl_2$ 30 min		Kinne et al. (1995)
Na^+-dependent D-glucose transport	Reduction in Na^+-dependent D-glucose uptake and phlorizin binding, i.e. units, no change in K_d	Renal cortical BBMV of intoxicated rabbits		Sc injections of $CdCl_2$ at a dose of 3 mg Cd^{2+}/kg body weight for about 3 weeks	Kim and Park (1995)
Na^+-dependent D-glucose transport	Similar results on direct exposure to free Cd^{2+}	Rabbit renal cortical BBMV of non-exposed rabbits	30 min preincubation in 50 μM $CdCl_2$		Kim and Park (1995)
Na^+-dependent D-glucose, L-alanine uptake	Dose dependent reduction in uptake, proportional with Cd^{2+} uptake, suggesting direct interaction with free Cd^{2+}	Rat renal cortical BBMV	60 min preincubation in 50, 100, 200 μM $CdCl_2$		Ahn et al. (1999)
Na^+-dependent transport of L-proline, L-alanine, and L-lysine	Marked attenuation of uptake	Rat renal cortical BBMV from exposed animals		Sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight for 2 weeks	Kim et al. (1990)
SGLT1 mRNA and Na^+-dependent glucose transport	SGLT1 mRNA decreased, followed by reduction in glucose transport itself	Primary culture of mouse renal cortical tubule cells on solid support	7.5 μM Cd_7MT for 24, 48 and 72 h		Blumenthal et al. (1998)

Table 9 continued

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
SGLT1 mRNA and Na^{+}-dependent glucose transport	Decrease in SGLT1 mRNA possibly due to transcriptional down-regulation of *SGLT1* mediated through modification of Sp1 binding to its promoter	Primary culture of mouse renal cortical tubule cells on solid support	5–10 μM $CdCl_2$ for 24 h		Tabatabai et al. (2005)
Na^{+}-dependent glucose uptake and mRNA of SGLT1, 2 and 3	Glucose transport and expression of SGLT1 and SGLT2 mRNA was reduced, SGLT3 mRNA increased fivefold	Primary culture of mouse renal cortical tubule cells on solid support	5, 7.5 or 10 μM $CdCl_2$ for 24 h		Tabatabai et al. (2001, 2003)
Apical Na^{+}-dependent glucose and amino acid transporters	Direct inhibition, detected via electrophysiological measurements, ED50 of luminal CdMT ~0.54 μM Cd^{2+})	in vitro luminally perfused isolated S2 segments of renal proximal tubules of female Japanese White rabbits	Luminal Cd^{2+} as CdMT: 10^{-9} to 10^{-5} g/ml (=~8.9 nM to 89 μM); $CdCl_2$ or basolateral CdMT are less potent		Tsuruoka et al. (2000)
GLUT4 transporter	90% reduction in GLUT4 mRNA followed by reduction in amount of protein	Adipocytes of rats		Sc injection of 2 mg CdCl2/kg daily for 4 days	Han et al. (2003)
GLUT1 transporter	Cd^{2+} increases glucose uptake via GLUT1 transporter	Mouse 3T3-L fibroblasts and adipocytes in culture	5 or 10 μM $CdSO_4$ for 18 h		Harrison et al. (1991)

(Kim and Park 1995). Because the inhibition of Na^+-dependent transport of glucose is not associated with changes in membrane permeability to Na^+ or substrates, it is apparent that there was a direct interaction with the cotransporter in the membrane in the experimental conditions used (Ahn et al. 1999). Furthermore, urine analysis of male Sprague–Dawley rats exposed daily to sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight during 2 weeks showed an increased excretion of 15 different amino acids, from neutral, acidic and basic families. In BBMVs from these rats, Na^+-dependent transport of L-proline, L-alanine and L-lysine was markedly attenuated, whereas transport through facilitated diffusion was not affected. Direct treatment of normal BBMVs to a medium containing free Cd^{2+} (ranging from 10 to 100 μM Cd^{2+}) induced similar changes in amino acid transport, suggesting that in long-term exposure to Cd^{2+}, the free Cd^{2+} ions liberated into the tubular cytoplasm impair BBM function (Kim et al. 1990). This cytoplasmic action of free Cd^{2+} ions was corroborated by Kinne et al., who showed that BBMVs isolated from male New Zealand white rabbits had to be preincubated with $CdCl_2$ (1 μM up to 1 mM) to inhibit L-glutamate transport. Furthermore, this inhibition was not reversed by EDTA, which has been shown to remove Cd^{2+} from its cellular binding sites (Templeton 1990), making it unlikely that Cd^{2+} acted from the periplasmic interface (Kinne et al. 1995).

In mice exposed to 100 mg Cd^{2+}/l (as $CdCl_2$) in the drinking water for up to 1 year, the relative gene expression of the SGLT1 and 2 in the kidney cortex was upregulated after 16 (SGLT1 and 2) or 24 weeks (SGLT1). But 1 year after exposure to Cd^{2+} the expression of both transporters was reduced again and so was the amount of protein as observed in a Western blot (Jolling 2008). To further elucidate the mechanisms underlying the effects of Cd^{2+} on Na^+-dependent glucose transport, Blumenthal et al. incubated primary cultures of mouse renal cortical cells, isolated from male C57bl6 mice in 7.5 μM Cd^{2+} up to 24 h and studied the expression of one of the apical Na^+-glucose cotransporters, SGLT1 (Blumenthal et al. 1998). Within 12 h, the relative concentration of SGLT1 mRNA decreased, resulting in a decreased synthesis and steady state level of the Na^+-glucose cotransporter which in turn led to a decreased cotransporter activity. These findings corroborate the previously mentioned kinetic finding that Cd^{2+} affects V_{max} but not K_m when studying a primary cell culture (Blumenthal et al. 1990) or intoxicated animals (Lee et al. 1990, 1991). Blumenthal et al. demonstrated that 7.5 μM CdMt decreased the expression of the SGLT1 in the cultured mouse renal cortical cells, suggesting a specific effect of Cd^{2+} on the expression of the Na^+-glucose cotransporter (Blumenthal et al. 1998). Curiously enough, effects of Cd^{2+} on BBMV isolated from non-treated animals lead to similar results, i.e. a reduction in V_{max} and not K_m (Lee et al. 1991; Kinne et al. 1995), which must have been a direct effect and not via expression of the transporters. It must be kept in mind however, that the Cd^{2+} concentrations used in the in vitro experiments may not necessarily be comparable to the in vivo situation and extrapolation may not always be justified. Tabatabai et al. demonstrated a decrease in the number of SGLT1 mRNA molecules parallel with a decrease in the uptake of glucose in response to Cd^{2+} exposure of primary cultures of mouse renal cortical cells (Tabatabai et al. 2005). Since there was no effect of Cd^{2+} on mRNA degradation, the decreased SGLT1 mRNA levels were due to effects on *SGLT1* gene transcription. The authors hypothesized that the transcription protein binding site composed by the zinc-finger protein Sp1 may be the factor affected by Cd^{2+} (Tabatabai et al. 2005). In the same model of primary mouse cortical cells, the mRNA expression of SGLT2 decreased between 0 and 5 μM $CdCl_2$ and reached a plateau level between 5 and 10 μM $CdCl_2$ (Tabatabai et al. 2001). These authors also discovered a third cotransporter in mouse renal cortical cells with 2 isoforms, SGLT3a and SGLT3b. The mRNA level of both isoforms increased upon exposure to 5–10 μM Cd^{2+} (Tabatabai et al. 2003), indicating that the *SGLT3* gene might be a stress-response gene, as reported previously in pigs (Plakidou-Dymock et al. 1994). The observed molecular response of the SGLT3 isoforms to Cd^{2+} may be indicative of a complementary transport activity in the kidney, suggesting a compensatory role in conditions where normal glucose uptake is compromised (Tabatabai et al. 2001, 2003).

Tsuruoka et al. studied the effect of CdMt (1 μg Cd^{2+}/ml ~8.9 μM) on glucose and amino acid

transport across the apical membrane via in vitro microperfusion of isolated renal tubules from female Japanese White rabbits by monitoring the transmural, the apical and the basolateral membrane voltage (Tsuruoka et al. 2000). Taking away glucose or alanine from the lumen stops the Na^+-glucose or Na^+-alanine cotransporter, respectively and hyperpolarizes the membrane. The results showed that luminal CdMt exposure abolished this hyperpolarizing effect, supposedly by affecting the transporters (Tsuruoka et al. 2000). The effect was pH dependent (Tsuruoka et al. 2008). Puzzling to us was the fact that CdMt in itself, and if it blocked the transporters, did not hyperpolarize the membrane. Possibly this may be ascribed to a second effect of CdMt, after having entered the cell, on the basolateral Na^+-K^+-ATPase. Luminal CdMt mimicked the depolarizing effect of ouabain applied from the basolateral side (Tsuruoka et al. 2000). Although a direct extracellular effect on the transporters from the lumen does not agree with the theory of a cytoplasmatic action of Cd^{2+} as reported by (Kim et al. 1990; Kinne et al. 1995), it is important to consider that different experimental models were used. On the other hand, the decreased Na^+-K^+-ATPase activity due to luminal CdMt perfusion confirms the findings of the group of Thévenod (Thévenod and Friedmann 1999), as described earlier. However, one must keep in mind that Tsuruoka et al. used a different form of Cd^{2+} administration, CdMt (Tsuruoka et al. 2000) instead of $CdCl_2$ which was used in most studies described above.

Cd^{2+} may also be diabetogenic and aggravate kidney problems in diabetic patients (Chen et al. 2006b reviewed by Edwards and Prozialeck 2009) and—as far as membrane transporters are concerned—in a study on rats exposed to $CdCl_2$ by a sc injection of 2 mg/kg daily for 4 days, Han et al. showed that the expression of GLUT4 transporters in adipocytes of rats was reduced by as much as 90%, the reduction in mRNA preceding the reduction in the amount of protein present (Han et al. 2003). But Cd^{2+} may also increase glucose uptake in mouse fibroblasts in culture exposed to 5 or 10 μM $CdSO_4$ (Harrison et al. 1991). A lot more research is needed to unravel the relationship between Cd^{2+} and diabetes. One of the players may be the GLUT transporters in cell membranes of tissues with or without insulin-dependent glucose uptake.

Cadmium and membrane transport of other organic substrates and metals

The membranes of kidney (Brenner and Levine 2008; Boron and Boulpaep 2009) and liver cells (Diaz 2000) contain several proteins involved in the uptake and/or excretion of xenobiotics and endogenous organic compounds: normally all that is not reclaimed is excreted. Metal transporters play a role in the uptake of essential metals into cells, the uptake via the intestine or the extrusion via the liver and the kidney.

Kidney tubules transport a variety of organic substances other than glucose and amino acids.

Organic anions (OA)

Many metabolites of endogenous compounds and administered drugs are reabsorbed or secreted (or both, e.g. urate). In kidney proximal tubule cells the secretion of many OA, that need to be removed from the body, depends on a tertiary active transport at the level of the basolateral membrane: (1) the Na^+K^+-ATPase creates a negative cell potential as a result of the K^+ gradient it generates and it sustains an inwardly directed Na^+ gradient, (2) both gradients drive the electrogenic $3Na^+$-dicarboxylate cotransporter (NaDC-3), (3) the intracellular alpha-ketoglutarate in turn drives the OAT1 and OAT3 OA-dicarboxylate exchanger, the alpha-ketoglutarate recycling across the basolateral plasma membrane to drive uptake of OA. The OA may leave the cell at the apical side via dicarboxylate-OA exchange. The p-aminohippuric acid (PAH) is a well known example of an OA that was described to use this system (Brenner and Levine 2008; Boron and Boulpaep 2009).

The impact of Cd^{2+} on OA secretion has been assessed. Experimental details of the papers discussed in this section can be followed in Table 10.

Several studies in the past observed a reduction in PAH excretion in vivo and a decrease in PAH uptake in kidney cortical slices exposed to Cd^{2+} in vitro (Suzuki and Cherian 1988) or after intoxicating animals with Cd^{2+}: mongrel dogs, iv dose of 200 μg/kg (Vander 1963); rats, sc injections of $CdCl_2$ (2 mg Cd^{2+}/kg day) for 16 days (Kim et al. 1988); rats, injection of CdMt, 0.3 mg Cd^{2+}/kg body weight (Suzuki and Cherian 1988). In the kidney slice

Table 10 Effects of Cd^{2+} on membrane transport systems: organic anions

Transport system	Cd^{2+} effect	Tissue/animal	[Cd^{2+}] in vitro	Cd^{2+} administration route in vivo	Reference
PAH excretion	Inhibition of PAH secretion by Cd^{2+}, prevented by dimercaptopropanol	Mongrel dogs		Iv dose of 200 μg Cd^{2+}/kg (as CdCysteine complex)	Vander (1963)
PAH transport	V_{max} drastically reduced, no change in K_m	Renal cortical slices of intoxicated rats		Sc injections of $CdCl_2$ at a dose of 2 mg Cd^{2+}/kg body weight for 16 days	Kim et al. (1988)
PAH accumulation in slices	Progressive decrease in uptake, 12–48 h after injection	Rat kidney cortical slices, 2, 4, 12, 24, 48 h after intoxication		Single injection of CdMT, 0.3 mg Cd^{2+}/kg body weight	Suzuki and Cherian (1988)
PAH accumulation in slices	Dose dependent decrease in uptake	Rat kidney cortical slices	5–200 μM $CdCl_2$ or CdMT, 90 min incubation		Suzuki and Cherian (1988)
PAH uptake in BLMV	Reduction of V_{max}, no effect on K_m	Rat kidney cortical basolateral BLMV of intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Kim et al. (1998)
Na^+-alpha-ketoglutarate cotransport	Not affected by 2-week cadmium treatment, but significantly inhibited by 3-week Cd^{2+} treatment	Rat kidney cortical basolateral BLMV of intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Kim et al. (1998)
Basolateral PAH uptake	2-fold increase at 1 μM Cd^{2+}	Microdissected nonperfused rabbit kidney S2 segments	1 nM up to 10 μM Cd^{2+} 15 min		Hohage et al. (1998)
Mrp2	Reduction of Mrp2 mediated transport	Proximal tubule of the killifish	10 μM Cd^{2+} short term exposure, 30 min		Terlouw et al. (2002)
Mrp2	Increase in transport and presence of Mrp2 protein at the luminal membrane	Proximal tubule of the killifish	0.5 and 1 μM Cd^{2+} 6–24 h pretreatment		Terlouw et al. (2002)
Na^+-dependent succinate transport	Reduction in Na^+ dependent uptake	Isolated renal proximal BLMV from $CdCl_2$ intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Lee et al. (1990)
Na^+-dependent succinate uptake	Dose dependent reduction in uptake, proportional with Cd^{2+} uptake, suggesting direct interaction with free Cd^{2+}	Rat renal cortical BBMV	60 min preincubation in 50, 100, 200 μM $CdCl_2$		Ahn et al. (1999)
Sulfate transporter sat-1	$^{35}SO_4^{2-}$ uptake, weak inhibitory effect only on the V_{max}	Expressed in *Xenopus* oocytes	100 and 500 μM Cd^{2+}, uptake of sulfate for 30 min in the absence or presence of Cd^{2+}		Markovich and Knight (1998), Markovich and James (1999)

experiments in vitro we expect the Cd^{2+} effect to be primarily situated at the basolateral membrane, because, as ultrafiltration is absent, the proximal tubules were probably collapsed, which would make it difficult for Cd^{2+} to reach the apical membrane. As mentioned above, at the basolateral side the uptake mechanism of OA consists of several steps which may have been affected. Also, PAH uses multiple OA-dicarboxylate exchangers (OAT1 and OAT3). As these transporters have a diverse substrate selectivity (Brenner and Levine 2008), this may imply that the transport of other organic anions carried by these membrane proteins is also affected by Cd^{2+}, although the concentrations used in the in vitro experiments (of the order of 5–200 μM) were rather high and the relevance for the in vivo situation, in humans at least, remains to be seen.

A study assessing transport across basolateral BLMV of kidney cortex of Cd^{2+} intoxicated rats (2 mg Cd^{2+}/kg day sc for 2–3 weeks) showed a reduction of the PAH uptake in the BLMV (V_{max} was reduced, no change in K_m). The uptake needed the presence of a Na^+ gradient and alpha-ketoglutarate as explained above. The Na^+-alpha-ketoglutarate cotransport itself in BLMV was also significantly inhibited after the 3rd week of Cd^{2+} exposure (Kim et al. 1998).

Hohage et al. on the other hand used lower Cd^{2+} concentrations (1 nM up to 10 μM) exposing micro-dissected nonperfused rabbit kidney S2 segments (Hohage et al. 1998). Incubation with Cd^{2+} induced a bell-shaped curve with a 2-fold increase of the PAH transport at 1 μM Cd^{2+}. Interestingly, these low Cd^{2+} concentrations also seem to stimulate cell growth. In rat liver epithelial cells, an increase in cell proliferation was observed at doses of 0.03 to 2.5 μM $CdCl_2$. At the same time, a decrease in the number of gap junctions between the cells was seen (Jeon et al. 2001). In LLC-PK-1 cells, kidney cells derived from pigs, DNA replication and cell growth is stimulated at low Cd^{2+} concentrations (nanomolar range up to 0.5 μM), higher concentrations suppress cell growth (von Zglinicki et al. 1992). In HEK293 cells (human embryonic kidney 293) a similar phenomenon was described (Hao et al. 2009). The latter authors suggest that activation of the MAPK pathway may be involved in the biphasic effect induced by Cd^{2+}. In trophoblast in culture 40 nM Cd^{2+} increased cell growth, measured as ^{3}H-thymidine uptake, 160 nM did not, 640 nM slowed it down. The higher concentrations also induced LDH release (Lin et al. 1997). It might well be therefore, that Cd^{2+}, although damaging cellular processes and transport systems in the end at these low concentrations, relevant to the general and the exposed population, also has a signaling function and induces cell dedifferentiation, cell growth/proliferation in an attempt of the cells to defend themselves. This aspect has not really been well studied up to now.

Endogenous metabolites such as the monocarboxylates (lactate, pyruvate, acetoacetate, beta-hydroxybutyrate), di- and tricarboxylates (alpha-ketoglutarate, malate, succinate and citrate) and bile salts are OA that need to be recovered. They are reabsorbed in the kidney proximal tubule, involving Na^+-dicarboxylate cotransport (e.g. NaDC-1). The system also needs the Na^+K^+-ATPase to maintain the necessary gradients as explained above. (Brenner and Levine 2008; Boron and Boulpaep 2009). NaDC-3 in the BLM as well as as the luminal NaDC-1 may also provide proximal tubule cells with di- and tricarboxylates required for energy metabolism and gluconeogenesis (Hagos et al. 2006). We found one study by Lee et al. (1990) showing that Na^+-dependent uptake of succinate by BLMV of the kidney cortex of intoxicated rats (2 mg Cd^{2+}/kg day sc for 2–3 weeks) was reduced by Cd^{2+} and in this way may interfere with the cell's function.

Taken together, although Cd^{2+} did affect OA transport, from the studies discussed above it is still not clear whether effects of Cd^{2+} are direct or indirect and are the result of the inhibition of the Na^+K^+-ATPase and/or the NADC-3 for instance.

From the family of ABC proteins, more specifically the ABCC or MRP/CFTR group, the multidrug resistance-associated protein Mrp2 is highly expressed in proximal tubule of the killifish. Terlouw et al. studied the effect of micromolar Cd^{2+} concentrations on this transporter (Terlouw et al. 2002). They found that, after an initial reduction in function, the Mrp2 mediated transport and its presence at the luminal membrane is increased. This seems to be in line with the induction of another member of the ABC family, i.e. the MDR1 by Cd^{2+} (see below).

Not much is known about Cd^{2+} effects on anion transporters in the intestine or in liver cells. The sulfate/bicarbonate anion transporter which occurs in the basolateral membrane of kidney proximal tubule

cells or in the canaliculus (apical) membrane of the liver cell was expressed in *Xenopus* oocytes (Markovich and Knight 1998; Markovich and James 1999). High Cd^{2+} concentrations (of the order of 100 and 500 μM) had a weak inhibitory effect on the V_{max} of the transporter.

Organic cations (OC)

The kidney is also capable of clearing the plasma of a vast array of endogenous and xenobiotic positively charged organic compounds. The transporters often have a very broad selectivity. Among the organic cation transporters of interest we distinguish the organic cation transporters (OCTs), extruding the smaller type I OC, and OC/H^+ exchangers, and P-glycoprotein or MDR1 of the family of ABC proteins, responsible for the extrusion of the larger type II OC (Brenner and Levine 2008). Experimental details of the papers discussed in this section can be followed in Table 11.

In BBMVs of intoxicated rats (Suzuki and Cherian 1988; Lee et al. 1990; Kim et al. 1998) or control animals (Suzuki and Cherian 1988) the proton gradient-dependent transport of tetraethylammonium, TEA, a substrate for the OC/H^+ exchanger, was inhibited with reduction in V_{max} and no change in K_m (Kim et al. 1998). The P-glycoprotein or MDR1 was studied in a rat proximal tubule cell line. It is upregulated and protects kidney proximal tubule cells from Cd^{2+} induced apoptosis (5–10 μM $CdCl_2$), although it is not responsible for the extrusion of Cd^{2+} out of the cells (Thévenod et al. 2000; Thévenod 2010). This upregulation may be part of the defense mechanisms of the cell that come into action when the cells are exposed to Cd^{2+} at first, especially when the dose is not too high.

Many cell membranes possess transporters for metals, i.e. Mn, Zn, Fe, Ca, Ni, Co. It has been shown that Cd^{2+} may use these transporters to enter the cells and therefore interfere with the uptake of the essential metals. This may interact with the normal functioning of the cell. ZIP8, ZIP14, DMT1, and Ca^{2+} channels and transporters are now listed as candidates for Cd^{2+} transporter (Himeno et al. 2009; Moulis 2010). As the expression of these transporters is high in the intestine, it seems likely that dietary intake of Fe, Ca, Zn, and Mn may influence the intestinal absorption of Cd^{2+} and vice versa. More details on Cd^{2+}

Table 11 Effects of Cd^{2+} on membrane transport systems: organic cations

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
P-glycoprotein Mdr1	Upregulation of expression, protects against apoptosis, and is probably triggered by Cd^{2+} induced ROS	Rat Immortalized cells (WKPT-0293 Cl.2) of the S1-segment	5–10 μM CdCl2 3–72 h		Thévenod et al. 2000, Thévenod 2010
TEA accumulation in slices	Decrease in uptake 48 h after injection	Rat kidney cortical slices, 2, 4, 12, 24, 48 h after intoxication		Single injection of CdMT, 0.3 mg Cd^{2+}/kg body weight	Suzuki and Cherian (1988)
TEA accumulation in slices	Decrease in uptake at high dose, CdMT more effective	Rat kidney cortical slices from non-exposed animals	5-200 μM $CdCl_2$ or CdMT, 90 min incubation		Suzuki and Cherian (1988)
H^+-driven organic cation antiport, measured via TEA uptake	Reduction of V_{max}, no effect on K_m	Kidney cortical apical BBMV of intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Kim et al. (1998)
H^+ dependent organic cation antiport system	Reduction in H^+ dependent uptake of TEA	Isolated renal proximal BBMV of intoxicated rats		2 mg Cd^{2+} as $CdCl_2$/kg/day for 2 or 3 weeks	Lee et al. (1990)

transport may be found elsewhere in this issue (Thévenod, this issue, 2010).

Cadmium effects on water channels and proton pathways

Voltage sensitive proton channels (DeCoursey and Cherny 2007) and epithelial proton and water channels (Harvey et al. 1991) are sensitive to Cd^{2+} and other metals. The basolateral membranes of frog skin and urinary bladder possess spontaneously open channels permeable to water and protons which can be blocked by micromolar concentrations of heavy metals. Cd^{2+} is also known to interfere with the renal vacuolar H^+-ATPase (reviewed by Wagner et al. 2004). V-ATPases play an important role in the acidification of endocytic vesicles and therefore in the endocytosis process. Cd^{2+} was shown to induce a strong decrease in the activity and amount of V-ATPase in the kidney proximal tubule BBM of intoxicated and control rats (Herak-Kramberger et al. 1998) and may therefore interfere with protein reabsorption, as will be explained in more detail in the next section. Furthermore the V_{max} of the Na^+/H^+-exchanger (NHE3) was reduced in intoxicated rats and the Na^+-bicarbonate cotransporter (NBC1) was slightly affected (Ahn et al. 2005).

Cd^{2+} intoxication can cause polyuria. The cause can be impaired solute uptake, but possibly also a blockage of antidiuretic hormone sensitive water channels (see e.g. Harvey et al. 1991). Effects on water channels and their role in fluid loss is a question that needs to be further explored. It has been shown that treatment with Cd^{2+} acidifies the cell (e.g. primary culture of rat proximal tubule cells after 12 h treatment with 2.5 and 5 μM $CdAc_2$) (Wang et al. 2009). Not many studies have been performed concerning proton transport (see Table 12). Interaction between Cd^{2+}, proton pathways and cell pH and its role in Cd^{2+} toxicity is another field that needs exploring.

Cadmium and endocytosis processes

Endocytosis is a process used by many cells to absorb large molecules by engulfing them with their cell membrane. Cd^{2+} has been shown to interfere with the

Table 12 Effects of Cd^{2+} on membrane transport systems: proton pathways

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
Basolateral epithelial proton and water channels	Blockage of H^+ permeation	Frog skin epithelium	100 μM Cd^{2+}		Harvey et al. (1991)
NHE3	Reduction in V_{max}, no change in K_{Na}, mRNA and amount of protein was reduced	Rat renal cortex, BBMVs of intoxicated animals, mRNA, immunoblots		2 mg Cd^{2+} as $CdCl_2$/kg/day for 3 weeks	Ahn et al. (2005)
NBC1	Slight reduction in amount of protein after 3 weeks	Rat renal cortex of intoxicated animals, immunoblots		2 mg Cd^{2+} as $CdCl_2$/kg/day for 3 weeks	Ahn et al. (2005)
V-ATPase activity	Diminished endocytosis, possibly via loss of activity and amount of V-ATPase in the proximal tubule BBMV	Renal cortical BBMV of exposed rats		Daily sc injections at a dose of 2 mg Cd^{2+}(as $CdCl_2$)/kg body weight for 14 days	Herak-Kramberger et al. (1998)
V-ATPase activity	Diminished endocytosis, possibly via loss of activity and amount of V-ATPase in the proximal tubule BBMV	Renal cortical BBMV of non-exposed rats	10–100 μM $CdCl_2$ for 10 or 20 min		Herak-Kramberger et al. (1998)

process in a wide range of organisms. A summary of the papers discussed is given in Table 13.

In unicellular organisms and invertebrates phagocytosis and endocytosis are used in acquisition of food, immune responses and are crucial for the survival of these lower life forms. Cd^{2+} exposure affects these processes. Shemarova et al. studied the effects of Cd^{2+} ions on activity of glutathione S-transferase, growth and endocytosis in culture of the infusorium Tetrahymena pyriformis (Shemarova et al. 2000). Exposure of cells of the infusorium *Tetrahymena pyriformis* of the micronucleus-free strain GL to Cd^{2+} cations both in the free and the bound chelated form inhibited growth, development and endocytosis (as assessed by the ratio of relative contents of phagocytizing cells in tested and control cultures) at concentrations ranging from 1 to 10 μM. During the first hour of exposure, the endocytosis was preserved in a significant part of the cell population (43–50%). However, by the third hour of exposure to the above-mentioned Cd^{2+} concentrations, the endocytosis level decreased to 33.3 and 16.6% for Cd^{2+}-EDTA and free Cd^{2+} cations, respectively. This decrease was probably due to a high rate of accumulation of metal ions during this period (Shemarova et al. 2000). Invertebrate species such as bivalve mollusks and worms were also used in immunotoxicological studies as they exist in direct contact with contaminated aquatic sediments and soil, respectively (Sauve et al. 2002a, b). As phagocytosis plays a central role in immune defense, hemocytes and coelomocytes, which play a role in the immune system of invertebrates, were isolated from bivalves (*Mya arenaria, Mytilus edulis, Mactromeris polynyma, Mya truncate, Cyrtodaria siliqua, Serripes groenlandicus, Mesosdesma arctatum, Siliqua costata*) and worms (*L. terrestris, E. fetida, A. turgida, T. tubifex*) and exposed to $CdCl_2$. Phagocytic activity was monitored using fluorescent spheres, and expressed as the percentage of cells containing fluorescent beads as assessed with flow cytometry. In some species of bivalves in very low concentrations of $CdCl_2$, ranging from 10 to 100 nM, phagocytosis by the hemocytes was slightly stimulated. This hormesis indicates stimulation of immune functions by low levels of the heavy metal, which confirms earlier findings (Cheng and Sullivan 1984; Bernier et al. 1995; Brousseau et al. 2000). Increasing $CdCl_2$ concentrations from 10 nM to 10 mM caused a decrease of phagocytosis by the hemocytes, with a 50% suppression of phagocytosis (EC_{50}) between 0.1 and 1 mM depending on the species (Brousseau et al. 2000; Sauve et al. 2002a). Similarly, the phagocytic activity of the coelomocytes isolated from different worm species was not influenced by $CdCl_2$ at concentrations ranging from 1 to 10 nM M. EC_{50} values were on average situated between 10 and 100 μM (Sauve et al. 2002a), leading to the conclusion that phagocytosis in worm as well as bivalve species is suppressed by $CdCl_2$ in this range of concentrations (Sauve et al. 2002a, b).

In vertebrates, in the kidney, endocytosis is essential in the reabsorption of plasma proteins from the renal ultrafiltrate. Chronic exposure to Cd^{2+} leads to proteinuria (Kim et al. 1999). The basic mechanism for protein reabsorption in renal tubular cells is receptor-mediated endocytosis. In order to unravel the effects of Cd^{2+} on this process, Choi et al. used albumin as a model substrate in opossum kidney (OK) cell cultures (Choi et al. 1999). The albumin uptake by the OK cells, as measured with FITC-labeled albumin, was consistent with receptor-mediated endocytosis. In addition, the endocytosis process was inhibited in OK cells exposed to 100 μM $CdCl_2$ for 60 min at 37°C. This inhibition seemed to be associated with a prevention of endosomal acidification and therefore a fall in ligand-receptor dissociation. This prevents receptor recycling and reduces the overall efficiency of endocytosis (Choi et al. 1999). These findings are in line with the results of (Herak-Kramberger et al. 1998). The authors exposed male Wistar rats to $CdCl_2$ by sc injections at a dose of 2 mg Cd^{2+}/kg body weight daily for 14 days. The control animals were injected with equivalent volumes of 0.9% NaCl during this period. The rats were anesthetized and 10 kDa FITC-labeled dextran was injected into the jugular vein. The dextran used was freely filtered by the glomerulus and endocytosed by the proximal tubule and other cells along the renal tubule (Sabolic et al. 1994). After fixation, the kidneys were washed, fixed and sliced. The levels of fluorescence in the slices clearly showed a diminished endocytosis, which coincided with the loss of endocytotic invaginations and subapical vesicles in the proximal tubule cells. Probably Cd^{2+} induced a strong decrease of the activity and amount of V-ATPase in the proximal tubule BBM, which was followed by a dissipation of

Table 13 Effects of Cd^{2+} on membrane transport systems: endocytosis

Transport system	Cd^{2+} effect	Tissue/animal	$[Cd^{2+}]$ in vitro	Cd^{2+} administration route in vivo	Reference
Receptor-mediated endocytosis	Inhibition, possibly by prevention of vesicle acidification and receptor recycling (effect on V-ATPase?)	OK cells	100 μM $CdCl_2$ for 60 min at 37°C		Choi et al. (1999)
Receptor-mediated endocytosis	Diminished endocytosis, possibly via loss of activity and amount of V-ATPase in the proximal tubule BBMV	Male Wistar rats	Injection of 35 mg 9,6 kD FITC-dextran per rat	Daily sc injections at a dose of 2 mg Cd^{2+}(as $CdCl_2$)/kg body weight for 14 days	Herak-Kramberger et al. (1998)
V-ATPase activity* see Proton pathways					Herak-Kramberger et al. (1998)
Megalin, V-ATPase, aquaporin-1 and type 3 Na^+/H^+ exchanger from the BBM	Time dependent loss, redistribution of transporters into vesicles scattered throughout the cytoplasm	Rat proximal tubule cells		Single subcutaneous dose of 0.4 mg of CdMT/kg body mass	Sabolic et al. (2002)
Endocytosis	Inhibition	Infusorium *Tetrahymena pyriformis*	Cd^{2+} cations (1–10 μM) both in the free and the bound chelated form		Shemarova et al. (2000)
Phagocytosis	Phagocytosis by the hemocytes was slightly stimulated	Hemocytes and coelomocytes from bivalves & worms	10–100 nM $CdCl_2$		Sauve et al. (2002a, b), Brousseau et al. (2000)
Phagocytosis	Decrease of phagocytosis, EC_{50} 0.1–1 mM in hemocytes, depending on the species; 10 and 100 μM in coelomocytes	Hemocytes and coelomocytes from bivalves & worms	10 nM to 10 mM $CdCl_2$		Sauve et al. (2002a, b),Brousseau et al. (2000)

the transmembrane pH gradient in the endocytic vesicles in the kidney cortex. The impaired endocytosis would lead to a decreased reabsorption of filtered proteins and thus proteinuria. Furthermore, it might also derange intracellular vesicle trafficking causing loss of specific transporters from the BBM (Herak-Kramberger et al. 1998). The mechanism underlying the Cd^{2+} effect was explored in an in vivo model of experimental Cd^{2+} nephrotoxicity induced by a single sc dose of 0.4 mg of CdMt/kg body mass in rats (Sabolic et al. 2002). Time-dependent redistribution of BBM transporters was assessed using fluorescence and gold-labeling immunocytochemistry on tissue sections and by immunoblotting of isolated renal cortical BBM. In the proximal tubule cells of CdMt treated rats, a time-dependent loss of megalin, V-ATPase, aquaporin-1 and type 3 Na^+/H^+ exchanger from the BBM combined with a redistribution of these transporters into vesicles scattered throughout the cytoplasm was observed. This internalization of BBM transporters was accompanied by fragmentation and loss of microtubules and occurred as early as 1 h after CdMt treatment with an increase in magnitude over the next 12 h. Thus, the early mechanism of Cd^{2+} toxicity in proximal tubule cells may include an impaired vesicle-dependent recycling of various BBM proteins (Sabolic et al. 2002), supporting the hypothesis of Herak-Kramberger et al. (1998). These processes may lead to a time-dependent loss of cell membrane components, resulting in reabsorptive and secretory defects that occur in Cd^{2+}-induced nephrotoxocity (Herak-Kramberger et al. 1998; Sabolic et al. 2002).

Cadmium and the integrity of the transporting epithelium

Cells of transporting epithelia and vascular endothelia are attached to each other by specialized junctional complexes which determine the transepithelial permeability and regulate the transport of substrates across the epithelium. These junctional complexes are composed of specific junction-associated proteins such as integrins, cadherins, connexins, etc. and are closely associated with the cytoskeleton (Alberts et al. 2009). Niewenhuis et al. showed that one of the earliest effects of Cd^{2+} in the LLC-PK1 cells, which is an established porcine renal epithelial cell line, is the loosening of the intercellular junctions. Immediately after addition of 10 μM Cd^{2+} to the basolateral surface, junctions start to break and by 30 min–1 h the entire cell monolayer is affected (Niewenhuis et al. 1997). At the same time, the transepithelial electrical resistance begins to decline by 30 min, and falls to near zero after about 6 h. This confirmed earlier results, reporting similar effects at micromolar Cd^{2+} levels and durations of exposure that did not affect viability of the cells, indicating that the junctional effects of Cd^{2+} were not a consequence of apoptosis (Prozialeck et al. 1995). In addition, the effects were even more pronounced when Cd^{2+} exposure occurred at the basolateral compared to the apical cell surface, and the severity of the effects was inversely related to the concentration of Ca^{2+} in the medium (Prozialeck and Niewenhuis 1991a, b, Prozialeck and Lamar 1993; Prozialeck et al. 1996). Electron microscopic analyses of the LLC-PK1 cells showed that the major ultrastructural change induced by exposure to 10 μM Cd^{2+} is a marked decrease in the electron density of the intracellular plaques that are associated with the adhering junctions. This observation suggested that Cd^{2+} might cause proteins, involved in linking membrane-associated cell adhesion molecules to the cytoskeleton, to disassociate and possibly diffuse into the cytosol. Thus, adhesion at this site might disappear, leading to a breakdown in the linkage between the junctional complexes and the cytoskeleton. Such a mechanism could explain the temporal relationship between the Cd^{2+}-induced breakdown of cell–cell junctions and the dramatic change in cell shape from a flat to a round appearance (Niewenhuis et al. 1997). A possible candidate site to be affected by Cd^{2+} is the Ca^{2+}-dependent cell adhesion molecule E-cadherin (Prozialeck 2000). In epithelial cells, E-cadherin is primarily localized at the adhesion belts of the adhering junctional complexes at the basolateral side, playing a key role in the homophilic Ca^{2+}-dependent cell–cell adhesion (Nelson et al. 1990; Takeichi 1990). Indeed, exposure to Cd^{2+} decreased the amount of E-cadherin that is associated with the contacts between LLC-PK1 cells, coinciding with the disruption of cell–cell junctions (Prozialeck and Niewenhuis 1991a). A later study has shown that Cd^{2+} binds to E-CAD1, a polypeptide analog of E-cadherin with a K_D of 20 μM, which is similar to the concentrations disrupting epithelial cell–cell junctions, ranging from 5 to 40 μM. These findings

support the hypothesis that E-cadherin may be a direct molecular target for Cd^{2+} toxicity in epithelial cells. Similar Cd^{2+} levels affected the transepithelial resistance in cell culture layers (Prozialeck et al. 1996). Subsequently, peptide B, a relatively simple polypeptide corresponding to one of the extracellular Ca^{2+} binding motifs of E-cadherin was shown to bind Cd^{2+}, which caused a distortion of the secondary structure of the peptide B molecule (Prozialeck and Lamar 1999). A similar distortion of a more complex polypeptide as E-CAD1 or E-cadherin itself could profoundly affect the spatial orientation of the adhesive regions of E-cadherin (Prozialeck and Lamar 1999). Disturbing effects of $CdCl_2$ (Cd^{2+}) on E-cadherin-dependent junctions were reported in rat Sertoli cells (Janecki et al. 1992; Chung and Cheng 2001), in intestinal epithelial cell lines (Duizer et al. 1999), in MDCK cells (Prozialeck and Lamar 1997; Zimmerhackl et al. 1998), in corneoscleral segments of bullfrogs (Weidner et al. 2000), in primary cultures of adult and, to a minor extent, of fetal rat hepatocytes (Bruscalupi et al. 2009). Furthermore, Cd^{2+} has been shown to have similar effects on several other cadherins, including N-cadherin in osteoblast-like cells (Prozialeck 2000) and VE-cadherin in vascular endothelial cells in the lung (Prozialeck 2000, Pearson et al. 2003), as well as on the actin cytoskeleton in various experimental models (Prozialeck and Lamar 1997; Zimmerhackl et al. 1998). However, investigating the effects of ionic Cd^{2+} on human proximal tubule cells, Hazen-Martin et al. reported the following observations, which differed partly from the findings described above (Hazen-Martin et al. 1989a, b). Exposing human proximal tubule cells to 0.5 μg/ml (4.5 μM) of Cd^{2+} at the basolateral cell surface reduced transepithelial resistance, confirming results in LLC-PK1 cells (Niewenhuis et al. 1997), in MDCK cells (Prozialeck and Lamar 1997; Zimmerhackl et al. 1998) and in rat Sertoli cells (Janecki et al. 1992). In a subsequent study, the effects of Cd^{2+} exposure on the cell membrane and junctional complex were investigated using freeze-fracture technology, which allows the study of junctions along their length (Hazen-Martin et al. 1993). The human proximal tubule cells were exposed to 0.5, 1.0 or 3.0 μg Cd^{2+}/ml (4.5, 9 and 27 μM) during 4 days and the Cd^{2+}-treated cell layers had a reduced transepithelial resistance. However, although Cd^{2+}-treated junctions exhibited a less uniform morphology along their length compared to control cells, the reduction in transepithelial resistance was not accompanied by gross alterations in the junctional structure, which is in contrast to the findings in LLC-PK1 cells as described earlier (Prozialeck et al. 1995; Niewenhuis et al. 1997). This contradiction might be explained by the freeze-fracture technique used in the study of Hazen-Martin et al. (Hazen-Martin et al. 1993), allowing the visualization of tight junctions of cells grown on permeable supports, whereas most other studies used cells grown on solid supports for visualization studies. A second reason could be the origin of the cells used. Hazen-Martin et al. concluded from their study that very subtle changes in morphology of the human proximal tubule cells could already have significant functional impact in terms of transepithelial resistance (Hazen-Martin et al. 1993).

Exposing male Sprague–Dawley rats to daily sc injections of 0.6 mg $CdCl_2$/kg, 5 days per week caused profound alterations in the patterns of N-cadherin, E-cadherin and β-catenin (which forms the link between cadherin and the cytoskeleton) localization in the epithelium of the proximal tubule. This Cd^{2+}-induced alteration of cadherin localization was not secondary to cell death, and in addition the Cd^{2+} exposure caused only a very low level of oxidative stress at the time these changes were occurring. Taken together, these findings suggest that the cadherin/catenin complex might be a very early target of Cd^{2+} toxicity in the proximal tubule in vivo (Prozialeck et al. 2003), which is in agreement with the findings with renal cell cultures as described above. Prozialeck et al. emphasize that although their results indicate that exposure to Cd^{2+} can disrupt cadherin-dependent cell–cell junctions in vivo, the underlying mechanisms have not been clarified (Prozialeck et al. 2003). Results of in vitro studies showed that the effect of Cd^{2+} on E-cadherin distribution is similar to that caused by the removal of extracellular Ca^{2+}. This suggests a direct effect of Cd^{2+} on E-cadherin, displacing Ca^{2+} from its binding site due to a higher affinity of Cd^{2+} for these surface-binding sites, thus changing the adhesive properties of the molecule and possibly its interaction with the actin cytoskeleton (Prozialeck and Lamar 1993, 1997; Prozialeck et al. 1996, Prozialeck 2000). Recently, Bathula et al. suggested a role for the third isoform of Mt (Mt-3) in the Cd^{2+}-induced effects on

cadherins. They studied human proximal tubule cells (HPT) with endogenous Mt-3 and HK-2 cells stably transfected with Mt-3 (HK-2(Mt-3)) and compared them with HK-2(blank) control cells (Bathula et al. 2008). The presence of Mt-3 in HK-2(Mt-3) was correlated with an increase in the transepithelial electrical resistance and in the expression status of E-, P- and Ksp-cadherin, that are involved in the mesenchymal to epithelial transition status of the epithelium. So Mt-3 presence seemed to go hand in hand with more E-cadherin and a tighter epithelium. After exposure of the epithelium to 1, 4.5 and 9 μM Cd^{2+} for 7 days, a dose-dependent reduction in transepithelial resistance and in the formation of domes occurred suggesting a loss in intercellular junctions. On the other hand it is known that Mt-3 undergoes a transient induction after exposure to Cd^{2+} before returning to control level (Kim et al. 2002). Bathula et al. hypothesized that the newly formed Mt-3 interacts with Cd^{2+} instead of Zn^{2+}. This could affect the properties of Mt-3 and therefore interact with the junctional proteins and alter the expression of cadherin and the adhesive properties of the proximal tubular cell (Bathula et al. 2008). This hypothesis needs to be investigated further however, as an extracellular action of Cd^{2+} on E-cadherin interfering with cell–cell interaction can not be excluded and could (also) occur and explain their results. Still another possible explanation for the loss in intercellular adhesion due to Cd^{2+} was studied by Park et al. (Park et al. 2008) in T47D breast cancer cells: the authors detected E-cadherin cleavage by a γ-secretase, after Cd^{2+} exposure, and an increased motility and invasion ability. This was preceded by Cd^{2+} induced ROS formation and changes in intracellular Ca^{2+}. Regardless of the causal mechanisms and given the importance of cadherins as regulators of epithelial function, the disruption of cell–cell junctions in the epithelium might help to explain some of the Cd^{2+}-induced changes in epithelial function (Chin and Templeton 1992; Prozialeck and Lamar 1997; Prozialeck et al. 2003). An interesting observation was made in rat liver cells, as mentioned earlier: $CdCl_2$ at low doses (0.03–2.5 μM) decreased the number of gap junctions between the cells and at the same time induced cell proliferation (Jeon et al. 2001). All studies described in this paragraph used the free, ionic form of Cd^{2+} to investigate epithelial integrity. However, in vivo, practically all Cd^{2+} that reaches the systemic circulation is bound to proteins and other materials in blood (Nordberg et al. 1971), which makes it difficult to extrapolate the results of in vitro studies to the in vivo situation. Studies on LLC-PK1 cells in culture showed that exposing the cells to 100 μM of the CdMt complex had no effect on the E-cadherin-dependent junctions between the cells, in contrast to exposure to 20 μM Cd^{2+}. Probably, the affinity of Cd^{2+} for Mt is higher than the binding site on E-cadherin (Prozialeck et al. 1993). However, although CdMt is generally more nephrotoxic in vivo compared to Cd^{2+}, it had almost no toxic effects on the renal epithelial cell line LLC-PK1 (Prozialeck et al. 1993). These findings emphasize the importance of the Cd^{2+} species, the concentrations and the experimental model used in different studies when discussing the results.

Concluding remarks

When discussing the literature and comparing the in vivo and in vitro situations we must keep in mind the range of Cd^{2+} concentrations that occur in an exposed or a general population or in the experimental models. Exposure and uptake of Cd^{2+} occurs via the digestive tract or the lungs. Blood values in humans, mostly measured in whole blood, have been measured in many countries. A few examples show that blood Cd^{2+} is almost always lower than 1 μM. The values measured were ~1 nM to 10 nM (e.g. in Sweden (Olsson et al. 2002; Elinder et al. 1983) or Belgium (Staessen et al. 1994)) or slightly higher in a study in China (Chen et al. 2006a) where mean values ranged from ~20 nM in a non-occupationally exposed, non-smoking group up to ~120 nM in an occupationally exposed, smoking group. They could reach values of the order of ~100 nM in elderly women in a highly polluted region in Japan (Nishijo et al. 2004) or in workers in Sweden, occupationally exposed to Cd^{2+} (range of 5–500 nM) (Hassler et al. 1983).

Most of the filtered Cd^{2+} is taken up by the kidney tubules, as was shown experimentally in mice (Barbier et al. 2004, Jacquillet et al. 2006). Often the proximal tubule and urinary concentrations are not extremely high, even in exposed individuals. Urine Cd^{2+} concentrations are mostly expressed as Cd^{2+}/g creatinine, but with an excretion of around 1 g creatinine per day (0.5–2 g) and a urine

production of 1.5 l/24 h in a healthy adult the concentration is of the order of ~10 nM (calculated for instance from Staessen et al. 1994) or may vary in individuals in occupational exposure from ~10 to 300 nM (Hassler et al. 1983).

A case of acute poisoning with oral intake of a high dose of Cd^{2+} (estimated at 1 g of Cd^{2+}) resulted in 222 nM Cd^{2+} in the blood on day 1 and 143 nM in the urine. After 7 days the blood and urine values returned to 1.8 and 1.8 nM, respectively (Hung and Chung 2004).

As far as experimental animals are concerned: blood values in chronically exposed mice (orally exposed via 100 mg $CdCl_2$/l in the drinking water) reached a peak value after 8–16 weeks of ~500 nM of Cd^{2+} and then stabilized at ~300 nM for the next few weeks (Thijssen et al. 2007b). The concentration in the ultrafiltrate reaching the proximal tubule cells is expected to be less. In rats, 50 mg $CdCl_2$/l in the drinking water resulted in a Cd^{2+} blood concentration of ~140 nM after 6 or 24 months of exposure (Brzoska et al. 2007). Cd^{2+} excretion in the urine was of the order of ~3 nmole/24 h.

Cd^{2+} can also be injected. The following examples give an idea of used procedures and doses and of the concentrations that may be reached in the blood of the experimental animals by a single or a continuous application of Cd^{2+}. In Sprague–Dawley rats, Petersson Grawe and Oskarsson measured Cd^{2+} separately in plasma and red blood cells after an infusion of the rats with 0.3 μg $CdCl_2$/kg/day (Petersson Grawe and Oskarsson 2000). After 16 days the respective concentrations were of the order of 18 and 91 nM. Min et al. (1995) injected one dose of CdMt in mice (2 mg Cd^{2+}/kg sc) and followed plasma and red blood cell Cd^{2+} content the following hours. The plasma value peaked at ~3.3 μM at 3 h but subsided well below 0.1 μM within the next few hours. Most of the Cd^{2+} was to be found in the red blood cells (plateau value reached at 48 h was ~2.7 μM).

In most cases the whole blood level was well below the micromolar range. The same goes for the urinary concentrations and presumably for the renal luminal concentrations, as Cd^{2+} is avidly taken up by the proximal tubule.

That micromolar plasma concentrations of free Cd^{2+} represent a massive dose for an organism was clearly demonstrated by Barbier et al. for instance (Barbier et al. 2004). The authors continuously perfused rats via iv infusion and managed to maintain a plasma free Cd^{2+} concentration of ~3 μM. Within 30 min this caused a Fanconi-like syndrome with severe loss of ions via the urine (Na^+, K^+, Ca^{2+}, Mg^{2+}, P_i, Cl^-).

Low Cd^{2+} exposure harms the organism and disturbs the functioning of organs, tissues, cells and molecular processes and causes illness and death in the end, as we learn from epidemiological studies (e.g. Nawrot et al. 2008). The Cd^{2+} is avidly taken up, especially by liver, kidney, red blood cells, fish gills, … although it may take a while in humans for chronic exposure to low doses to impair cell membrane transport and damage kidney function for instance. It seems the cells need to accumulate a certain amount of Cd^{2+} before signs of dysfunction start to appear. Also, in several studies, after exposing the animals or cells to Cd^{2+} it was found that V_{max} of the transporters was reduced, and not necessarily the K_m (Blumenthal et al. 1990; Lee et al. 1990; Lee et al. 1991; Kim and Park 1995), which could be explained by a change in expression and/or insertion of the transporters in membranes, rather than a change in their properties.

Therefore acute in vitro experiments trying to discover direct effects of Cd^{2+} on cell membrane transporters need to be considered with caution. With a few exceptions the correlation with the in vivo situation may not be entirely justified. The doses applied in the in vitro experiments are often a few orders of magnitude higher than those encountered in vivo. Possibly Cd^{2+} does not affect the transport proteins and transport directly, but rather indirectly via oxidative stress, destruction of the cytoskeleton and/or disruption of cell–cell contacts, suppression or stimulation of expression of transporters, cell death, or still other indirect pathways. To unravel the molecular mechanisms of changes in membrane transport functions due to Cd^{2+}, experimental work is necessary in conditions that are much closer to the in vivo situation.

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