ELSEVIER

Aquatic Toxicology 72 (2005) 119–133

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Renal function in the freshwater rainbow trout (*Oncorhynchus mykiss*) following acute and prolonged exposure to waterborne nickel

Eric F. Pane*, Carol Bucking, Monika Patel, Chris M. Wood

Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ont., Canada L8S 4K1

Received 21 June 2004; received in revised form 3 November 2004; accepted 27 November 2004

Abstract

Renal function was investigated in adult rainbow trout following acute and prolonged exposure to waterborne Ni in moderately hard Lake Ontario water (~140 mg L^{-1} as $CaCO_3$). Fish were exposed for 36 days to a sublethal concentration of 442 μg Ni L^{-1}, followed by 96 h of exposure to 12,850 μg Ni L^{-1} (approximately 33% of the 96 h LC50). Prolonged exposure markedly affected only the renal handling of Ni, with no substantial effect on the plasma concentration, urinary excretion rate (UER) or clearance ratio (CR) of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, inorganic phosphate (P_i), glucose, lactate, total ammonia (T_{amm}), protein and free amino acids (FAA). Glomerular filtration rate (GFR) was reduced by 75% over 96 h of acute Ni challenge in both fish previously exposed to Ni and naïve fish, with no significant change in urine flow rate (UFR), suggesting a substantial reduction in water reabsorption to maintain urine flow and water balance. Renal Mg^{2+} handling was specifically impaired by acute Ni challenge, leading to a significantly increased $UER_{Mg^{2+}}$ and significantly decreased plasma [Mg^{2+}] only in naïve fish. Previously-exposed fish were well-protected against Ni-induced Mg^{2+} antagonism, indicating true acclimation to Ni. Only in naïve, acutely challenged fish was there an increased UER of titratable acidity (TA-HCO_3), net acidic equivalents, P_i, T_{amm} and K^+. Again, all of these parameters were well-conserved in previously-exposed fish during acute Ni exposure, strongly suggesting that prolonged, sublethal exposure protected against acute Ni-induced respiratory toxicity.

Keywords: Nickel; Rainbow trout; Renal physiology; Prolonged exposure; Acclimation

1. Introduction

The kidney of freshwater fish accumulates substantial amounts of Ni during both waterborne (Calamari et al., 1982; Ghazaly, 1992; Pane et al., 2003a, 2004a,b) and dietary (Ptashynski et al., 2002; Ptashynski and Klaverkamp, 2002) Ni exposure. To our knowledge,

* Corresponding author. Tel.: +1 905 525 9140x23237; fax: +1 905 522 6066.
E-mail address: michanderic@yahoo.com (E.F. Pane).

0166-445X/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2004.11.020

the only diagnostic work examining the physiological effects of extensive renal Ni accumulation following waterborne Ni exposure is that of Ghazaly (1992), who documented a dose-dependent increase in Fe content of the kidney of *Tilapia nilotica*, and that of Sreedevi et al. (1992), who reported proteolytic activity in the kidney of acutely exposed carp (*Cyprinus carpio*), including decreased protein content and increased protease activity and free amino acid content of the kidney. Following chronic dietary Ni exposure in the lake whitefish (*Coregonus clupeaformis*), Ptashynski et al. (2002) observed time and dose-dependent histopathological lesions in the glomeruli, tubules, collecting ducts, and hematopoietic tissue.

Injection of Ni into mammals also leads to substantial accumulation in the kidney (Smith and Hackley, 1968; Clary, 1975; Mathur et al., 1978). The effects of Ni accumulation on the physiological (Gitlitz et al., 1975) and biochemical (Abdulwajid and Sarkar, 1983; Ciccarelli and Wetterhahn, 1984; Templeton and Sarkar, 1985; Templeton, 1987) function of the mammalian kidney have been investigated. The study of Gitlitz et al. (1975) reported significant proteinuria and aminoaciduria in Ni-injected rats, the former consistent with fused foot processes of glomerular epithelial cells.

The primary goal of the present study was to comprehensively examine renal function in the rainbow trout during acute and prolonged waterborne Ni exposure. Following 36 days of exposure to 442 μg Ni L^{-1}, renal function was examined and compared to that of unexposed fish. Subsequently, both groups of fish were acutely challenged with Ni (96 h; 12,850 μg Ni L^{-1}) in order to investigate the effects of acute Ni exposure on renal function, and the potential modification of these effects by prior, sublethal exposure. The concentration used for the prolonged exposure (442 μg Ni L^{-1}) is sublethal and only ∼1.5% of the 96 h LC_{50} for adult rainbow trout (Segner et al., 1994). This concentration falls in the upper range of Ni concentrations found in watersheds heavily impacted by mining and industrial activity (Chau and Kulikovsky-Cordeiro, 1995; Eisler, 1998). The acute exposure concentration used in the present study (12,850 μg Ni L^{-1}) is well outside the range of environmental relevance, yet is only ∼33% of the 96 h LC_{50} for adult trout. This diagnostic Ni level has been used previously by us to document mechanisms of branchial and hematological pathology in rainbow trout (Pane et al., 2003a, 2004b). At 12,850 μg L^{-1}, Ni is a potent respiratory toxicant, causing extensive branchial damage leading to a blockade of gas transfer and subsequent hypoxemia and respiratory acidosis (Pane et al., 2003a, 2004b). We therefore hypothesized that investigation of renal function during acute Ni challenge would reveal specific renal compensatory processes to combat this acute, Ni-induced blood acidosis.

2. Materials and methods

2.1. Experimental animals

Adult rainbow trout (200–350 g) were purchased from Humber Springs Trout Farm, Orangeville, Ontario. Fish were acclimated for at least 2 weeks to aerated, flowing dechlorinated Hamilton tap water from Lake Ontario at 12–14 °C and fed ad libitum several times weekly with commercial trout pellets. Water composition was (in mM) $Ca^{2+} \cong 1$, $Mg^{2+} \cong 0.2$, $Na^{+} \cong 0.6$, $Cl^{-} \cong 0.8$, $SO_4{}^{2-} \cong 0.25$, titratable alkalinity to pH 4.0 ≅ 1.9, background Ni ≅ 4 μg L^{-1}, dissolved organic carbon (DOC) ≅ 3 mg L^{-1}, total hardness (as $CaCO_3$) ≅ 140 mg L^{-1} and pH 7.9–8.0. Fish were not fed at least 48 h prior to and throughout all experiments.

2.2. Experimentation

2.2.1. Prolonged Ni exposure

In all exposures, Ni was delivered as $NiSO_4{\cdot}6H_2O$ by gravity from a concentrated stock solution in a flow-through set-up with dechlorinated Hamilton tap water. For prolonged waterborne exposure, a concentration of 442 μg Ni L^{-1} was used for 36 days. Fish were held in 500 L tanks, 15 fish per tank, receiving a flow of 1 L min^{-1}. During this period, fish were fed 1% of their body weight daily. The composition of the food was: crude protein ≅ 40%, crude fat ≅ 11%, crude fiber ≅ 3.5%, Ca ≅ 1.0%, P ≅ 0.85%, Na ≅ 0.45%, and Ni ≅ 3.9 mg kg^{-1} dry weight. Water samples for dissolved Ni were taken five times weekly, 0.45 μm filtered, acidified with trace metal grade HNO_3 (Fisher Scientific), and analyzed for dissolved Ni by graphite furnace atomic absorption spectrophotometry (GFAAS; 220 SpectrAA; Varian, Aus-

tralia) against certified atomic absorption standards (Fisher Scientific).

2.2.2. Surgery

Fish previously exposed to 442 μg Ni L^{-1} for 36 days and naïve fish were anesthetized with 0.075 g L^{-1} of MS222 (neutralized with NaOH to pH 8.0) and fitted with indwelling dorsal aortic catheters (Soivio et al., 1972) and urinary catheters (Wood and Patrick, 1994). Insertion of an indwelling urinary catheter implanted in the urinary bladder allows for continuous gravimetrical collection of urine, commonly referred to as "ureteral urine", as constant siphoning prevents any physiological modification of the urine by the urinary bladder (Wood and Patrick, 1994).

During surgery, the anesthetic solution irrigating the gills of fish previously-exposed fish to Ni was spiked with $NiSO_4 \cdot 6H_2O$ to yield a Ni concentration comparable to that to which these fish had been exposed (442 μg Ni L^{-1}). After surgery, fish were transferred to individual darkened plexiglass chambers (3 L) served with a water flow of 100 mL min^{-1} and continuous aeration, and allowed to recover for 48 h before acute Ni exposure. During this recovery period, boxes housing fish previously exposed to Ni received a comparable solution delivered from a stock solution by gravity flow as described above. Urine flow was continually collected throughout recovery at a pressure differential relative to the water surface of 3 cm of H_2O.

2.2.3. Acute Ni challenge

After 48 h of post-surgical recovery, both previously-exposed and naïve fish were acutely challenged with 96 h of exposure to 12,850 μg Ni L^{-1} delivered by adjustment of the Ni exposure system described above. Additionally, a control treatment included naïve fish receiving Ni-free water for the 96 h acute challenge period. The three treatments are therefore referred to throughout as UnCon (previously-unexposed, unchallenged; $n = 7$), UnNi (previously-unexposed, acutely-challenged; $n = 7$) and ExNi (previously-exposed and acutely-challenged; $n = 7$).

2.2.4. Sampling

Urine flow rate (UFR; mL kg^{-1} h^{-1}) was determined gravimetrically on urine continuously collected over 12 h intervals. To determine glomerular filtration rate (GFR), 24 h before acute Ni challenge, fish were injected individually via the dorsal aortic catheter with 17 μCi of [^{3}H] polyethyleneglycol-4000 (PEG-4000; Perkin-Elmer; specific activity 1.56 mCi g^{-1}). Radiolabelled PEG-4000 is the preferred marker of GFR in teleost fish, as it yields more accurate and conservative GFR values than other markers (see Beyenbach and Kirschner, 1976; Wood and Patrick, 1994 for a discussion of various filtration markers). The radiotracer was delivered in 0.66 mL of Cortland saline (Wolf, 1963) and allowed to equilibrate within the blood of the trout for 12 h prior to the first 12 h collection of urine for analysis (−12 to 0 h pre-challenge period).

Blood samples (50 μL), taken every 12 h from 12 h before acute challenge through 96 h, and urine collected in 12 h intervals, were used to determine GFR. All blood samples were drawn anaerobically via the arterial catheter into an ice-cold, Li-heparinized (50 i.u. mL^{-1}; Sigma–Aldrich), gas-tight Hamilton syringe. For determination of GFR, plasma was separated by centrifugation at 14,000 × *g* for 1 min, and 25 μL of plasma was added to a scintillation vial along with 4.975 mL of double-distilled water and 10 mL of scintillation fluid (ACS; Amersham). Samples were then counted for β activity (1217 Rackbeta; LKB Wallac, Turka, Finland). Urine samples (0.5 mL aliquots) were similarly processed by addition of 4.5 mL of double-distilled water and 10 mL of scintillation fluid prior to β counting.

Additional 1 mL blood samples were taken at 24 h before and 96 h after acute Ni exposure for determination of plasma concentrations of ions (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Ni and inorganic phosphate (P_i)) and metabolites (glucose, lactate, urea, total ammonia (T_{amm}), protein, and free amino acids (FAA)). Blood was drawn and plasma separated by centrifugation (see above), followed by snap-freezing of plasma aliquots in liquid nitrogen and storage at −80 °C for later analysis. Erythrocytes were resuspended in an appropriate volume of Cortland saline and reinjected into the fish. Subsamples of urine collected over the 12 h periods ending at −12, 0, 24, 48, 72, 84 and 96 h were also aliquoted, snap-frozen and stored at −80 °C for later analysis of the same ions and metabolites.

2.2.5. Analyses of plasma and urine parameters

Before all analyses, plasma aliquots were sonicated on ice for 5 s at 5 W (Microson; Misonix Inc., Farm-

ingdale, NY) to ensure homogeneity. Plasma and urine K^+, Ca^{2+}, and Mg^{2+} concentrations were measured by flame atomic absorption spectrophotometry (FAAS; 220FS SpectrAA; Varian, Australia). Due to technical limitations with plasma Na^+ and Cl^- samples, Na^+ and Cl^- concentrations were determined only in the urine by FAAS and the mercuric thiocyanate method (Zall et al., 1956), respectively. Plasma and urine Ni concentration were determined by GFAAS as described above.

Urine pH was measured at 24 h intervals using Radiometer (Copenhagen, Denmark) electrodes, meters, and precision buffer solutions thermostatted to the experimental temperature. Plasma and urine protein concentrations were assayed using Bradford reagent (Bradford, 1976) and bovine serum albumin standards (Sigma–Aldrich). Plasma total ammonia concentration was measured using the glutamate dehydrogenase/NADP enzyme system (Sigma), while urine T_{amm} concentration was determined by the salicylate/hypocholite method (Verdouw et al., 1978) using Sigma reagents. Plasma and urine glucose and lactate concentrations were measured on deproteinized samples (6% perchloric acid) using Infinity reagents (Sigma) and the L-lactate dehydrogenase/NADH enzyme system (Sigma), respectively. Plasma and urine urea were measured using the diacetyl monoxime method of Rahmatullah and Boyde (1980), while total inorganic phosphate (P_i) was determined by the phosphomolybdate reduction method using Sigma reagents. Plasma and urine were assayed for free amino acids with ninhydrin reagent (Sigma) using glycine standards. The T_{amm} concentration of plasma and urine samples was then subtracted from the ninhydrin-derived values to yield the FAA concentration as the ninhydrin reaction is sensitive to ammonia nitrogen.

2.2.6. Urine titration

Urine samples from UnNi and ExNi fish collected from 84 to 96 h were titrated to determine net titratable acidity [TA-HCO_3]. Urine [TA-HCO_3] was measured using a double titration procedure (Hills, 1973; Wood et al., 1999), first titrating the urine down to pH < 3.80, then back up to blood pH as the titration endpoint. Standardized 0.02N HCl and NaOH solutions (Sigma) were used as titrants, delivered by Gilmont microburettes, and pH was measured during the titration process with a Radiometer combination pH electrode and acid–base analyzer. The UER of TA-HCO_3 was calculated by multiplying the [TA-HCO_3] of urine by the UFR measured between 84 and 96 h.

2.3. Calculations

To calculate the GFR over 12 h intervals, the ^3H counts in the continuously collected urine were compared to the ^3H counts in the plasma:

$$\text{glomerular filtration rate (mL kg}^{-1}\text{ h}^{-1}) = \frac{(\text{cpm}_u)(\text{UFR})}{(\text{cpm}_p)} \quad (1)$$

where (cpm_u) is the counts per minute per millilitre of urine and (cpm_p) is the average counts per minute per millilitre of two plasma samples bracketing the 12 h urine collection period.

The urinary excretion rates (UER) of all ions and metabolites were calculated on a mass-specific basis using the formula:

$$\text{urinary excretion rate} = [X]_u(\text{UFR}) \quad (2)$$

where $[X]_u$ is the concentration of a substance in urine collected over a 12 h period, and UFR is the urine flow rate per kg over that same period.

Clearance ratios (CR) of ions and metabolites were calculated according to the formula:

$$\text{clearance ratio} = \frac{[X]_u\ (\text{UFR})}{[X]_p\ (\text{GFR})} \quad (3)$$

where $[X]_u$, UFR and GFR are as described above, and $[X]_p$ is the concentration of a particular substance in the plasma. A clearance ratio is an indicator of whether a substance undergoes net reabsorption (CR < 1) or net secretion (CR > 1) within the kidney.

The clearance rate of Ni at −12 and 96 h of acute exposure was calculated from the data of Fig. 4C and D by dividing UER_{Ni} by the plasma Ni concentration ($[Ni]_p$).

2.4. Blood pressure and heart rate experiments

In a separate experiment to measure blood pressure and heart rate during acute Ni challenge, 14 naïve fish were implanted with dorsal aortic cannulae and allowed to recover as described above. Following the recovery period, seven fish were acutely challenged for 84 h with

Ni (12,605 μg Ni L^{-1}) in flow through conditions as described above, while seven fish served as controls. Blood pressure and heart rate were measured at 0, 12, 24, 48, 72 and 84 h of exposure. Data were collected by attaching saline filled dorsal aortic catheters to saline filled pressure transducers, followed by signal amplification and transfer to a chart recorder (Harvard Apparatus; Holliston, MA). No blood was removed from the fish during the data collections. Traces were taken for 2 min each to ensure steady responses from each fish. Pressure recordings were calibrated gravimetrically with columns of water, while heart rate was calculated from the wave form of each trace at a known chart speed.

2.5. Statistical analyses

All measured and calculated values are presented as mean ± 1 standard error of the mean (S.E.M., n = number of fish). During the acute challenge experiments, time-dependent responses in both control and experimental groups were tested against their respective pre-challenge values by a one-way ANOVA with a two-sided Dunnett's post-hoc multiple comparison test. Comparisons among treatments at each time point during the acute challenge were made with a one-way ANOVA with a Bonferroni post-hoc multiple comparison test. For simplicity, symbols appear only at time points at which significant differences occurred between treatments. All pre-challenge comparisons between treated and control fish were made by an unpaired two-tailed Student's t-test. In Fig. 2, simultaneous control and experimental means were compared with an unpaired two-tailed Student's t-test. Statistical significance in all cases was accepted at $P < 0.05$.

3. Results

3.1. Mortality

There was no mortality during prolonged (36 days) exposure to 442 μg Ni L^{-1}. In control fish (UnCon) and ExNi fish, the decrease from a pre-challenge $n = 7$ to an $n = 5$ during acute Ni exposure was due to catheter failure, as there was no mortality in these groups during the 96 h time course. The same reduction in UnNi fish was due to one catheter failure and one death just prior to the 96 h sampling.

3.2. Effects of prolonged Ni exposure on renal function

Table 1 compares renal parameters from fish exposed for 36 days to sublethal Ni with those of naïve

Table 1
The effects of prolonged, sublethal Ni exposure on renal function of adult rainbow trout

	Control	Ni-exposed
GFR	4.02 ± 0.34	3.89 ± 0.55
UFR	1.81 ± 0.26	1.65 ± 0.09
Plasma		
Na^+ (mM)	146.3 ± 1.0[a]	145.6 ± 0.5[a]
Cl^- (mM)	134.1 ± 1.0[a]	131.5 ± 0.9[a]
K^+ (mM)	2.80 ± 0.09	2.67 ± 0.12
Ca^{2+} (mM)	2.20 ± 0.05	2.36 ± 0.05
Mg^{2+} (mM)	0.72 ± 0.02	0.75 ± 0.02
Ni (μg L^{-1})	287.5 ± 28.5	2617.2 ± 321.8*
P_i (mM)	2.70 ± 0.14	2.56 ± 0.05
Glucose (mM)	8.30 ± 1.96	11.86 ± 2.39
Lactate (mM)	1.34 ± 0.17	1.29 ± 0.17
T_{amm} (mM)	0.152 ± 0.023	0.125 ± 0.013
Urea (mM)	2.19 ± 0.13	2.67 ± 0.25
Protein (g dL^{-1})	2.00 ± 0.16	2.18 ± 0.12
FAA (mM)	11.76 ± 0.62	13.34 ± 0.72
Urine		
Na^+ (mM)	7.64 ± 1.47	7.69 ± 2.77
Cl^- (mM)	8.52 ± 1.21	8.45 ± 2.30
K^+ (mM)	0.95 ± 0.14	0.55 ± 0.05*
Ca^{2+} (mM)	1.18 ± 0.19	1.16 ± 0.19
Mg^{2+} (mM)	0.75 ± 0.32	0.62 ± 0.17
Ni (μg L^{-1})	16.54 ± 3.20	1295.0 ± 297.0*
P_i (mM)	0.53 ± 0.14	0.15 ± 0.04*
Glucose (mM)	0.48 ± 0.18	0.46 ± 0.17
Lactate (mM)	0.12 ± 0.02	0.16 ± 0.03
T_{amm} (mM)	1.52 ± 0.24	0.95 ± 0.36
Urea (mM)	1.98 ± 0.13	3.76 ± 0.70
Protein (g dL^{-1})	0.0049 ± 0.0006	0.0032 ± 0.0008
FAA (mM)	5.56 ± 1.15	1.89 ± 0.73*

Fish were exposed to 442 μg Ni L^{-1} for 36 days. Data are expressed as mean ± 1S.E.M. (n = 5–7). GFR: glomerular filtration rate (mL kg^{-1} h^{-1}); UFR: urine flow rate (mL kg^{-1} h^{-1}); P_i: inorganic phosphate; T_{amm}: total ammonia; FAA: free amino acids.

[a] Not measured in the present study (see Section 2) and taken from Pane et al. (2004a) in which rainbow trout were exposed for 42 days to 384 μg Ni L^{-1}.

* Significant difference ($P < 0.05$; two-tailed Student's t-test) between control and treated fish.

Table 2
The impact of prolonged Ni exposure on the clearance ratios (CR) of ions and metabolites in adult rainbow trout

Clearance ratios	Control	Ni-exposed
Na^+	N/A	N/A
Cl^-	N/A	N/A
K^+	0.14 ± 0.02	0.09 ± 0.02
Ca^{2+}	0.24 ± 0.07	0.17 ± 0.02
Mg^{2+}	0.63 ± 0.37	0.23 ± 0.07
Ni	0.022 ± 0.003	0.189 ± 0.054*
P_i	0.067 ± 0.020	0.026 ± 0.008
Glucose	0.033 ± 0.013	0.013 ± 0.004
Lactate	0.045 ± 0.013	0.042 ± 0.008
T_{amm}	5.64 ± 1.11	3.17 ± 1.39
Urea	0.47 ± 0.10	0.52 ± 0.11
Protein	0.0010 ± 0.0003	0.0006 ± 0.0002
FAA	0.20 ± 0.06	0.04 ± 0.01

CR < 1: net reabsorption, while a CR > 1: net secretion. Exposure details, symbols and abbreviations are as in Table 1.

* Significant difference ($P < 0.05$; two-tailed Student's *t*-test) between control and treated fish.

fish. Prolonged Ni exposure had a marked impact only on Ni homeostasis, as the plasma and urine concentration of Ni were substantially elevated, as were the urinary excretion rate (UER) of Ni (Fig. 4C) and the clearance ratio (CR) of Ni (CR_{Ni}; Table 2) (note that the pre-challenge UER data for ions and metabolites appear on the left-hand edge of the UER plots of Figs. 3, 4 and 6 as −12 to 0 h UER). Ni was strongly reabsorbed (CRs of 0.022–0.189) in both treated and control fish (Table 2).

Although there were significant decreases in the urine concentration of K^+, inorganic phosphate, and free amino acids, the plasma concentrations (Table 1) and clearance ratios (Table 2) of these substances were not significantly impacted by Ni exposure. Nor were the urinary excretion rates (UER) of K^+ (Fig. 6D), P_i (Fig. 6A) and FAA (data not shown) affected by prolonged Ni exposure. Prior Ni exposure had no significant impact on other aspects of renal function, including glomerular filtration rate and urine flow rate (Table 1), as well as plasma concentrations (Table 1), urine concentrations (Table 1) CRs (Table 2) and UERs (Figs. 3, 4 and 6) of Na^+, Cl^-, Ca^{2+}, Mg^{2+}, glucose, lactate, total ammonia, urea, and protein.

3.3. Renal water handling during acute Ni challenge

GFR was strongly affected by acute Ni exposure, being significantly reduced in both UnNi and ExNi fish from 48 h onward (Fig. 1A). Interestingly, despite the decreased GFR in acutely exposed fish, UFR was not substantially altered by acute Ni challenge (Fig. 1B). Despite two transient time points (12 and 60 h) where there were significant differences among the UFRs of the three treatments, UFR at 96 h was very similar both between treatments and within treatments. Because transient variation in UFR has been well documented as a common occurrence (Hickman and Trump, 1969; Cameron, 1980), and because there was no clear pattern of variation among the three treatments, we do not attach any physiological significance to these variations in UFR.

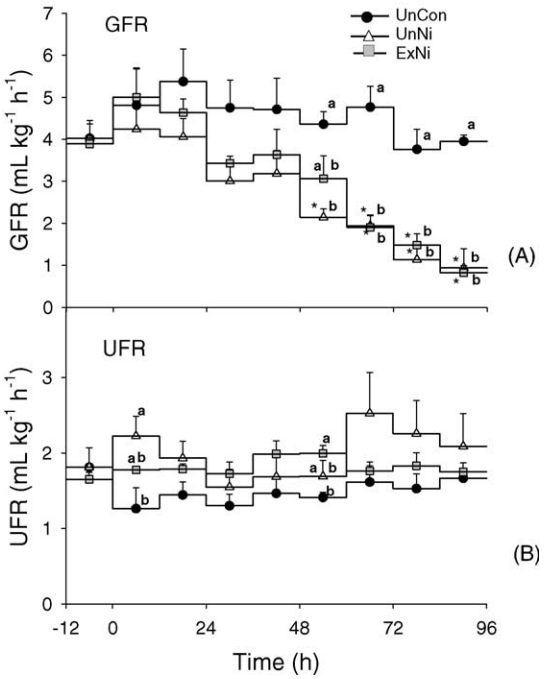

Fig. 1. Renal water handling in adult rainbow trout during acute and prolonged exposure to Ni. Prolonged exposure was to 442 μg Ni L^{-1} for 36 days; acute exposure was to 12,850 μg Ni L^{-1} for 96 h. UnCon: previously-unexposed, unchallenged; UnNi: previously-unexposed, acutely-challenged; and ExNi: previously-exposed and acutely-challenged. Data are expressed as mean ± 1S.E.M. (n = 5–7). Means from the period of −12 to 0 h represent pre-challenge values. Asterisk (*) means within a treatment indicates significant difference from pre-challenge mean. Simultaneous means not sharing the same letter are significantly different. (A) Glomerular filtration rate (GFR). (B) Urine flow rate (UFR).

Cardiac function was not markedly impacted by acute Ni challenge (Fig. 2). Heart rate was only reduced significantly at 12 h in UnNi fish, and well-

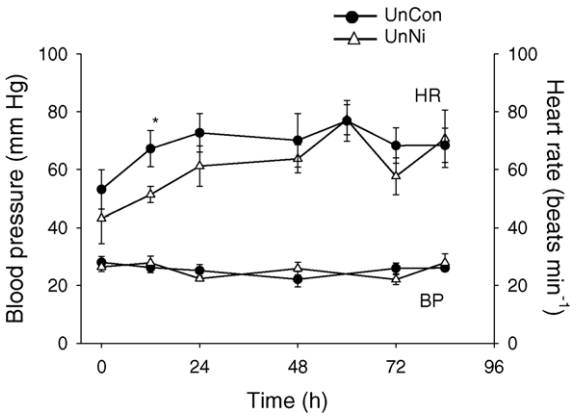

Fig. 2. Cardiac function in adult rainbow trout during acute Ni exposure. Fish were exposed for 84 h to either control or 12,605 μg Ni^{-1}. Asterisk (*) indicates an experimental mean significantly different from its simultaneous control mean. Abbreviations are given in Fig. 1.

conserved from 48 h onward. Similarly, blood pressure was not significantly affected over an 84 h exposure period (Fig. 2).

3.4. Renal ion and metabolite handling during acute Ni challenge

The general trend during acute Ni exposure was toward increased UER of ions and metabolites in UnNi fish. Non-significant elevations of UER of Na^+ (Fig. 3A), glucose (Fig. 3B) and protein (Fig. 3C) in UnNi fish are also representative of similar trends in the renal handling of Cl^- (data not shown). There were significant elevations in the UER of Mg^{2+} (Fig. 4A), P_i (Fig. 6A), T_{amm} (Fig. 6B) and K^+ (Fig. 6D) in UnNi fish. This effect was specific to UnNi fish only, as the UER of these ions and metabolites were well-conserved in ExNi fish during acute Ni exposure (see Figs. 3, 4 and 6).

Urinary excretion rates of Ca^{2+}, lactate and free amino acids measured over 96 h of acute Ni challenge showed no significant differences or consistent patterns among the three treatments (data not shown).

3.5. Renal handling of Mg and Ni during acute Ni challenge

Specific antagonism of renal Mg^{2+} reabsorption during acute Ni exposure is shown in Fig. 4. From

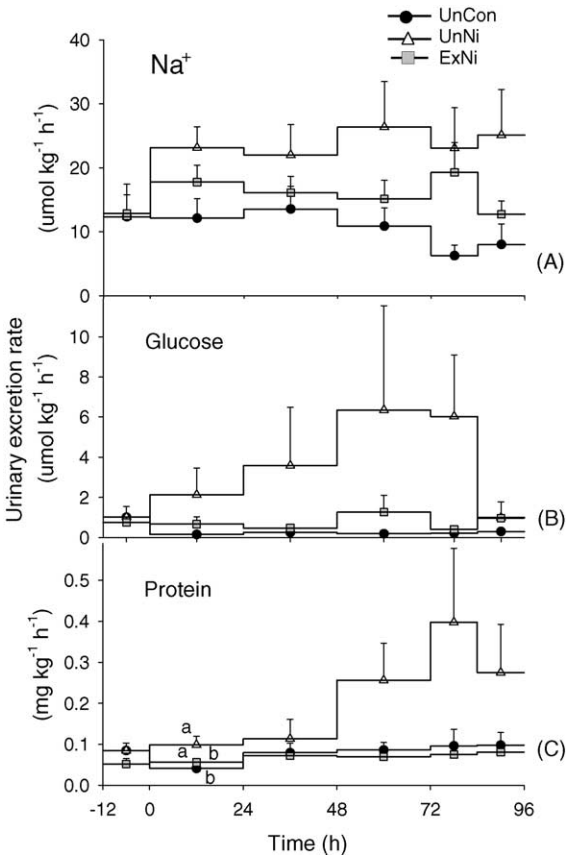

Fig. 3. Renal ion and metabolite handling in adult rainbow trout during acute and prolonged exposure to Ni. Data are expressed as mean ± 1S.E.M. (n = 5–7). Means from the period of −12 h to 0 h represent pre-challenge values. Exposure conditions, symbols and abbreviations are given in Fig. 1. (A) Urinary excretion rate (UER_{Na^+}) of Na^+; (B) $UER_{glucose}$; (C) $UER_{protein}$.

48 to 84 h, the $UER_{Mg^{2+}}$ of UnNi fish was significantly higher than the $UER_{Mg^{2+}}$ of either ExNi or UnCon fish (Fig. 4A). The same trend occurred at 96 h. The increase in Mg^{2+} excretion in the urine of UnNi fish is consistent with a significantly decreased plasma [Mg^{2+}] in these fish at 96 h of acute Ni exposure (Fig. 4B). Prior Ni exposure was an important factor in renal Mg^{2+} handling during acute Ni challenge, as both the $UER_{Mg^{2+}}$ (Fig. 4A) and plasma [Mg^{2+}] (Fig. 4B) of ExNi fish (but not UnNi fish) were well conserved over 96 h of acute Ni exposure.

The pre-challenge UER_{Ni} of ExNi fish was significantly elevated compared to that of control fish (Fig. 4C, Table 1), and remained relatively unchanged

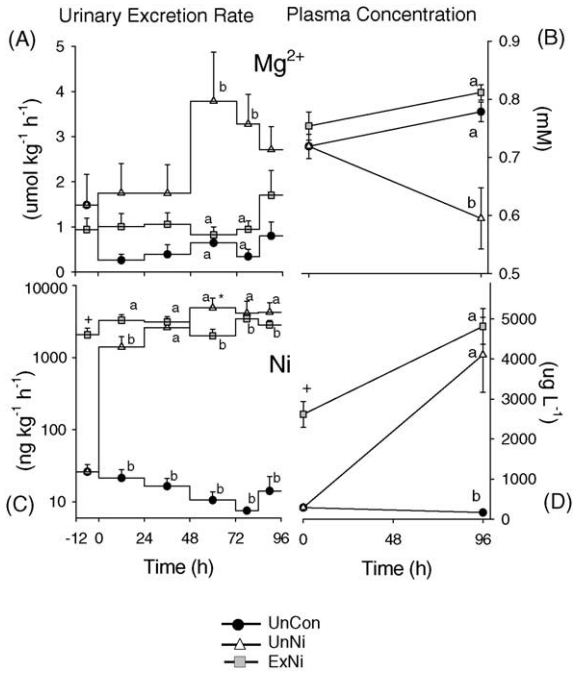

Fig. 4. Renal Mg and Ni handling in adult rainbow trout during acute and prolonged exposure to Ni. Data are expressed as mean ± 1S.E.M. (n = 5–7). Means from the period of −12 h to 0 h represent pre-challenge values. (+): pre-challenge values significantly different from one another. Note log scale in Fig. 4C. Exposure conditions, symbols and abbreviations are given in Fig. 1. (A) $UER_{Mg^{2+}}$; (B) plasma [Mg^{2+}]; (C) UER_{Ni}; (D) plasma [Ni].

throughout the 96 h Ni exposure (Fig. 4C). The UER_{Ni} of UnNi fish increased approximately 100-fold within 48 h, reaching the level of the UER_{Ni} of ExNi fish (Fig. 4C). A similar pattern was observed with plasma [Ni] (Fig. 4D). Here, plasma [Ni] at 96 h was similar in both UnNi and ExNi fish, being about 25–30-fold higher than the plasma [Ni] concentration of UnCon fish (Fig. 4D), despite the significantly different starting points in the two treatments (Table 1).

3.6. Renal compensation of acid–base disturbance

In the present study, renal compensation of a blood acidosis in UnNi fish was evident from the data presented in Figs. 5 and 6. Urine pH of UnNi fish was significantly depressed by 48 h of acute Ni challenge (Fig. 5), falling to a level about 0.5 units below that of UnCon and ExNi fish. Consistent with this drop in urine pH of UnNi fish, titration of urine produced by both UnNi and ExNi fish from 84 to 96 h revealed

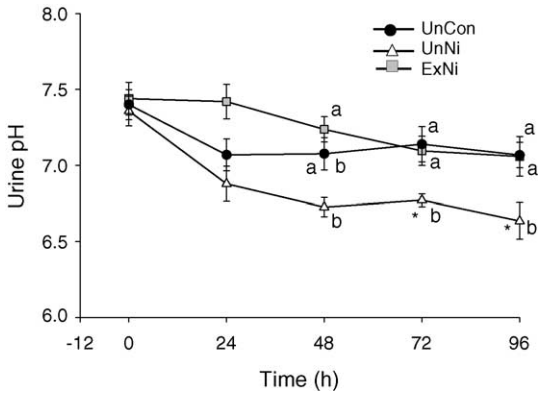

Fig. 5. Urine pH of adult rainbow trout during acute and prolonged exposure to Ni. Data are expressed as mean ± 1S.E.M. (n = 5–7). Time 0 h means represent pre-challenge values. Exposure conditions, symbols and abbreviations are given in Fig. 1.

a elevated urinary excretion rate of titratable acidity ($TA\text{-}HCO_3$) in UnNi fish ($5.06 \pm 2.07\ \mu mol\ kg^{-1}\ h^{-1}$ for UnNi fish versus $-0.55 \pm 0.99\ \mu mol\ kg^{-1}\ h^{-1}$ for ExNi fish; $P < 0.05$).

The UER of the primary urine buffer of freshwater fish, inorganic phosphate P_i, was significantly elevated in UnNi fish from 24 h onward (Fig. 6A). The UER of $TA\text{-}HCO_3$ and P_i in UnNi fish at 96 h were approximately 5.6 (as calculated above) and $6.4\ \mu mol\ kg^{-1}\ h^{-1}$ (see bracket and arrow in Fig. 6A) higher than those of ExNi fish, respectively, highlighting the close coupling of simultaneously increased buffering capacity and acid excretion in the urine of UnNi fish. The UER of total ammonia, another important urine buffer, was also significantly elevated at 84 h in UnNi fish (Fig. 6B), further indicating increased urinary excretion of acid equivalents in UnNi fish. Net excretion rate of acid equivalents in the urine is the sum of the UER of $TA\text{-}HCO_3$ and the UER of T_{amm} (Hills, 1973; Truchot, 1987). Taken together, then, UnNi fish excreted net acid equivalents at a rate of $9.90 \pm 2.64\ \mu mol\ kg^{-1}\ h^{-1}$, compared to only $2.85 \pm 1.52\ \mu mol\ kg^{-1}\ h^{-1}$ in ExNi fish. The overall effects of acute Ni challenge on renal acid–base status are summed up in Table 3.

That the increased UER of T_{amm} in UnNi fish was not a product of impaired nitrogen metabolism is supported by the lack of significant increase in the UER_{urea} of UnNi fish over the 96 h course of acute Ni challenge (Fig. 6C). Indeed, the only significant difference in the UER_{urea} among the three treatments occurred at 84 h,

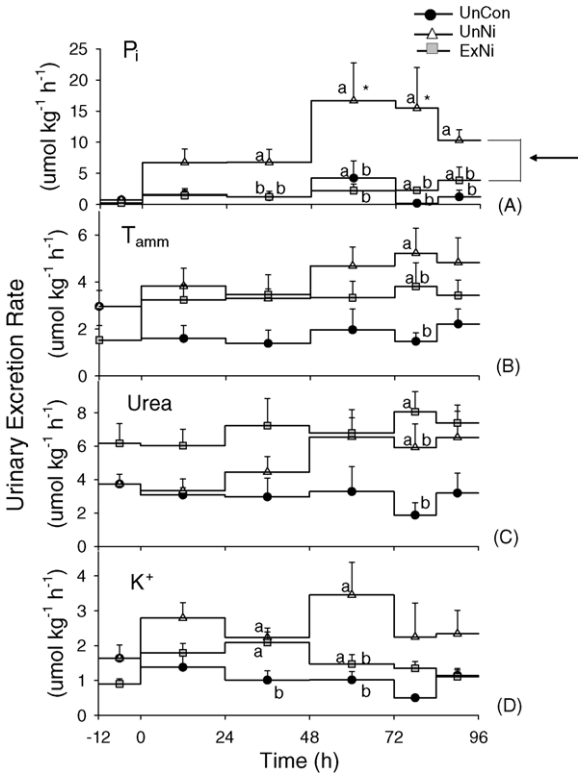

Fig. 6. Renal compensation of acidosis in adult rainbow trout during acute and prolonged exposure to Ni. Data are expressed as mean ± 1S.E.M. (n = 5–7). Means from the period of −12 h to 0 h represent pre-challenge values. Exposure conditions, symbols and abbreviations are given in Fig. 1. Arrow and bracket indicate the 96 h difference in UER_{P_i} between UnNi and ExNi fish (see text for details). (A) UER_{P_i}; (B) $UER_{T_{amm}}$; (C) UER_{urea}; and (D) UER_{K^+}.

where the UER_{urea} of ExNi fish was actually higher than that of UnCon fish (Fig. 6C).

As shown in Fig. 6D, from 24 to 72 h UER_{K^+} was also significantly higher in UnNi fish than in UnCon fish.

4. Discussion

4.1. The effects of prolonged Ni exposure on renal function

Prior to acute challenge, prolonged (36 days) exposure to a sublethal Ni concentration (442 μg Ni L^{-1}) markedly impacted only Ni handling by the kidney of the rainbow trout. The lack of substantial effects of prolonged Ni exposure on most parameters measured (Table 1) is consistent with an earlier study in which a similar prolonged Ni exposure (384 μg Ni L^{-1}; 42 days) had no effect on a suite of physiological parameters in resting rainbow trout, including blood gases, plasma ions and metabolites, hematology and ventilation (Pane et al., 2004a). It appears, then, that resting rainbow trout accumulate Ni in the plasma and urine (Table 1; Fig. 4C and D), as well as in the kidney, gill, heart and intestine (Pane et al., 2004a) during prolonged, sublethal Ni exposure with no great physiological consequence. It should be noted, though, that an acute stress, in the form of exhaustive exercise, imposed on rainbow trout in the study of Pane et al. (2004a) revealed limitations in high performance gas transfer in chronically Ni-exposed fish. In the present study, acute Ni challenge of fish previously exposed to sublethal Ni also revealed marked differences in physiological function suggestive of acclimation effects occurring during the prolonged exposure period.

4.2. Renal water handling during acute Ni challenge

Freshwater fish must constantly deal with the osmotically driven uptake of water from the freshwater medium by production of urine hypo-osmotic to the blood plasma (Hickman and Trump, 1969). The

Table 3
A summary of renal acid–base status following 96 h of acute Ni challenge

	UnCon	UnNi	ExNi
Urine pH	7.07 ± 0.08	6.64 ± 0.12	7.06 ± 0.13
UER of TA-HCO_3^-	N/A	5.06 ± 2.07	−0.55 ± 1.00
UER of T_{amm}	2.21 ± 0.63	4.83 ± 1.05	3.40 ± 0.80
UER of Net acidic equivalents	N/A	9.90 ± 2.64	2.85 ± 1.52
UER of P_i	1.22 ± 1.09	10.32 ± 1.69	3.90 ± 2.11

Abbreviations are as in Table 1 and Fig. 1. UER: urinary excretion rate (μmol kg^{-1} h^{-1}); TA−HCO_3^-: titratable acidity-HCO_3^-; UER of net acidic equivalents is the sum of the UERs of TA-HCO_3^- and T_{amm}.

freshwater kidney is, therefore, primarily a water excretory organ, generally accomplishing water excretion by fractional tubular reabsorption of solutes and water filtered at the glomerulus (Hickman and Trump, 1969). The primary means of controlling UFR is via changes in GFR, underscoring the tight relationship observed between GFR and UFR over a wide range of values in freshwater fish (Hickman, 1965). Because GFR and UFR tend to be directly related, it is inferred that a nearly constant proportion of filtered water should be reabsorbed over a range of filtration rates.

A surprising finding of the present study, however, was the uncoupling of GFR and UFR during acute Ni exposure, independent of Ni pre-exposure, evidenced by the sharp drop in GFR in acutely Ni-exposed fish (Fig. 1A) coupled with a constant UFR (Fig. 1B). In theory, UFR is adjusted to achieve overall net water balance by matching water loss (via urine production) to water uptake.

Although GFR is responsive to changes in systemic blood pressure (Hickman and Trump, 1969), there is no pressure effect underlying the marked GFR reduction during acute Ni exposure, as blood pressure (and heart rate) were unaffected by acute Ni challenge (Fig. 2). How such changes in GFR could occur in the absence of a pressure response, then, is not known at this point in time, and certainly presents an area for future study.

One possible explanation for maintenance of a constant UFR in the face of this falling GFR is a substantial decrease in the fractional tubular reabsorption of water in UnNi and ExNi fish in order to conserve UFR. Although 50% of filtered water is typically reabsorbed in the tubules, reduction of this fraction to as low as 5% can drive changes in UFR (Hickman, 1965). When such a small fraction of water is being reabsorbed, most of filtered water is excreted as urine and the UFR essentially equals the GFR. Under such conditions, one might expect to observe solute loss as decreased rates of reabsorption of solutes would presumably be necessary to drive this increased fractional water excretion.

Changes in fractional water reabsorption can be driven by altered permeability of the apical surface of the renal epithelium to water, particularly in the distal regions of the nephron (Hickman and Trump, 1969). Changes in the permeability of the renal epithelium to water can be accomplished either by alteration of mucus properties or levels of hormones such as arginine vasotocin or urophyseal hormones. Of particular interest is the possibility that Ni damages the ultrastructure (and subsequent function) of the renal epithelium in a manner analogous to the toxic action of Ni within the gill of rainbow trout (Pane et al., 2003a, 2004b).

Although the mean GFR in UnNi and ExNi fell sharply and linearly with time over the 96 h time course, in both of these treatments, the mean GFR exceeded or equalled the mean UFR through 84 h. At 96 h, however, the mean GFR of both treatments was actually significantly lower than the UFR (paired student's *t*-test; $P < 0.05$). Given this aberration, and our inability to explain the reduction in GFR via a pressure effect, the drop in GFR in both groups of Ni-exposed fish may be an artifact, possibly mediated by very high levels of Ni in the plasma and urine (Fig. 4). PEG-4000 was chosen as a GFR marker because it is freely filtered from the blood plasma, yet neither reabsorbed nor secreted. It is possible that Ni interferes with normal PEG distribution either at the level of the glomerulus by impairing filtration, or in the tubule by stimulating either reabsorption or secretion of PEG-4000. Accordingly, these are areas for future study to determine if GFR measurement by PEG-4000 is compatible with high-level metal exposures.

4.3. Renal ion and metabolite handling during acute Ni challenge

The kidney of freshwater fish conserves certain ions and metabolites such as Na^+ and glucose with almost complete tubular reabsorption (Hickman and Trump, 1969). Although there was no effect of prolonged Ni exposure on the handling of Na^+ or glucose (Table 1; Fig. 3A and B), acute Ni challenge resulted in a consistent trend of elevated UER of these substances in UnNi fish (Fig. 3A and B). As UER is the product of UFR and urine ion concentration, and both of these parameters have been shown to vary substantially with time and among fish in healthy control populations (Hickman and Trump, 1969; Cameron, 1980), determination of any clear effect of acute Ni challenge on reabsorptive capacity in UnNi fish appears to necessitate a higher sampling number than the *n* of 5–7 used in the present study. Additionally, fish may be variable in the amount of time needed to exhibit increased UERs of ions and metabolites indicative of Ni-induced renal damage, as evidenced by the fact that four of the five UnNi fish sam-

pled at 96 h experienced a marked increase in UER_{Na^+} averaging 340%.

A similar phenomenon occurred with the UER of protein (Fig. 3C) and substantial variance precluded a clear conclusion about the effects of acute Ni challenge on the integrity of glomerular ultrastructure and the filtration barrier. Here, three of five UnNi fish sampled at 96 h exhibited proteinuria, with an average increase in $UER_{protein}$ of 420%. Based on the mammalian literature, we hypothesized that the normal production of a protein-free filtrate (Brown et al., 1993) would be compromised by Ni exposure, as Ni-injected rats exhibit proteinuria and aminoaciduria mediated by Ni-induced damage to the glomerular filtration barrier (Gitlitz et al., 1975).

4.4. Interactions between Ni and Mg in the trout kidney

Of the ions investigated, only Mg^{2+} was significantly affected by acute Ni exposure, though only in UnNi fish. The data presented in Fig. 4 suggest that Ni inhibited Mg^{2+} reabsorption in the renal tubules of UnNi fish, leading to significantly increased $UER_{Mg^{2+}}$ of and decreased plasma [Mg^{2+}] (Fig. 4). The kidney of freshwater fish reabsorbs ultrafiltered Mg^{2+} (Oikari and Rankin, 1985; Hickman and Trump, 1969). From Table 1, the clearance ratio of Mg^{2+} was approximately 0.6 in control fish, in rough agreement with a CR_{Mg} of approximately 0.4 calculated from the data of Oikari and Rankin (1985) for naïve rainbow trout. Following 36 days of Ni exposure, but prior to 96 h of acute challenge, CR_{Mg} fell non-significantly to 0.23 (Table 1). Fractional Ni reabsorption was far greater than Mg reabsorption under control conditions ($CR_{Ni} = 0.02$; Table 1).

Although we previously reported no significant effects of similar acute Ni exposure on plasma concentrations of the major ions in rainbow trout (Pane et al., 2003a), plasma [Mg^{2+}] in the previous study decreased by about 10% over 96 h of Ni exposure, an effect we have consistently observed (Pane and Wood, personal observation). Plasma [Mg^{2+}] was significantly decreased in the present study by ~20% (Fig. 4B).

Specific antagonism of Mg^{2+} handling by Ni has also been documented by us in another aquatic animal, the freshwater invertebrate, *Daphnia magna* (Pane et al., 2003b). The unidirectional uptake rates of Na^+, Cl^- and Ca^{2+} from the ambient water by *D. magna* were unaffected by acute Ni exposure, despite a 50% reduction of the unidirectional uptake rate of Mg^{2+} (Pane et al., 2003b). Additionally, there is a wealth of literature reporting specific interactions between Ni and Mg in bacteria (Kaltwasser and Frings, 1980; Smith et al., 1995; Eisler, 1998), mold (Adiga et al., 1962), yeast (Ross, 1995), amphibians (Brommundt and Kavaler, 1987) and mammals (Enyedi et al., 1982; Kasprzak and Poirier, 1985; Kasprzak et al., 1986; Miki et al., 1987; Sunderman, 1989; Costa, 1991; Costa et al., 1993). Indeed, Ni uptake in prokaryotes is markedly dependent on Mg^{2+} transport systems, with the two cations transported interchangeably. In fact, unidirectional Mg^{2+} uptake rates in some bacterial species have been approximated using ^{63}Ni as a surrogate radioisotope (Snavely et al., 1991; Smith et al., 1995).

Putative Ni-induced inhibition of Mg^{2+} reabsorption in the renal tubules of freshwater fish is presumably the result of extensive urine accumulation of Ni during acute Ni exposure (Fig. 4C). In the urine of a naïve rainbow trout, a typical Mg to Ni molar ratio is approximately 2700:1. Following 96 h of acute Ni exposure, however, the molar Mg to Ni ratio in the urine of UnNi fish fell to ~40:1. Interestingly, the inhibition of unidirectional uptake rate of Mg^{2+} from freshwater in *D. magna* was observed when the normal freshwater molar Mg to Ni ratio of 2500:1 was experimentally decreased to 8:1 during acute Ni exposure (Pane et al., 2003b).

It should be noted, however, that antagonism of renal Mg^{2+} handling observed in the present study was purely an acute phenomenon. Despite an approximate 30:1 Mg to Ni molar ratio in the urine both before and throughout the 96 h acute Ni exposure, ExNi fish showed a well-conserved $UER_{Mg^{2+}}$ and plasma [Mg^{2+}] both before (Fig. 4A and B: Table 1) and after (Fig. 4A and B) acute Ni exposure.

Although not statistically significant due to the high variability in the response of control fish, the lower $CR_{Mg^{2+}}$ following prolonged Ni exposure (0.23 ± 0.07 versus 0.63 ± 0.37 in control fish) suggests that Mg reabsorption is rendered more efficient by prolonged Ni exposure. $CR_{Mg^{2+}}$ of Ni-exposed fish was clearly well below 1 indicating strong reabsorption of Mg^{2+} despite substantial urine Ni loading. Prolonged Ni exposure could have conferred an acclimation effect by

protecting Mg^{2+} reabsorption against the competitive effects of Ni either by increasing the copy number of key Mg^{2+} transporters in the renal tubules, or by changing their kinetic properties, i.e. the substrate affinity (K_m) or transport capacity (J_{max}). Alterations of these two kinetic properties have been observed for branchial Na^+ transport following prolonged, sublethal exposure to waterborne Cu (Lauren and McDonald, 1987; McDonald and Wood, 1993).

In addition to the possibility that Ni may directly compete with Mg for reabsorption pathways within the renal tubules, the increased $UER_{Mg^{2+}}$ of UnNi fish (Fig. 4A) may be driven in part by Ni-induced perturbation of the cellular machinery powering active Mg^{2+} reabsorption from the urine. According to a trapped plasma analysis of the substantial Ni burden accumulated by the kidney of rainbow trout during acute Ni exposure, the majority of renally accumulated Ni is intracellularly located at levels that may be cytotoxic (>100 μmol kg^{-1}; Pane et al., 2004b). The specific renal interactions between these two cations are currently being investigated using in vitro preparations of kidney brush border membrane vesicles from fish acutely and chronically exposed to Ni.

4.5. Renal excretion of accumulated Ni

Calculation of clearance rate allows for estimation of the efficiency of renal clearance of plasma constituents (Wood and Patrick, 1994). Clearance rates of Ni in UnCon fish were predictably low (0.09 ± 0.02 mL kg^{-1} h^{-1}), increasing to 0.89 ± 0.35 mL kg^{-1} h^{-1} in UnNi fish after 96 h of acute exposure. ExNi fish had a pre-challenge Ni clearance rate of 0.56 ± 0.08 mL kg^{-1} h^{-1} and a post-challenge rate of 0.80 ± 0.24 mL kg^{-1} h^{-1}.

Despite the 10-fold increase over control rates, 96 h clearance rates of Ni in UnNi and ExNi were only approximately half of 96 h UFR in these fish (~1.8–2.0 mL kg^{-1} h^{-1}). As UFR is essentially a measure of the clearance rate of water, the urinary route appears to be a relatively poor means of Ni excretion in acutely challenged fish, with Ni being cleared from the blood plasma less efficiently than water. The poor renal clearance of Ni may be attributed in part to strong renal Ni reabsorption. Acting as a Mg analog, Ni would move in the same direction as Mg, a large fraction of which is reabsorbed by the kidney of freshwater fish (Oikari and Rankin, 1985; Hickman and Trump, 1969).

The inefficiency of renal Ni clearance and the marked accumulation of Ni in the kidney of rainbow trout following waterborne Ni exposure (Calamari et al., 1982; Pane et al., 2003a, 2004a,b) may also be due to Ni binding in the plasma. Ni in human blood is bound to three carriers, one of which is the low molecular weight amino acid histidine (Abdulwajid and Sarkar, 1983; Eisler, 1998). Plasma Ni, therefore, is presumably subject to high rates of ultrafiltration when bound to histidine. The efficiency of renal FAA absorption (see Table 1), however, may prevent the excretion of histidine–Ni complexes and the implication that reabsorptive Ni transport in renal tubules is facilitated by histidine–Ni binding needs to be tested experimentally. Interestingly, the absorption of Zn by the rainbow trout intestine is strongly modified by amino acids, particularly histidine (Glover and Hogstrand, 2002; Glover et al., 2003).

4.6. Renal compensation of acid–base disturbance

Although the gill of a freshwater fish is typically a more important acid–base regulatory organ than the kidney (Truchot, 1987), there are circumstances in which renal contribution to acid–base regulation can be substantial (Wood and Caldwell, 1978). Regardless of the stimulus of the acid–base disturbance, the kidney generally responds to acidosis by increased net acid excretion, resulting from increased UER of TA-HCO_3 and T_{amm} (Cameron, 1980; Truchot, 1987; see Table 3). This is consistent with Ni-induction of respiratory acidosis observed during acute exposure (Pane et al., 2003a). Acute Ni exposure similar to that of the present study causes acute respiratory toxicity in rainbow trout via a blockade of gas exchange culminating in a profound hypoxemia and a subsequent respiratory acidosis (Pane et al., 2003a). The extensive gill damage that impairs gas exchange during acute Ni exposure (see Pane et al., 2004b) presumably necessitates a greater reliance on renal compensation of the ensuing respiratory acidosis.

The importance of increasing buffering capacity to facilitate net acid excretion in the kidney is underscored by the commitment of a substantial fraction of tubular, mucosally-directed proton pumping to bicarbonate reabsorption from the urine, a process which results in

proton cycling, not net excretion (Truchot, 1987). Inorganic phosphate (Fig. 6A; Table 3) is an important urine acid buffer in freshwater fish, serving to excrete protons as $H_2PO_4^-$, while a typical urine pH that is 2–3 units below the pK of ammonia (∼9.2) dictates that almost all of the increase in excreted T_{amm} (Fig. 6B; Table 3) is in the form of ammonium ion, further serving net excretion of acid equivalents.

In addition to eliminating excess acidic equivalents via branchial and renal routes, rainbow trout can buffer excess protons during an extracellular acidosis by exchange of extracellular protons with intracellular K^+ (McDonald and Wood, 1981). This electroneutral exchange causes a K^+ "washout" (McDonald and Wood, 1981) potentially accounting for the increased UER_{K^+} of UnNi fish (Fig. 6D).

An intriguing result of the present study is the apparent marked protection against acute Ni-induced respiratory acidosis afforded by prolonged, sublethal Ni exposure. In conjunction with the renal data presented herein strongly suggesting that ExNi fish are protected to some degree against acute respiratory toxicity, we also have preliminary blood gas and acid–base data confirming this phenomenon (Pane and Wood, unpublished data). If indeed this is the case, Ni would join several other metals for which an acclimation effect has been described whereby prolonged sublethal exposure protects against acute toxicity (McDonald and Wood, 1993).

Unique to Ni, acclimation to waterborne Ni may be a consequence of immune function. In mammals, Ni is considered a moderate contact allergen (Kligman, 1966). We have speculated previously that extensive hypertrophy of the branchial epithelium and subsequent blockade of gas transfer during acute Ni exposure may be mediated in part by an allergic reaction (Pane et al., 2004b). Accordingly, we hypothesize that prolonged Ni exposure might desensitize the acute immune response, via a classic acquired immunity process, thereby preventing swelling of respiratory lamellae otherwise seen during acute Ni challenge (Pane et al., 2004b).

Acknowledgments

This work was supported by the NSERC Strategic Grants Program, the Nickel Producers Environmental Research Association, the International Copper Association, the Copper Development Association, the International Lead Zinc Research Organization, Teck Cominco and Noranda-Falconbridge. EFP is supported by an Ontario Graduate Scholarship. CMW is supported by the Canada Research Chair Program.

References

Abdulwajid, A.W., Sarkar, B., 1983. Nickel-sequestering renal glycoprotein. Proc. Natl. Acad. Sci. U.S.A. 80, 4509–4512.

Adiga, P.R., Sastry, K.S., Sarma, P.S., 1962. The influence of iron and magnesium on the uptake of heavy metals in metal toxicities in *Aspergillus niger*. Biochim. Biophys. Acta 64, 548–551.

Beyenbach, K.W., Kirschner, L.B., 1976. The unreliability of mammalian glomerular markers in the teleostean renal studies. J. Exp. Biol. 64, 369–378.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brommundt, G., Kavaler, F., 1987. La^{3+}, Mn^{2+}, and Ni^{2+} effects on Ca^{2+} pump and on Na^+–Ca^{2+} exchange in bullfrog ventricle. Am. J. Physiol. 253, C45–C51.

Brown, J.A., Rankin, J.C., Yokota, S.D., 1993. Glomerular haemodynamics and filtration in single nephrons of non-mammalian vertebrates. In: Brown, J.A., Balment, R.J., Rankin, J.C. (Eds.), New Insights in Vertebrate Kidney Function, Society for Experimental Biology Seminar Series 52. Cambridge University Press, Cambridge, UK.

Calamari, D., Gaggino, G.F., Pacchetti, G., 1982. Toxicokinetics of low levels of Cd, Cr, Ni and their mixture in long-term treatment on *Salmo gairdneri* rich. Chemosphere 11, 59–70.

Cameron, J.N., 1980. Body fluid pools, kidney function, and acid–base regulation in the freshwater catfish *Ictalurus punctatus*. J. Exp. Biol. 86, 171–185.

Chau, Y.K., Kulikovsky-Cordeiro, O.T.R., 1995. Occurrence of nickel in the Canadian environment. Environ. Rev. 3, 95–117.

Ciccarelli, R.B., Wetterhahn, K.E., 1984. Nickel-bound chromatin, nucleic acids, and nuclear proteins from kidney and liver of rats treated with nickel carbonate in vivo. Cancer Res. 44, 3892–3897.

Clary, J.J., 1975. Nickel chloride-induced metabolic changes in the rat and guinea pig. Toxicol. Appl. Pharmacol. 31, 55–65.

Costa, M., 1991. Molecular mechanisms of nickel carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 31, 321–337.

Costa, M., Zhitkovich, A., Toniolo, P., 1993. DNA-protein cross-links in welders: molecular implications. Cancer Res. 53, 460–463.

Eisler, R., 1998. Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. US Geological Survey, Biological Resources Division, Biological Science Report 1998, 0001.

Enyedi, A., Sarkadi, B., Nyers, A., Gardos, G., 1982. Effects of divalent metal ions on the calcium pump and membrane phosphorylation in human red cells. Biochim. Biophys. Acta 690, 41–49.

Ghazaly, K.S., 1992. Sublethal effects of nickel on carbohydrate metabolism, blood and mineral contents of *Tilapia nilotica*. Water Air Soil Pollut. 64, 525–532.

Gitlitz, P.H., Sunderman Jr., F.W., Goldblatt, P.J., 1975. Aminoaciduria and proteinuria in rats after a single intraperitoneal injection of Ni(II). Toxicol. Appl. Pharmacol. 34, 430–440.

Glover, C.N., Hogstrand, C., 2002. Amino acid modulation of in vivo intestinal zinc absorption in freshwater rainbow trout. J. Exp. Biol. 205, 151–158.

Glover, C.N., Bury, N.R., Hogstrand, C., 2003. Zinc uptake across the apical membrane of freshwater rainbow trout intestine is mediated by high affinity, low affinity, and histidine-facilitated pathways. Biochim. Biophys. Acta 1614, 211–219.

Hickman, C.P., 1965. Studies on renal function in freshwater teleost fish. Tran. R. Soc. Can. Sect. III [4] 3, 213–236.

Hickman, C.P., Trump, B.F., 1969. The kidney. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 1. Academic Press, New York, NY.

Hills, A.G., 1973. Acid–Base Balance. Chemistry, Physiology, Pathophysiology. Williams and Wilkens, Baltimore, MD.

Kaltwasser, H., Frings, W., 1980. Transport and metabolism of nickel in microorganisms. In: Nriagu, J.O. (Ed.), Nickel in the Environment. Wiley, New York, NY.

Kasprzak, K.S., Poirier, L.A., 1985. Effects of calcium(II) and magnesium(II) on nickel(II) uptake and stimulation of thymidine incorporation into DNA in the lungs of strain A mice. Carcinogenesis 6, 1819–1821.

Kasprzak, K.S., Waalkes, M.P., Poirier, L.A., 1986. Antagonism by essential divalent metals and amino acids of nickel(II)-DNA binding in vitro. Toxicol. Appl. Pharmacol. 82, 336–343.

Kligman, A.M., 1966. The identification of contact allergens by human assay. III. The maximization test: a procedure for screening and rating contact sensitizers. J. Invest. Dermatol. 47, 393–409.

Lauren, D.J., McDonald, D.G., 1987. Acclimation to copper by rainbow trout: physiology. Can. J. Fish Aquat. Sci. 44, 99–104.

Mathur, A.K., Dikshith, T.S.S., Lal, M.M., Tandon, S.K., 1978. Distribution of nickel and cytogenetic changes in poisoned rats. Toxicology 10, 105–113.

McDonald, D.G., Wood, C.M., 1981. Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. J. Exp. Biol. 93, 101–118.

McDonald, D.G., Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman & Hall, London.

Miki, H., Kasprzak, K.S., Kenney, S., Heine, U.I., 1987. Inhibition of intercellular communication by nickel(II): antagonistic effect of magnesium. Carcinogenesis 8, 1757–1760.

Oikari, A.O.J., Rankin, J.C., 1985. Renal excretion of magnesium in a freshwater teleost, *Salmo gairdneri*. J. Exp. Biol. 117, 319–333.

Pane, E.F., Richards, J.G., Wood, C.M., 2003a. Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than ionoregulatory mechanism. Aquat. Toxicol. 63, 65–82.

Pane, E.F., Smith, C., McGeer, J.C., Wood, C.M., 2003b. Mechanisms of acute and chronic waterborne nickel toxicity in the freshwater cladoceran, *Daphnia magna*. Environ. Sci. Technol. 37, 4382–4389.

Pane, E.F., Haque, A., Goss, G.G., Wood, C.M., 2004a. The physiological consequences of exposure to chronic, sublethal waterborne Ni in the rainbow trout (*Oncorhynchus mykiss*): exercise vs. resting physiology. J. Exp. Biol. 207, 1249–1261.

Pane, E.F., Haque, A., Wood, C.M., 2004b. Mechanistic analysis of acute, Ni-induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon. Aquat. Toxicol. 69, 11–24.

Ptashynski, M.D., Klaverkamp, J.F., 2002. Accumulation and distribution of dietary nickel in lake whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 58, 249–264.

Ptashynski, M.D., Pedlar, R.M., Evans, R.E., Baron, C.L., Klaverkamp, J.F., 2002. Toxicology of dietary nickel in lake whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 58, 229–247.

Rahmatullah, M., Boyde, T.R.C., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. Clin. Chim. Acta 107, 3–9.

Ross, I.S., 1995. Reduced uptake of nickel by a nickel resistant strain of *Candida utilis*. Microbios 83, 261–270.

Segner, H., Lenz, D., Hanke, W., Schuurmann, G., 1994. Cytotoxicity of metals toward rainbow trout R1 cell line. Environ. Toxicol. Water Qual. 9, 273–279.

Smith, J.C., Hackley, B., 1968. Distribution and excretion of nickel-63 administered intravenously to rats. J. Nutr. 95, 541–546.

Smith, R.L., Thompson, L.J., Maguire, M.E., 1995. Cloning and characterization of MgtE, a putative new class of Mg^{2+} transporter from *Bacillus firmus*OF4. J. Bacteriol. 177, 1233–1238.

Snavely, M.D., Gravina, S.A., Cheung, T.T., Miller, C.G., Maguire, M.E., 1991. Magnesium transport in *Salmonella typhimurium*. J. Biol. Chem. 266, 824–829.

Soivio, A., Westman, K., Nyholm, K., 1972. Improved method of dorsal aorta catheterization: hematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*). Finn. Fish Res. 1, 11–21.

Sreedevi, P., Sivaramakrishna, B., Suresh, A., Radhakrishnaiah, K., 1992. Effects of nickel on some aspects of protein metabolism in the gill and kidney of the freshwater fish *Cyprinus carpio* L. Environ. Pollut. 77, 59–63.

Sunderman Jr., F.W., 1989. Mechanistic aspects of nickel carcinogenicity. Arch. Toxicol. Suppl. 13, 40–47.

Templeton, D.M., Sarkar, B., 1985. Peptide and carbohydrate complexes of nickel in human kidney. Biochem. J. 230, 35–42.

Templeton, D.M., 1987. Interaction of toxic cations with the glomerulus: binding of Ni to purified glomerular basement membrane. Toxicology 43, 1–15.

Truchot, J.P., 1987. Comparative Aspects of Extracellular Acid–Base Balance. Springer-Verlag, Berlin.

Verdouw, H., van Echteld, C.J.A., Dekkers, E.M.J., 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12, 399–402.

Wolf, K., 1963. Physiological salines for freshwater teleosts. Prog. Fish Cult. 25, 135–140.

Wood, C.M., Caldwell, F.H., 1978. Renal regulation of acid–base balance in a freshwater fish. J. Exp. Zool. 205, 301–307.

Wood, C.M., Patrick, M.L., 1994. Methods for assessing kidney and urinary bladder function in fish. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier, New York, NY.

Wood, C.M., Milligan, C.L., Walsh, P.J., 1999. Renal responses of trout to chronic respiratory and metabolic acidosis and metabolic alkalosis. Am. J. Physiol. 277, R482–R492.

Zall, D.M., Fisher, M.D., Garner, Q.M., 1956. Photometric determination of chlorides in water. Anal. Chem. 28, 1665–1678.