Impact of Bicarbonate, Ammonium Chloride, and Acetazolamide on Hepatic and Renal SLC26A4 Expression

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Key Words

SLC26A4 • Acidosis • Alkalosis • Liver • Kidney

Abstract

SLC26A4 encodes pendrin, a transporter exchanging anions such as chloride, bicarbonate, and iodide. Loss of function mutations of SLC26A4 cause Pendred syndrome characterized by hearing loss and enlarged vestibular aqueducts as well as variable hypothyroidism and goiter. In the kidney, pendrin is expressed in the distal nephron and accomplishes HCO₃⁻ secretion and Cl⁻ reabsorption. Renal pendrin expression is regulated by acid-base balance. The liver contributes to acid-base regulation by producing or consuming glutamine, which is utilized by the kidney for generation and excretion of NH₄⁺, paralleled by HCO₃⁻ formation. Little is known about the regulation of pendrin in liver. The present study thus examined the expression of Slc26a4 in liver and kidney of mice drinking tap water without or with NaHCO₃ (150 mM), NH₄Cl (280 mM) or acetazolamide (3.6 mM) for seven days. As compared to *Gapdh* transcript levels, *Slc26a4* transcript levels were moderately lower in liver than in renal tissue. *Slc26a4* transcript levels were not significantly affected by NaHCO₃ in liver, but

significantly increased by NaHCO₃ in kidney. Pendrin protein expression was significantly enhanced in kidney and reduced in liver by NaHCO₃. Slc26a4 transcript levels were significantly increased by NH₄Cl and acetazolamide in liver, and significantly decreased by NH₄Cl and by acetazolamide in kidney. NH₄Cl and acetazolamide reduced pendrin protein expression significantly in kidney, but did not significantly modify pendrin protein expression in liver. The observations point to expression of pendrin in the liver and to opposite effects of acidosis on pendrin transcription in liver and kidney.

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Introduction

SLC26A4 encodes pendrin, an anion exchanger transporting chloride, bicarbonate, iodide and a variety of other anions [1]. Loss of function defects of SLC26A4 [2] result in autosomal-recessive Pendred syndrome (PDS), characterized by sensorineural hearing loss and enlarged vestibular aqueducts [1, 3, 4] as well as an iodide organification defect with enhanced susceptibility to develop goiter and hypothyroidism [1, 5, 6]. The development of goiter and hypothyroidism vary and

partially depend on nutritional iodide intake [6, 7].

SLC26A4 expression is most abundant in the thyroid gland, the inner ear, and the kidney [5, 7]. In the thyroid, SLC26A4 participates in the transport of iodide [5, 7]. SLC26A4 is further expressed in lung tissue and participates in the regulation of mucus production [8]. It has been suggested that IL-13 induced SLC26A4 expression contributes to the pathophysiology of bronchial asthma [8].

In the kidney, SLC26A4 is expressed in the apical membrane of type B and non-A, non-B intercalated cells of the distal convoluted tubule, connecting tubule and cortical collecting duct and thus accomplishes HCO_3^- secretion and Cl^- reabsorption in the distal nephron [9–12]. SLC26A4 expression and function is increased at enhanced aldosterone or angiotensin II levels and SLC26A4 function contributes to the hypertensive effect of those hormones [12, 13]. SLC26A4 modifies the expression of the epithelial Na^+ channel ENaC, an effect attributed, at least in part, to its impact on luminal HCO_3^- concentration [12].

SLC26A4 activity and expression in the kidney is regulated by the acid-base status [11, 14, 15]. Generation of HCO_3^- in type B intercalated cells is accomplished by a cellular carbonic anhydrase (CAII) and a H^+ -ATPase at the basolateral membrane [9, 16]. Carbonic anhydrase II deficiency decreases SLC26A4 expression in the kidney [16].

Regulation of systemic acid-base balance requires coordinated functions of the liver and the kidneys [17]. Acid-base regulation involves renal ammoniagenesis and hepatic urea production, which are tied together by interorgan glutamine flux. Glutamine could be used either in the liver for urea formation or in the kidney for generation and excretion of ammonia [17]. Renal ammonia excretion but not hepatic urea formation is paralleled by generation of HCO_3^- . Accordingly, metabolic acidosis is followed by a shift from the use of glutamine for hepatic urea formation to the use of glutamine for renal ammonium formation and excretion [17–19].

Little is known about expression of SLC26A4 in the liver. The present study thus explored whether liver tissue expresses SLC26A4 and whether hepatic SLC26A4 expression is influenced by acid-base balance in a similar way as in the kidney. To this end, SLC26A4 transcript and protein levels were quantified in liver and kidney from mice treated with bicarbonate to induce metabolic alkalosis [20], ammonium chloride to trigger metabolic acidosis [21] and acetazolamide to inhibit carbonic anhydrase [22].

Materials and Methods

Animals

Experiments were performed in 8–10 week old female and male C57Bl/6J mice. All animal experiments were conducted according to German and Swiss laws for the welfare of animals and were approved by local authorities. The animals had free access to food (C1310, Altromin, Heidenau, Germany) and tap water. Where indicated NaHCO_3 (150 mM), NH_4Cl (280 mM) or acetazolamide (3.6 mM) were added to the drinking water for 7 days. Drinking volumes and body weight were determined prior to and during the experimental period.

Determination of blood pH and pCO_2

Blood samples were collected by submandibular bleeding utilizing a lancet for mandibular cheek punch (Goldenrod lancet, New York, USA) to determine pH and pCO_2 . The pH was determined utilizing an electrode and pCO_2 utilizing a blood gas analyzer (System C 2000, Eschweiler, Kiel, Germany). Plasma HCO_3^- concentration was calculated from pCO_2 and pH utilizing the Henderson Hasselbach equation [23].

RT-PCR analysis

To determine *Slc26a4* mRNA abundance in mouse liver and kidney RNA was extracted from both tissues using Trifast Reagent (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. Reverse transcription of 2 μg RNA was performed using oligo(dT)_{12–18} primers (Invitrogen, Karlsruhe, Germany) and SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). cDNA samples were treated with RNase H (Invitrogen, Karlsruhe, Germany). Quantitative RT-PCR was performed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and iTaq™ Sybr Green Supermix with ROX (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The following primers were used (5'→3' orientation): SLC26A4 s: TTC GGT CTC TAC TCT GCC TTT; SLC26A4 as: CCC ACC ATT AAA CTG ACC ACG; Gapdh s: AGG TCG GTG TGA ACG GAT TTG; Gapdh as: TGT AGA CCA TGT AGT TGA GGT CA. The specificity of the PCR products was confirmed by analysis of the melting curves and in addition by agarose gel electrophoresis. All PCRs were performed in duplicate, and mRNA fold changes were calculated by the $\Delta\Delta\text{Ct}$ method using Gapdh as internal reference.

Membrane preparation and western blot analysis

For total membrane preparation kidney and liver samples were homogenized in an ice-cold K-HEPES buffer (200 mM mannitol, 80 mM HEPES, 41 mM KOH, pH 7.5) containing a protease inhibitor mix (Complete Mini, Roche Diagnostics, Germany) at a final concentration of 1 tablet in a volume of 10 ml solution. Samples were centrifuged at 2000 rpm for 20 min at 4°C. Subsequently, the supernatant was transferred to a new tube and centrifuged at 41000 rpm for 1 h at 4°C. The resultant pellet was resuspended in K-HEPES buffer containing protease inhibitors. After measurement of the total protein concentration (Bio-Rad D_c Protein Assay; Bio-Rad, Hercules, CA, USA), 10 (kidney) or 50 (liver) μg of crude membrane proteins were

Drinking fluid	pH	pCO ₂	Na ⁺	K ⁺	Cl ⁻	cHCO ₃ ⁻	n
Water	7.39 ± 0.01	41.5 ± 0.8	143.2 ± 0.4	4.9 ± 0.2	111.4 ± 1.3	24.9 ± 0.7	5
NaHCO ₃	7.38 ± 0.01	41.0 ± 2.0	143.2 ± 0.4	4.8 ± 0.1	111.2 ± 0.6	23.9 ± 1.5	5
NH ₄ Cl	7.29 ± 0.03**	41.2 ± 1.2	147.6 ± 1.2**	4.7 ± 0.1	119.6 ± 0.7***	19.4 ± 1.0**	5
Acetazolamide	7.22 ± 0.01***	50.6 ± 1.3***	148.6 ± 0.7***	4.8 ± 0.1	122.0 ± 0.9***	19.9 ± 0.4**	5

Table 1. Plasma pH, plasma CO₂ partial pressure (pCO₂, mmHg), as well as plasma Na⁺, K⁺, and Cl⁻ concentration (mM) in water, NaHCO₃ (150 mM), NH₄Cl (280 mM) and acetazolamide (3.6 mM) drinking animals (arithmetic means ± SEM, n = 5). **(*p* < 0.01), ***(*p* < 0.001) indicate statistically significant differences to water drinking animals.

solubilized in Laemmli sample buffer, and SDS-PAGE was performed on 8% polyacrylamide gels. For immunoblotting, proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA, USA). After blocking with 5% milk powder in Tris-buffered saline/0.1% Tween-20 for 60 min, the blots were incubated with the respective primary antibodies (rabbit anti-pendrin 1:5000 [14] and mouse monoclonal anti-β-actin antibody (42 kDa; Sigma, St. Louis, MO, USA) 1:5000, diluted in 1% milk/TBS-T) either for 2 h at room temperature or overnight at 4°C. After washing and subsequent blocking, the membranes were incubated for 1 h at room temperature with the secondary antibody: goat anti-rabbit or anti-mouse IgG-conjugated with alkaline phosphatase 1:5000 (Promega, WI, USA). Antibody binding was detected with the CDP-Star Western chemiluminescence kit (Roche Diagnostics, Mannheim, Germany) using the DIANA III-chemiluminescence detection system (Raytest; Straubenhardt, Germany). All images were analyzed using appropriate software (Advanced Image Data Analyzer, Raytest, Straubenhardt, Germany) to calculate the protein of interest/β-actin ratio.

Statistical analysis

As indicated, data are provided as means ± SEM; *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed t-test, or ANOVA, where applicable. Only results with *(*p* < 0.05), **(*p* < 0.01) or ***(*p* < 0.001) were considered statistically significant.

Results

In order to determine the abundance of mRNA encoding *Slc26a4* in hepatic and renal tissue, semi-quantitative reverse transcription polymerase chain reaction (RT-qPCR) was employed. As illustrated in Fig. 1A, *Slc26a4* mRNA was detected in both, hepatic and renal tissue. mRNA expression was moderately lower in liver than in kidney. The *Slc26a4* transcript levels were normalized to the transcript levels of the house keeping gene *Gapdh*. Again, the *Slc26a4/Gapdh* transcript level ratio was moderately lower in liver (0.51 ± 0.04, *n* = 4) than in kidney (1.00 ± 0.04, *n* = 4). Immunoblotting with antibodies raised against pendrin detected bands in kidney and

Fig. 1. *Slc26a4* mRNA abundance in liver and kidney of water and NaHCO₃ drinking animals. A. Representative original bands of mRNA encoding SLC26A4 (upper bands) in kidney (left bands) and liver (right bands). The housekeeping gene *Gapdh* (lower bands) served as a calibrator and control. B. Detection of pendrin protein by immunoblotting of mouse kidney and liver. The signal was abolished by preincubation of the immune-serum with the immunizing peptide. The house-keeping protein β-actin served as positive control. C. Arithmetic means ± SEM of *Slc26a4* mRNA abundance in liver (left) (*n* = 6–8) and kidney (right) (*n* = 8) of animals drinking water (white bars) or drinking 150 mM NaHCO₃ (black bars) for seven days. *(*p* < 0.05) indicates statistically significant difference to water drinking animals.

liver that were not found when the antibody was preincubated with the immunizing peptide (Fig. 1B). Of note, pendrin in liver displayed a slightly higher molecular weight than in liver.

Further experiments were performed to explore the influence of alkalosis and acidosis on *Slc26a4* transcript levels in liver and kidney. To this end animals were drinking either plain tap water or either, 150 mM NaHCO₃, 280 mM NH₄Cl or 3.6 mM acetazolamide for 7 days. The daily fluid intake approached 4.3 ± 0.1 ml (*n* = 4), 4.8 ± 0.1 μl (*n* = 4), 4.1 ± 0.1 μl (*n* = 4), and 2.1 ± 0.1 μl (*n* = 4), respectively, in water, NaHCO₃, NH₄Cl and

Fig. 2. Slc26a4 protein abundance in liver and kidney from mice exposed to NaHCO_3 in drinking water. Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals prior to (control) and following (NaHCO_3) addition of NaHCO_3 in drinking water for 7 days. All blots were stripped and reprobed for β -actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β -actin on the same blot. Arithmetic means $\pm$ SEM are shown for $n = 5$ animals/group. **($p < 0.01$) and ***($p < 0.001$) indicate statistically significant differences to water drinking animals.

acetazolamide drinking animals. The body weight change during the experimental period approached 0.6 ± 0.2 g ($n = 4$), 0.6 ± 0.2 g ($n = 4$), 0.4 ± 0.1 g ($n = 4$), and 6.4 ± 0.4 g ($n = 4$), in water, NaHCO_3 , NH_4Cl and acetazolamide drinking animals. Plasma pH did not significantly change following drinking of NaHCO_3 , but significantly decreased following drinking of NH_4Cl or acetazolamide (Table 1).

The switch from water to NaHCO_3 did not significantly modify *Slc26a4* mRNA expression in liver, but significantly increased *Slc26a4* mRNA expression in kidney (Fig. 1C). NaHCO_3 significantly increased pendrin protein abundance in kidney and significantly decreased pendrin protein expression in liver (Fig. 2, $n = 5$ /group).

The switch from water to NH_4Cl was followed by a profound increase of *Slc26a4* mRNA expression in liver, but to a similarly profound decrease of *Slc26a4* mRNA expression in the kidney (Fig. 3). On the protein level, NH_4Cl treatment did not significantly modify pendrin protein expression in liver but significantly reduced pendrin expression in kidney (Fig. 4).

Treatment of the animals with acetazolamide was again followed by a profound increase of SLC26A4 mRNA expression in liver and to a similarly profound decrease of *Slc26a4* mRNA expression in the kidney (Fig. 5). Acetazolamide strongly and significantly reduced pendrin protein levels in kidney but did not significantly modify pendrin protein expression in liver (Fig. 6).

Discussion

The present study demonstrates that *Slc26a4* transcript and protein levels could be detected in hepatic tissue. The transcript level was moderately lower in liver than in kidney, but the data strongly suggest that substantial amounts of pendrin are expressed in hepatic tissue. Western blotting confirmed the expression of pendrin protein in the liver.

Fig. 3. *Slc26a4* mRNA abundance in liver and kidney of water and NH_4Cl drinking animals. Arithmetic means $\pm$ SEM of *Slc26a4* mRNA abundance in liver (left) ($n = 7$) and kidney (right) ($n = 6-7$) of animals drinking water (white bars) or drinking 280 mM NH_4Cl (black bars) for seven days, *($p < 0.01$), *** ($p = 0.001$) indicates statistically significant difference to water drinking animals.

Fig. 4. *Slc26a4* protein abundance in liver and kidney from mice receiving NH_4Cl in drinking water. Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals prior to (control) and following (NH_4Cl) addition of NH_4Cl in drinking water for 7 days. All blots were stripped and reprobed for β -actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β -actin on the same blot. Arithmetic means $\pm$ SEM are shown for $n = 5$ animals/group. ***($p < 0.001$) indicate statistically significant differences to water drinking animals.

Fig. 5. *Slc26a4* mRNA abundance in liver and kidney of animals following drinking of water without or with acetazolamide. Arithmetic means $\pm$ SEM of *Slc26a4* mRNA abundance in liver (left) ($n = 7-8$) and kidney (right) ($n = 7-8$) of animals drinking water (white bars) or drinking 3.6 mM acetazolamide (black bars) for seven days. *($p < 0.05$), ***($p = 0.001$) indicate statistically significant difference to water drinking animals.

The present observations further demonstrate sensitivity of hepatic and renal *Slc26a4* mRNA and protein expression to acidosis and alkalosis. Both, NH_4Cl [21] and acetazolamide [22] are known to cause acidosis, which in turn down-regulates renal *Slc26a4* expression [11]. Following acidosis, renal H^+ secretion is up-regulated and renal bicarbonate secretion is down-regulated [11, 14]. Since pendrin is inserted into the apical cell membrane and accomplishes HCO_3^- secretion, its operation would be counterproductive for renal acid elimination during acidosis [9-12]. Along those lines, renal *Slc26a4* transcript levels are decreased in animals lacking carbonic anhydrase II [16] and its protein abundance is reduced in rats treated with the carbonic anhydrase inhibitor acetazolamide [24].

The effect of acidosis on the *Slc26a4* transcript levels in liver were opposite to what was observed in the kidney. Both, NH_4Cl and acetazolamide significantly increased pendrin transcript levels in the liver. However, neither NH_4Cl nor acetazolamide significantly modified pendrin protein expression. Thus, it remains uncertain, whether the enhanced pendrin transcript levels indeed translate into the respective alterations of protein expression.

In theory, SLC26A4 may serve a second function in the liver, i.e. cell volume regulation. Parallel activation of Na^+/H^+ exchangers and $\text{Cl}^-/\text{HCO}_3^-$ exchangers accomplish regulatory cell volume increase [25, 26], as they mediate cellular uptake of NaCl , which in turn osmotically drives water entry. The extrusion of H^+ and HCO_3^-

Fig. 6. *Slc26a4* protein abundance in liver and kidney from mice treated with acetazolamide. Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals without (control) or with acetazolamide in drinking water for 7 days. All blots were stripped and reprobed for β -actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β -actin on the same blot. Arithmetic means $\pm$ SEM are shown for $n = 5$ animals/group. ***($p < 0.001$) indicates statistically significant differences to water drinking animals.

by the Na^+/H^+ exchanger and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger, respectively, is osmotically not relevant, as they are replenished in the cell from CO_2 via H_2CO_3 [25, 26]. Na^+ accumulated by the Na^+/H^+ -exchanger is pumped out by the Na^+/K^+ -ATPase in exchange for K^+ . Thus, the transporters eventually accomplish cellular KCl -uptake [3, 25].

Cell volume is in turn a decisive regulator of hepatic metabolism. Cell shrinkage stimulates proteolysis and inhibits protein synthesis in the liver, and, conversely, cell swelling stimulates protein synthesis and inhibits proteolysis [27-30]. Similarly, cell swelling inhibits and cell shrinkage stimulates glycogenolysis [31]. As a result, following cell swelling, the intracellular amino acids and glucose are incorporated into and upon cell shrinkage they are released from the osmotically less active macromolecules [26]. Cell volume further influences glycolysis, flux through the pentose phosphate pathway, lipogenesis from glucose, gluconeogenesis, oxidation of glycine and alanine, degradation of glutamine, degradation of amino acids to NH_4^+ and urea, ketoisocaproate oxidation, acetyl CoA carboxylase, lipogenesis, carnitine palmitoyltransferase I activity, cytosolic ATP and phosphocreatine concentrations, respiration as well as RNA and DNA synthesis [26, 29, 32]. Whether or not pendrin indeed participates in cell volume regulatory transport, remains, however, to be tested.

In conclusion, *Slc26a4* transcripts and protein were observed in liver tissue. The abundance of both was only moderately lower than in the kidney. The hepatic *Slc26a4*

transcript levels were upregulated by NH_4Cl and acetazolamide. On the protein level, hepatic *Slc26a4* decreased in response to NaHCO_3 . Thus, pendrin transcription and/or protein expression is presumably governed by acid-base balance. The present observations point to a novel role of pendrin, i.e. Cl/HCO_3^- exchange in liver cells.

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References

- Choi BY, Muskett J, King KA, Zalewski CK, Shawker T, Reynolds JC, Butman JA, Brewer CC, Stewart AK, Alper SL, Griffith AJ: Hereditary hearing loss with thyroid abnormalities. *Adv Otorhinolaryngol* 2011;70:43-49.
- Dossena S, Rodighiero S, Vezzoli V, Nofziger C, Salvioni E, Boccazzi M, Grabmayer E, Botta G, Meyer G, Fugazzola L, Beck-Peccoz P, Paulmichl M: Functional characterization of wild-type and mutated pendrin (*SLC26A4*), the anion transporter involved in Pendred syndrome. *J Mol Endocrinol* 2009;43:93-103.
- Lang F, Foller M, Lang K, Lang P, Ritter M, Vereninov A, Szabo I, Huber SM, Gulbins E: Cell volume regulatory ion channels in cell proliferation and cell death. *Methods Enzymol* 2007;428:209-225.
- Maciaszczyk K, Lewinski A: Phenotypes of *SLC26A4* gene mutations: Pendred syndrome and hypoacusis with enlarged vestibular aqueduct. *Neuro Endocrinol Lett* 2008;29:29-36.
- Bizhanova A, Kopp P: Minireview: The sodium-iodide symporter NIS and pendrin in iodide homeostasis of the thyroid. *Endocrinology* 2009;150:1084-1090.
- Calebiro D, Porazzi P, Bonomi M, Lisi S, Grindati A, De Nittis D, Fugazzola L, Marino M, Botta G, Persani L: Absence of primary hypothyroidism and goiter in *SLC26A4*^{-/-} Mice Fed on a Low Iodine Diet. *J Endocrinol Invest* 2011; in press.
- Bizhanova A, Kopp P: Genetics and phenomics of Pendred syndrome. *Mol Cell Endocrinol* 2010;322:83-90.
- Izuhara K, Ohta S, Shiraishi H, Suzuki S, Taniguchi K, Toda S, Tanabe T, Yasuo M, Kubo K, Hoshino T, Aizawa H: The mechanism of mucus production in bronchial asthma. *Curr Med Chem* 2009;16:2867-2875.
- Carraro-Lacroix LR, Malnic G: Acid-base transport by the renal distal nephron. *J Nephrol* 2010;23:S19-S27.
- Sindic A, Schlatter E: Renal electrolyte effects of guanylin and uroguanylin. *Curr Opin Nephrol Hypertens* 2007;16:10-15.
- Wagner CA, Devuyst O, Bourgeois S, Mohebbi N: Regulated acid-base transport in the collecting duct. *Pflugers Arch* 2009;458:137-156.
- Wall SM, Pech V: Pendrin and sodium channels: relevance to hypertension. *J Nephrol* 2010;23:S118-S123.
- Eladari D, Chambrey R, Frische S, Vallet M, Edwards A: Pendrin as a regulator of ECF and blood pressure. *Curr Opin Nephrol Hypertens* 2009;18:356-362.
- Hafner P, Grimaldi R, Capuano P, Capasso G, Wagner CA: Pendrin in the mouse kidney is primarily regulated by Cl -excretion but also by systemic metabolic acidosis. *Am J Physiol Cell Physiol* 2008;295:C1658-C1667.
- Wagner CA, Finberg KE, Stehberger PA, Lifton RP, Giebisch GH, Aronson PS, Geibel JP: Regulation of the expression of the Cl/anion exchanger pendrin in mouse kidney by acid-base status. *Kidney Int* 2002;62:2109-2117.
- Sun X, Soleimani M, Petrovic S: Decreased expression of *Slc26a4* (Pendrin) and *Slc26a7* in the kidneys of carbonic anhydrase II-deficient mice. *Cell Physiol Biochem* 2008;21:95-108.
- Guder WG, Haussinger D, Gerok W: Renal and hepatic nitrogen metabolism in systemic acid base regulation. *J Clin Chem Clin Biochem* 1987;25:457-466.
- Karim Z, Attmane-Elakeb A, Bichara M: Renal handling of NH_4^+ in relation to the control of acid-base balance by the kidney. *J Nephrol* 2002;15:S128-S134.
- Nissim I: Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am J Physiol* 1999;277:F493-F497.
- Huber S, Asan E, Jons T, Kersch C, Puschel B, Drenckhahn D: Expression of rat kidney anion exchanger 1 in type A intercalated cells in metabolic acidosis and alkalosis. *Am J Physiol* 1999;277:F841-F849.
- Nowik M, Kampik NB, Mihailova M, Eladari D, Wagner CA: Induction of metabolic acidosis with ammonium chloride (NH_4Cl) in mice and rats- species differences and technical considerations. *Cell Physiol Biochem* 2010;26:1059-1072.
- Lonnerholm G, Wistrand PJ, Barany E: Carbonic anhydrase isoenzymes in the rat kidney. Effects of chronic acetazolamide treatment. *Acta Physiol Scand* 1986;126:51-60.
- Harrison RA: Acid-base balance. *Respir Care Clin N Am* 1995;1:7-21.
- Welsh-Bacic D, Nowik M, Kaissling B, Wagner CA: Proliferation of acid-secretory cells in the kidney during adaptive remodelling of the collecting duct. *Plos One* 2011;in press.
- Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. *Physiol Rev* 2009;89:193-277.
- Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 1998;78:247-306.
- vom Dahl S, Haussinger D: Cell hydration and proteolysis control in liver. *Biochem J* 1995;312:988-989.
- vom Dahl S, Dombrowski F, Schmitt M, Schliess F, Pfeifer U, Haussinger D: Cell hydration controls autophagosome formation in rat liver in a microtubule-dependent way downstream from p38MAPK activation. *Biochem J* 2001;354:31-36.
- Haussinger D, Lang F: Cell volume in the regulation of hepatic function: a mechanism for metabolic control. *Biochim Biophys Acta* 1991;1071:331-350.
- Stoll B, Gerok W, Lang F, Haussinger D: Liver cell volume and protein synthesis. *Biochem J* 1992;287:217-222.
- Lang F, Stehle T, Haussinger D: Water, K^+ , H^+ , lactate and glucose fluxes during cell volume regulation in perfused rat liver. *Pflugers Arch* 1989;413:209-216.
- Haussinger D: Osmosensing and osmosignaling in the liver. *Wien Med Wochenschr* 2008;158:549-552.