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Identification of a chloride channel resembling ClC-K2 in the mouse connecting tubule

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The mechanisms of chloride transport within the connecting tubule (CNT), a segment interposed between the distal convoluted tubule and the collecting tubule, is presently indetermined. We investigated basolateral chloride channels in the mouse CNT, using the cell-attached and inside-out variants of the patch-clamp method. Microdissected CNTs were isolated from collagenase-treated kidneys (Lourdel et al. 2003) after mice were humanely killed. The pipette solution contained (mM): 145 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes at pH 7.4. The bath solution was similar except that it contained 5 mM KCl and 140 NaCl.
In cell-attached patches, we found a channel with a conductance of 10.6 ± 0.9 pS (mean ± S.E.M.; n = 8) which is chloride-selective : the reversal potential significantly changed from 1.9 ± 4.0 mV in the control (n = 8), to 25.7 ± 1.2 mV in the presence of 100 mM Na-gluconate (n = 4), whereas there was no significant change (-1.1 ± 2.0 mV, n = 8) with 140 mM NMDG-chloride. We estimated the influence of extracellular Ca^{2+} and pH on channel activity by measuring the NP_o on separate patches. The NP_o dramatically increased from 23 ± 6 (n = 11) with 1 mM Ca^{2+} in the pipette to 101 ± 12 (n = 10) with 5 mM Ca^{2+}. The NP_o also significantly increased with extracellular pH, from 26 ± 7 (n = 13) at pH 7.0 to 71 ± 12 at pH 7.8 (n = 12). The anionic selectivity was investigated quantitatively in the excised configuration and showed the relative permeability sequence $Cl^- > Br^- \approx NO_3^- > F^-$ (n = 4-6). The properties of the channel appear similar to a previously reported Cl^- channel in the DCT (Lourdel et al. 2003) and are compatible with the currently known properties of the ClC-K channels (Estevez et al. 2001).
The unpaired Student's t test was used. $P < 0.05$ was considered significant.

Lourdel S et al. (2003). J Gen Physiol 121, 287-300.

Estevez R et al. (2001). Nature 414, 558-561.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Loop of Henle sodium and chloride transport molecules increase in parallel with urine concentrating capacity in rat kidneys during postnatal development

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In rats, urine concentrating ability develops progressively from birth and reaches the adult level at weaning in the fourth postnatal week. We addressed intrarenal mechanisms for this phenomenon. We determined the abundance and cellular localization of Na and Cl-transporters in the thick ascending limb of Henle's loop (TAL) during postnatal development and tested whether their expression was stimulated by glucocorticoid, which exhibits a marked surge at weaning. Moreover, we determined whether proliferation in TAL cells during postnatal development excludes expression of NaCl transport molecules.
Rat pups were humanely killed and for immunohistochemistry they were anaesthetized with sodium pentobarbitone (0.5 mg/10 g rat) and perfused with 4% paraformaldehyde through the left cardiac ventricle. Expression level and localization of NaCl transporters NKCC-2, NHE-3, ROMK, CLCK2 and Na^+-K^+-ATPase-α1 was determined by ribonuclease protection, Western blotting and immunohistochemistry in a developmental series of kidneys. The effect of dexamethasone (DEX) at the nadir of endogenous glucocorticoid (postnatal day (P)8-P12, 100 μg/kg) on transporter proteins and urinary concentrating ability was tested (n=5-8). Cell proliferation was assessed by immunostaining and Western blot for proliferating cell nuclear antigen (PCNA).
Messenger RNAs for all tested NaCl transporters were significantly increased with postnatal development with a peak at weaning (P21). There were significant increase of Na^+-K^+-ATPase-α1, NHE-3 and ROMK between P7 and 21 (2234 ± 55 vs 2986 ± 84 cpm, 210 ± 20 vs 320 ± 10 cpm, and 88 ± 4 vs 129 ± 7 cpm, n = 4-5, $P<0.01$, respectively). For NKCC2 there was a significant increase between P0 and P21 (201 ± 12 vs 356 ± 22 cpm, n = 4-5, $P<0.001$). After P21, mRNA levels stabilized or decreased. Immunostaining for NKCC-2, ROMK and Na^+-K^+-ATPase-α1 confirmed a marked increase in labelling intensity and more wide distribution along loops of Henle with development. DEX injections increased significantly the abundance of NHE-3, NKCC2, Na^+-K^+-ATPase-α1 and ROMK (201 ± 19 vs 282 ± 33 cpm, 495 ± 31 vs 707 ± 16 cpm, 5451 ± 109 vs 6951 ± 228 cpm, and 109 ± 8 vs 175 ± 7 cpm, n = 7-8, $P<0.001$, respectively) mRNAs. Immunostaining confirmed up-regulation of NKCC2 and Na^+-K^+-ATPase-α1 by DEX. DEX treatment led to a significant increase of urinary concentrating ability (871 ± 17 vs 643 ± 77 mosm/kg, thirst 12 h) at P12. Kidney outer medulla was increasingly labelled for PCNA with development with a peak around P10-14. PCNA co-localized with the TAL marker Tamm-Horsfall glycoprotein. PCNA labelling was almost exclusively restricted to TAL cells not expressing NKCC-2. PCNA labelling was decreased in response to DEX. We conclude that development of urinary concentrating capacity is associated with increased expression and more wide distribution of crucial NaCl transporter proteins and fewer proliferating cells in TAL. As proliferating TAL cells differentiate they acquire NaCl transporters and this process is accelerated by glucocorticoid during postnatal kidney development.

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Effect of mineralocorticoid deficiency on renocortical COX-2 expression and kidney growth in rats during postnatal development

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Cyclooxygenase type 2 (COX-2) expression is enhanced in cortical loops of Henle (cTAL) during postnatal rat kidney development. The cTAL proliferates significantly in the postnatal

period and COX-2 is crucial for normal development of the kidneys. We asked whether COX-2 expression and kidney proliferation was regulated by salt balance and/or by mineralocorticoids in the postnatal period. During brief anesthesia (ketamin 0.25 mg/10 g rat and xylasin 0.05 mg/10 g rat) at postnatal day 10, rats were either adrenalectomized (ADX) or sham operated. All rats were supplied with cholesterol pellets containing only cholesterol (sham and ADX), corticosterone (ADX-C) and corticosterone and DOCA (ADX-CD). ADX rats were injected with isotonic NaCl to sustain survival. At postnatal day 20 (P20), the rats were humanely killed. For immunohistochemistry rats were anaesthetized with mebumal (0.5 mg/10 g rat), then perfused with 4% paraformaldehyde through the left ventricle for 5 min. ADX prevented the peak in postnatal corticosterone seen between P13-P21 (18.5 ± 1.9 vs 137.3 ± 30 ng/ml plasma, n = 7-11, P<0.05), reduced body growth rate and led to a 5-fold increase in urinary Na/K ratio (0.18 ± 0.05 vs 0.99 ± 0.16, n = 6-7, P < 0.002). The kidney/body weight ratio was slightly elevated in the ADX-CD group compared to the other groups (10.46 ± 0.18 vs 9.83 ± 0.10 mg/g body weight, n = 8-11, P = 0.005). Growth rate was reduced in ADX and ADX-C compared to sham and ADX-CD (body weight increase P10-P20; 13.5 ± 0.9 g; 13.4 ± 1.2 g; 20.9 ± 0.7 g, respectively, n = 7-10, P<0.001). COX-2 expression was significantly elevated in ADX and ADX-C rats despite NaCl supplementation (mRNA 4-fold ($1.99\times10^{-4} \pm 2.27\times10^{-5}$ and $6.23\times10^{-4} \pm 1.51\times10^{-4}$ vs $8.71\times10^{-4} \pm 2.51\times10^{-4}$ cpm, n = 7-8, P<0.02, respectively); protein 2-fold (2.7 ± 0.7 and 6.6 ± 1.9 vs 6.1 ± 1.2 OD u × mm2, n = 4, P<0.05, respectively), whereas COX-2 expression was not changed in ADX-CD rats ($1.65\times10^{-4} \pm 3.99\times10^{-5}$ cpm and 1.2 ± 0.6 OD u × mm2). Immunohistochemical labelling for COX-2 revealed a wide distribution along the full length of cTAL in ADX and ADX-C while in sham and ADX-CD rats COX-2 was hardly detectable. Urine concentrating ability was decreased in ADX and ADX-C animals, measured as lower papillary osmolality (311.1 ± 21 and 392.4 ± 29 vs 552.5 ± 62 mosm/kg, n = 7-9, P<0.002, respectively) and Na concentration (44.7 ± 4 and 48.0 ± 4 vs 85.5 ± 10 mmol/l, n = 7-9, P<0.002, respectively) after 22 h dehydration. We did not detect any change in TAL transporter mRNAs; NKCC-2, ROMK and Na-K-ATPase-α1.

In control kidneys at various stages, COX-2 and the proliferation marker PCNA were always mutually exclusive along TAL and there were no obvious differences in PCNA labelling distribution at postnatal day 20 between sham and ADX animals. Thus we conclude that NaCl substitution in the absence of adrenal steroids does not normalize cortical COX-2 expression; that mineralocorticoid exerts a stronger negative influence on COX-2 compared to corticosterone; that COX-2 is not localized to proliferating TAL cells; and that mineralocorticoids and NaCl supply are both required to sustain normal growth and postnatal rat kidney development.

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