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Ontogeny of the Regulation of Na^+,K^+-ATPase Activity in the Renal Proximal Tubule Cell[1]

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ABSTRACT. This study examines the ontogeny of the regulation of Na^+,K^+-ATPase activity in the proximal tubule (PT) by a first messenger, dopamine (DA), and by direct stimulation of a third messenger, protein kinase C (PKC). PT segments dissected from 10- (PT10), 15- (PT15), 20- (PT20), and 40- (PT40) d-old rats were preincubated with DA 10^{-5} M, diacylglycerol (DAG) 10^{-5} M (an endogenous activator of PKC), or phorbol 12,13-dibutyrate (PDBu) 10^{-6} M (an exogenous activator of PKC). DA inhibited Na^+,K^+-ATPase activity in PT40. In PT20, DA also inhibited Na^+,K^+-ATPase activity, but the inhibitory effect in PT20 was less pronounced than in PT40. In PT15, DA had no effect on Na^+,K^+-ATPase activity. DAG significantly inhibited Na^+,K^+-ATPase activity in PT40. DAG also inhibited Na^+,K^+-ATPase activity in PT20, but the inhibition was slightly less pronounced than in PT40. DAG had no effect on Na^+,K^+-ATPase activity in PT15. Na^+,K^+-ATPase activity in PT40 and PT20 preincubated with PDBu was significantly lower than with vehicle. The inhibitory effect in PT20 was less pronounced than in PT40. When PT40 and PT20 were preincubated with both PDBu and 5×10^{-5} M sphingosine, an inhibitor of PKC activation, the inhibitory effect of PDBu was abolished. In both PT40 and PT20 incubated with 4-α-12,13 phorbol didecanoate 10^{-7} M, a phorbol ester that will not activate PKC, Na^+,K^+-ATPase activity was not different from the control. In PT10, Na^+,K^+-ATPase activity was the same after PDBu incubation and after vehicle incubation. Na^+,K^+-ATPase activity in PT10 incubated with both PDBu and sphingosine was not different from the control. PDBu inhibited Na^+,K^+-ATPase activity in a dose-dependent way in PT40 and PT20 but not in PT10. We conclude that the short-term regulation of renal tubular Na^+,K^+-ATPase activity undergoes postnatal changes. (*Pediatr Res* 30: 131–134, 1991)

Abbreviations

DA, dopamine
DAG, diacylglycerol analogue
Na^+,K^+-ATPase, sodium potassium adenosine triphosphatase
PDBu, phorbol 12,13-dibutyrate
PKC, protein kinase C
PT, proximal tubule
MHM, modified Hanks' medium

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Infants have a lower capacity than adults to adapt sodium excretion to sodium intake (1–4). Several factors may contribute to the poor control of sodium balance in infancy. There is a postnatal increase of the V_{max} of Na^+,K^+-ATPase, the enzyme that yields energy to active sodium transport out of the cell (5). The developmental increase in the V_{max} of Na^+,K^+-ATPase activity is associated with increases in the Na reabsorptive and the renal concentrating capacities (6–10).

Under physiologic conditions, Na^+,K^+-ATPase operates at a fraction of its V_{max}. Because the intracellular concentration of sodium is lower (<20 mM) than that required to reach the V_{max} for Na^+,K^+-ATPase (>50 mM) (11), it may be questioned whether the low V_{max} of Na^+,K^+-ATPase in immature kidney tubule cells is sufficient to explain the poor renal tolerance to sodium that we find in the infant.

The activity of Na^+,K^+-ATPase in the renal tubule cell is controlled by a number of hormones. Dopamine, which has a natriuretic effect, inhibits Na^+,K^+-ATPase activity (12–14). Norepinephrine, which is antinatriuretic, stimulates Na^+,K^+-ATPase activity (15). Other hormones, such as prostaglandin (16), AVP (17), and endothelin (18), have also been shown to modulate renal Na^+,K^+-ATPase activity. Direct activation of second and third messengers, such as cAMP (19) and PKC (20, 21), can also cause a change of Na^+,K^+-ATPase activity. Both phorbol esters and diacylglycerol inhibit proximal tubular Na^+,K^+-ATPase activity in the adult kidney (22). There is also evidence that dopamine inhibition of proximal tubular Na^+,K^+-ATPase activity involves activation of PKC (22).

This study addresses the question of whether the short-term regulation of renal Na^+,K^+-ATPase activity is blunted in the infant kidney and whether this may be due to immaturity of the systems that transduce the signal from an activated receptor to the effector. We have used single permeabilized tubules from 10- to 40-d-old Sprague-Dawley rats and examined the effects of both dopamine, phorbol esters, and diacylglycerol, an endogenous activator of PKC on Na^+,K^+-ATPase.

MATERIALS AND METHODS

The experiments were performed on male Sprague-Dawley rats aged 10, 15, 20, and 40 d. The rats were kept with their dams until the age of 20 d. Rat food containing 21% protein (Ewos, Södertälje, Sweden) and tap water were given (8) *ad libitum*.

Preparation of tubules. Kidney perfusion and tubule microdissection were performed as described (23). The rats were anesthetized with an intraperitoneal injection of Inactin, (Byk-Gulden, Konstanz, Germany) 80 mg/kg. After a midline incision, the left kidney was exposed and perfused with a cold solution containing (in mM): 137 NaCl; 5 KCl; 0.8 $MgSO_4$; 0.33 Na_2HPO_4; 0.44 KH_2PO_4; 1 $CaCl_2$; 1 $MgCl_2$; 10 Tris-HCl, to which collagenase 0.05% (Sigma Chemical Co., St. Louis, MO) and BSA 0.1% (Behringwerke, Marburg, Germany) had been added. The pH was adjusted to 7.4. The kidney was removed and thin pyramids (5–10 pieces) were cut along the cortical papillary axis. The

pyramids were incubated at 35°C for 20 min in 10 mL of a modified Hanks' solution containing collagenase. The solution was continuously bubbled with air. The tissue was then transferred to the same solution at 4°C, except that collagenase and BSA were omitted and the $CaCl_2$ concentration was reduced to 0.25 mM (MHM). The PT segments were dissected from the superficial cortex under a stereomicroscope at 4°C. The tubule segments were individually transferred to the concavity of the bacteriologic slide and were inspected and photographed for length determinations by means of an inverted microscope at ×100 magnification. The tubules were then stored on ice for 30 to 60 min.

Determination of Na^+,K^+-ATPase activity. The tubules that were stored on ice were transferred to room temperature. When the tubules had reached this temperature, the MHM was removed by mouth pipette and replaced by 1 μL MHM containing the drugs or vehicle [DA (incubation time 30 min); L-α-1-oleoyl-2-acetoyl-sn-3-glycerol (DAG), DMSO < 0.1%; PDBu, DMSO < 0.01%; 4-α-12-13-phorbol didecanoate, DMSO < 0.01%; and sphingosine, ethanol < 0.1% (incubation time, 20 min)]. The preincubation period was terminated by rapid cooling of the tubule segments to 4°C. After preincubation, the medium was removed by mouth pipette and each segment was permeabilized with a hypotonic shock to allow ATP and sodium free access to the interior of the cell, then incubated (in 1 μL final volume) for 15 min at 37°C in a medium containing the following (in mM): 50 NaCl, 5 KCl, 10 $MgCl_2$, 1 EGTA, 100 Tris-HCl, 10 Na_2ATP (grade II, Sigma Chemical Co.), and 5 μCi/mL [^{32}P]ATP (New England Nuclear, Boston, MA; 2–10 Ci/mmol). For the determination of ouabain-insensitive (Mg-dependent) ATPase activity, NaCl and KCl were omitted, Tris-HCl was 150 mM, and 1 mM ouabain (Merck, Darmstadt, Germany) was added. The pH of both media was 7.4. The media containing the tubule segments and the media alone (blanks) were incubated at 37°C for 15 min. After absorption of the unhydrolyzed nucleotide on activated charcoal, the ^{32}P liberated by the hydrolysis of [^{32}P]ATP was separated by filtration through a Millipore filter (France). The radioactivity was counted in a liquid scintillation spectrophotometer. In each study, we used six to 10 segments individually to determine both total ATPase and ouabain-insensitive ATPase. In each assay, the nonspecific release of ^{32}P was determined in five samples without tubule segments (blanks). Na^+,K^+-ATPase activity was calculated as the difference between the mean value for total ATPase and the mean value for ouabain-insensitive ATPase and expressed per unit of tubule length in single tubules.

Statistical analysis. Values are given as mean ± SEM. When appropriate, the data were examined by means of the *t* test or analysis of variance. A *p* value less than 0.05 was considered significant.

RESULTS

Effect of DA on PT Na^+,K^+-ATPase activity. PT segments from 15- (PT15), 20- (PT20), and 40- (PT40) d-old rats were preincubated with DA 10^{-5} M or vehicle for 30 min at room temperature. This dose was chosen because it causes maximal inhibition of Na^+,K^+-ATPase in adult rats (12). The effect of DA 10^{-5} M on Na^+,K^+-ATPase activity (pmol Pi/mm tubule/h) in PT15, PT20, and PT40 segments is illustrated in Figure 1. DA 10^{-5} M inhibited Na^+,K^+-ATPase activity in PT40, but the inhibitory effect in PT20 was less pronounced than in PT40. In PT15, dopamine had no effect on proximal tubular Na^+,K^+-ATPase activity.

DA did not influence ouabain-insensitive ATPase activity (data not shown).

Effect of PKC activation on proximal tubule Na^+,K^+-ATPase activity. In one protocol, PT segments from 15-, 20-, and 40-d-old rats were incubated with the diacylglycerol analogue, DAG. Diacylglycerol is an endogenous activator of PKC. PT segments were incubated with DAG 10^{-5} M or vehicle for 20 min at room

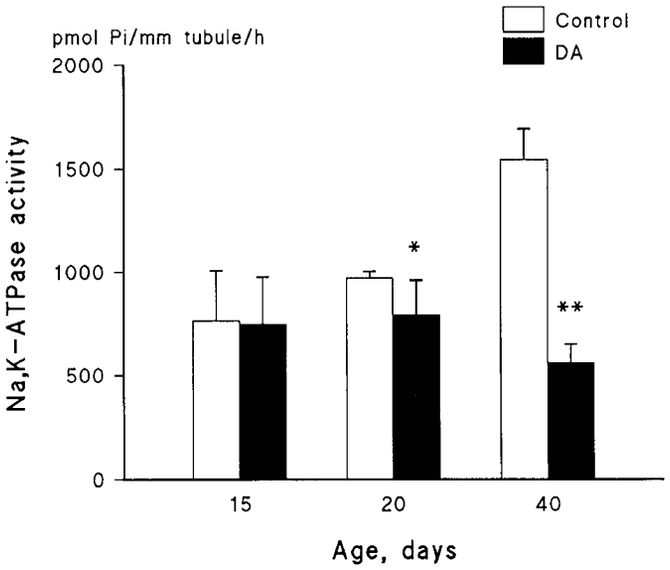

Fig. 1. Effect of 10^{-5} M DA on Na^+,K^+-ATPase activity in 15-, 20-, and 40-d-old rats. Single proximal convoluted tubule segments were incubated with DA or vehicle for 30 min at room temperature. Each *bar* represents the mean value of three to six experiments. The *line* indicates SEM. *, $p < 0.05$; **, $p < 0.01$.

temperature. DAG 10^{-5} M inhibited Na^+,K^+-ATPase activity in PT40 significantly (1403 ± 128 *versus* 673 ± 51, $n = 6$, $p < 0.01$), but in PT20 the inhibition was slightly less pronounced (1119 ± 101 *versus* 573 ± 104, $n = 5$, $p < 0.05$).

DAG 10^{-5} M had no effect on Na^+,K^+-ATPase activity in PT15 (673 ± 71 *versus* 786 ± 48; $n = 5$).

Phorbol esters with a diacylglycerol-like structure are specific activators of PKC. We investigated the effect of the phorbol ester PDBu on PT Na^+,K^+-ATPase activity. These studies were performed on PT from 10- (PT10), 20-, and 40-d-old rats. PT segments were preincubated with 10^{-6} M PDBu or vehicle for 20 min at room temperature.

Na^+,K^+-ATPase activity in PT40 incubated with PDBu 10^{-6} M was significantly lower than in vehicle-incubated PT40. When PT segments were preincubated with both 10^{-6} PDBu and 5×10^{-5} M sphingosine, an inhibitor of PKC activation, the inhibitory effect of PDBu was abolished (Fig. 2*A*). In PT40 segments incubated with 4-α-12,13 phorbol didecanoate 10^{-7} M, a phorbol ester that does not activate PKC, Na^+,K^+-ATPase activity was 1450 ± 164 pmol Pi/mm tubule/h, which is not different from the control.

Na^+,K^+-ATPase activity in PT20 incubated with PDBu 10^{-6} M was significantly lower than in vehicle-incubated PT20. The inhibitory effect was less pronounced than in PT40. When PT20 segments were incubated with both PDBu and sphingosine, the inhibitory effect of PDBu was abolished (Fig. 2*B*). Na^+,K^+-ATPase activity in PT20 segments incubated with 4-α-12,13 phorbol didecanoate was 988 ± 101 pmol Pi/mm tubule/h, which is not different from the control.

In PT dissected from 10-d-old rats, Na^+,K^+-ATPase activity was the same after PDBu incubation and after vehicle incubation. Na^+,K^+-ATPase activity in PT10 incubated with both PDBu and sphingosine was 534 pmol Pi/mm tubule/h, which was not different from the control (Fig. 2*C*).

The response to PDBu was also examined as shown in Figure 3. PDBu inhibited Na^+,K^+-ATPase activity in a dose-dependent way in PT40 and PT20 but not in PT10. It is apparent that PT40 segments were more sensitive to the inhibitory effect than PT20 segments. Inhibition by PDBu was maximal from 10^{-8} M in both PT40 and PT20.

Neither DAG nor PDBu influenced ouabain-insensitive ATPase activity (data not shown).

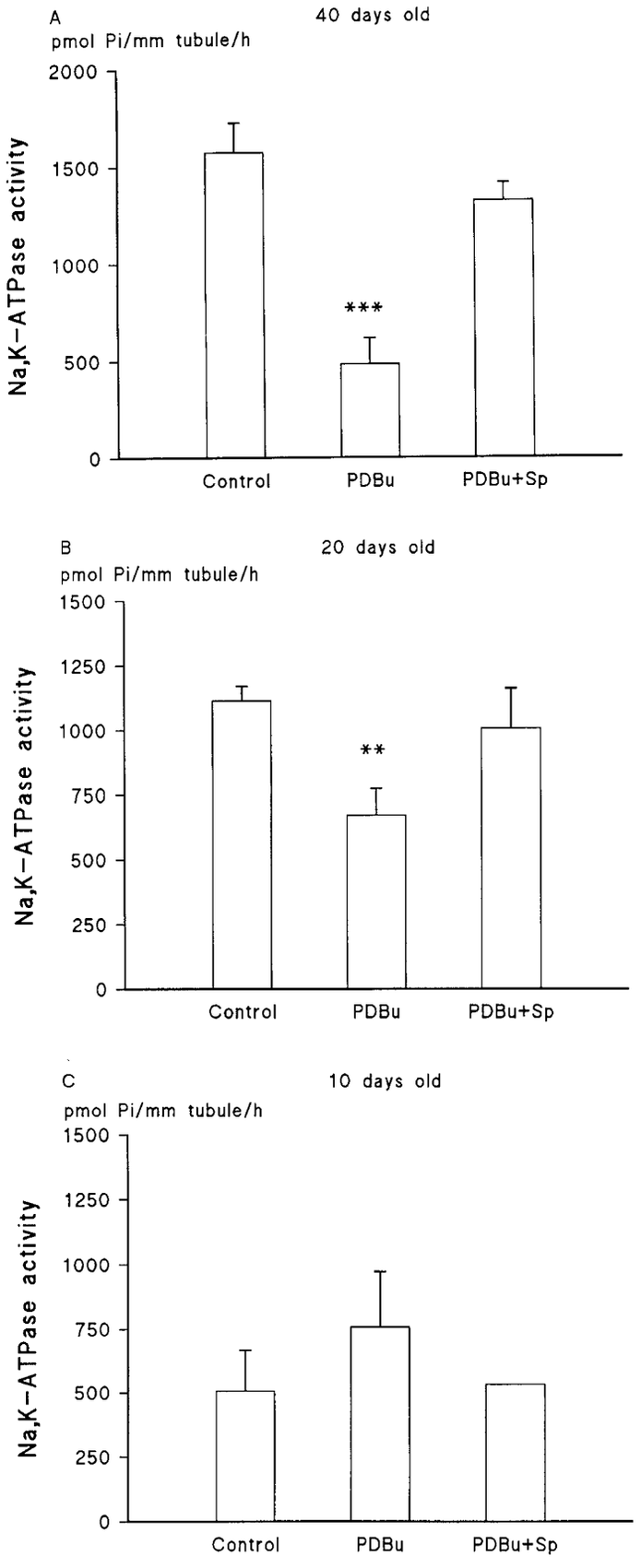

Fig. 2. Effect of 10^{-6} M PDBu on Na^+,K^+-ATPase activity in 40- (*A*), 20- (*B*), and 10- (*C*) d-old rats. Single proximal convoluted tubule segments were incubated with vehicle (*Control*), PDBu alone (*PDBu*), and PDBu together with sphingosine 5×10^{-5} M (*PDBu+Sp*) for 20 min at room temperature. PDBu was diluted in DMSO (final concentration <0.01%) and sphingosine was diluted in ethanol solution (final concentration <0.1%); an equal amount was therefore present in control studies. *Bars* represent the mean value of three to six determinations. At the age of 10 d (*C*), the bar of PDBu together with sphingosine represents one experiment. *Lines* indicate SEM. **, $p < 0.01$; ***, $p < 0.001$.

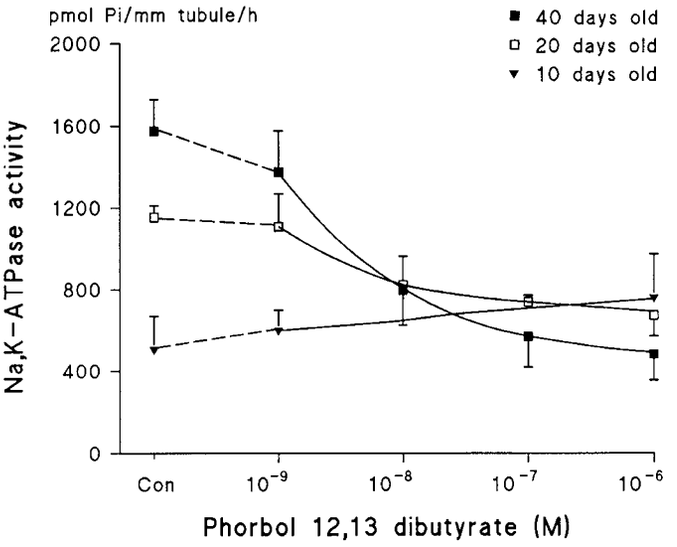

Fig. 3. Na^+,K^+-ATPase activity in proximal convoluted tubules incubated with different concentrations of PDBu and control (*Con*) in 40-, 20-, and 10-d-old rats. Each *point* represents the mean values of three to six experiments. The line indicates SEM. In 40-d-old rats, at 10^{-8} $p < 0.01$, at 10^{-7} $p < 0.01$, and at 10^{-6} $p < 0.01$ compared to control. In 20-d-old rats, at 10^{-8} $p < 0.05$, at 10^{-7} $p < 0.01$, and at 10^{-6} $p < 0.01$ compared to control.

DISCUSSION

Our study confirms the finding that DA and PKC activations lead to an inhibition of Na^+,K^+-ATPase activity in adult PT cells and demonstrates that this short-term regulation of active Na transport develops gradually during postnatal life.

DA had a well-documented natriuretic effect (13, 14, 24, 25). The PT cells are the source of renal dopamine production (26–28). DA produced in PT cells acts as a paracrine factor and inhibits Na reabsorption in both proximal and distal tubular segments (29, 30). In adult rats fed a high salt diet, urinary Na excretion is increased and PT Na^+,K^+-ATPase is decreased. If the rats are fed benzerazide, an inhibitor of L-amino acid decarboxylase, the enzyme that converts L-DOPA to DA, this natriuretic response is attenuated and Na^+,K^+-ATPase activity increases to normal values (13). These observations imply that locally produced DA acts as a natriuretic hormone during a high salt diet and that the inhibition of renal tubule Na^+,K^+-ATPase activity contributes to this natriuretic effect.

Several observations suggest that the renal effects of DA are age-dependent (24, 25, 31). Pelayo *et al.* (31) reported that the infusion of DA increases sodium excretion more in older puppies than in young ones. The results of our study are well in line with the observation by Pelayo *et al.* DA inhibition of Na^+,K^+-ATPase, the enzyme that yields energy to transcellular Na transport in the tubule cell, is blunted in 20-d-old rats and absent in 10-d-old rats.

The blunted response to DA in infant rats may thoretically be due to a number of factors such as *1*) low DA receptor density, *2*) immaturity of the systems that transduce a signal from an activated receptor to the effector, and *3*) the expression of different isoforms of the catalytic and/or the regulatory unit of Na^+,K^+-ATPase that would be less sensitive to the inhibitory effect of DA. There is no evidence to support the first or the third explanation. The DA receptor density decreases with age (32). Analysis of Na^+,K^+-ATPase α- and β-subunit mRNA

expressions in rat kidney tissue has consistently shown that α1-mRNA is the predominant isoform transcript in both the infant and the adult kidneys (33–35). Studies using specific antisera have also shown that the α1-subunit and β-subunit polypeptides, but not the α2- and α3-subunits, are present in kidneys from 2-wk-old rats as well as from adult rats (36). The second possibility—that the intracellular signal transduction systems are different in immature and mature cells—is a strong one. DA inhibition of Na^+,K^+-ATPase involves activation of both the DA1 and the DA2 receptors (37, 38). cAMP generated by DA1 receptor activation appears to play a permissive role in the signal transduction from the DA2 receptor. DA1 receptor stimulation of adenylate cyclase activity is age-dependent (32). The signal systems coupled to the DA2 receptor have not been clarified, but they may involve PKC activation because sphingosine, an inhibitor of PKC, abolishes DA inhibition of Na^+,K^+-ATPase.

Direct activation of PKC inhibits Na^+,K^+-ATPase in mature PT cells (22) (Figs. 2 and 3). PKC also reduces water and electrolyte reabsorption in PT segments (39, 40). PKC-mediated inhibition of Na^+,K^+-ATPase was found to be age-dependent (Figs. 2 and 3). The specificity of the PKC activators that we used was confirmed by the following observations. The active phorbol PDBu inhibition of Na^+,K^+-ATPase activity was dose-dependent (Fig. 3). The inhibition by PDBu was abolished in the presence of sphingosine, one specific and characterized inhibitor of PKC activation (41). The 4α-12-13 phorbol didecanoate that does not activate PKC similarly did not inhibit Na^+,K^+-ATPase activity.

The final step involved in the short-term regulation of Na^+,K^+-ATPase probably involves protein kinase-mediated phosphorylation either of an Na^+,K^+-ATPase subunit or of an interacting protein. The lack of effect of phorbols and DAG on Na^+, K^+-ATPase activity in immature cells may be due to several factors, such as the expression of functionally different isoforms of PKC in immature and mature cells, developmental differences in the regulation of dephosphorylation of the proteins phosphorylated by PKC, and the absence of a protein interacting with the Na^+,K^+-ATPase molecule in immature cells. Studies on nerve cells show that the relative RNA expression of at least three different PKC isoforms (α, β, and τ) changes postnatally (42). These isoforms are known to have different substrate affinities, different sensitivities to calcium and lipids, and a different rate of degradation. The expression of PKC genes has not yet been studied in the developing kidney.

In conclusion, inhibition of proximal tubule Na^+, K^+-ATPase by dopamine receptor activation and PKC activation is blunted or absent during early postnatal life. These observations imply that the infant renal tubule cells have a low capacity to rapidly alter sodium transport and may explain the low capacity of the infant to regulate sodium balance. They also imply that the intracellular systems that transduce a signal from an activated membrane receptor undergo important functional changes during postnatal life.

REFERENCES

1. Aperia A, Broberger O, Herin P, Zetterström R 1979 Sodium excretion in relation to sodium intake and aldosterone excretion in newborn preterm and full-term infants. Acta Paediatr Scand 68:813–817
2. Ekblad H, Aperia A 1991 Renal function in the neonate. In: Grünfeld J-P (ed) Oxford Textbook of Clinical Nephrology. Oxford University Press (in press)
3. Al-Dahhan J, Haycock GB, Chantler C, Stimmler L 1983 Sodium homeostasis in term and preterm neonates. I. Renal aspects. Arch Dis Child 58:335–342
4. Sulyok E, Varga F, Györy E, Jobst K, Csaba IF 1979 Postnatal development of renal sodium handling in premature infants. J Pediatr 95:787–792
5. Schmidt U, Horster M 1977 Na-K-activated ATPase: activity maturation in rabbit nephron segments dissected *in vitro*. Am J Physiol 233:F55–F60
6. Schwartz GJ, Evan AP 1983 Development of solute transport in rabbit proximal tubule. I. HCO_3 and glucose absorption. Am J Physiol 245:F382–F390
7. Schwartz GJ, Evan AP 1984 Development of solute transport in rabbit proximal tubule. III. Na-K-ATPase activity. Am J Physiol 246:F845–F852
8. Aperia A, Larsson L 1979 Correlation between fluid reabsorption and proximal tubule ultrastructure during development of the rat kidney. Acta Physiol Scand 105:11–22
9. Rane S, Aperia A 1985 Ontogeny of Na^+-K^+-ATPase activity in thick ascending limb and of concentrating capacity. Am J Physiol 249:F723–F728
10. Larsson SH, Rane S, Fukuda Y, Aperia A, Lechene C 1990 Changes in Na influx precede post-natal increase in Na,K-ATPase activity in rat renal proximal tubular cells. Acta Physiol Scand 138:99–100
11. Katz AI 1982 Renal Na^+-K^+-ATPase: its role in tubular sodium and potassium transport. Am J Physiol 242:F207–F219
12. Aperia A, Bertorello A, Seri I 1987 Dopamine causes inhibition of Na^+-K^+-ATPase activity in rat proximal convoluted tubule segments. Am J Physiol 252:F39–F45
13. Bertorello A, Hökfelt T, Goldstein M, Aperia A 1988 Proximal tubule Na^+,K^+-ATPase activity is inhibited during high salt diet: evidence for DA-mediated effect. Am J Physiol 254:F795–F801
14. Seri I, Kone BC, Gullans SR, Aperia A, Brenner BM, Ballermann BJ 1988 Locally produced dopamine inhibits Na^+-K^+-ATPase activity in rat renal cortical tubule cells. Am J Physiol 255:F666–F673
15. Beach RE, Schwab SJ, Brazy PC, Dennis VW 1987 Norepinephrine increases Na^+-K^+-ATPase and solute transport in rabbit proximal tubules. Am J Physiol 252:F215–F220
16. Jabs K, Zeidel ML, Silva P 1989 Prostaglandin E2 inhibits Na^+-K^+-ATPase activity in the inner medullary collecting duct. Am J Physiol 257:F424–F430
17. Reznik VM, Shapiro RJ, Mendoza SA 1985 Vasopressin stimulates DNA synthesis and ion transport in quiescent epithelial cells. Am J Physiol 249:C267–C270
18. Zeidel ML, Rrany HR, Kone BC, Gullans SR, Brenner BM 1989 Endothelin, a peptide inhibitor of Na^+,K^+-ATPase in intact renal tubular epithelial cells. Am J Physiol 257:C1101–C1107
19. Marver D, Lear S, Marver LT, Silva P, Epstein FH 1986 Cyclic AMP-dependent stimulation of Na,K-ATPase in shark recta gland. J Membr Biol 94:205–215
20. Lynch CJ, Wilson PB, Blackmore PF, Exton JH 1986 The hormone-sensitive hepatic Na^+-pump. Evidence for regulation by diacylglycerol and tumor promoters. J Biol Chem 261:14551–14556
21. Smart JL, Deth RC 1988 Influence of α1-adrenergic receptor stimulation and phorbol esters on hepatic Na^+/K^+-ATPase activity. Pharmacology 37:94–104
22. Bertorello A, Aperia A 1989 Na^+,K^+-ATPase is an effector protein or protein kinase C in renal proximal tubule cells. Am J Physiol 256:F370–F373
23. Doucet A, Katz I, Morel F 1979 Determination of Na^+-K^+-ATPase activity in single segments of the mammalian nephron. Am J Physiol 237:F105–F113
24. Jose PA, Felder RA, Holloway RR, Eisner GM 1986 Dopamine receptors modulate sodium excretion in denervated kidney. Am J Physiol 250:F1033–F1038
25. Pelayo JC, Fildes RD, Eisner GM, Jose PA 1983 Effect of dopamine blockade on renal sodium excretion. Am J Physiol 245:F245–F253
26. Adam WR, Culvenor AJ, Hall J, Jarrott B, Wellard RM 1986 Aromatic L-amino acid decarboxylase: histochemical localization in rat kidney and lack of effect of dietary potassium or sodium loading on enzyme distribution. Clin Exp Pharmacol Physiol 13:47–53
27. Richet G, Wahbe F, Hagge J, Weimeyer W 1987 Extraneuronal production of dopamine by kidney slices in normo- and hypertensive rats. Clin Exp Hypertens [A] 9(suppl 1):127–134
28. Hayashi M, Yamaji Y, Kitajima W, Saruta T 1990 Aromatic L-amino acid decarboxylase activity along the rat nephron. Am J Physiol 258:F28–F33
29. Siragy HM, Felder RA, Howell NL, Chevalier RL, Peach MJ, Carey RM 1988 Intrarenal dopamine acts as a paracrine substance at the renal tubule. Clin Res 36:617A(abstr)
30. Hughes JM, Ragsdale NV, Felder RA, Chevalier RL, King B, Carey RM 1988 Diuresis and natriuresis during continuous dopamine-1 stimulation. Hypertension 2(suppl 1):I-60–I-74
31. Pelayo JC, Fildes RD, Jose A 1984 Age-dependent renal effects of intrarenal dopamine infusion. Am J Physiol 247:R212–R216
32. Kinoshita S, Jose PA, Felder RA 1989 Ontogeny of the dopamine 1 (DA1) receptor in rat renal proximal convoluted tubule (PCT). Pediatr Res 25:68A(abstr)
33. Emanuel JR, Garete S, Stone L, Levenson R 1987 Differential expression of Na^+,K^+-ATPase α- and β-subunit mRNAs in rat tissues and cell line. Proc Natl Acad Sci USA 84:9030–9034
34. Schneider JW, Mercer RW, Gilmore-Hebert M, Utset MF, Lai C, Greene A, Benz Jr EJ 1988 Tissue specificity localization in brain and cell-free translation of mRNA encoding the α3 isoform of Na^+,K^+-ATPase. Proc Natl Acad Sci USA 85:284–288
35. Orlowski J, Lingrel JB 1988 Tissue-specific and developmental regulation of rat Na^+,K^+-ATPase catalytic isoform and β subunit mRNAs. J Biol Chem 263:10436–10442
36. Shyjan AW, Levenson R 1989 Antisera specific for the α1, α2, α3, and β subunits of the Na^+,K^+-ATPase: differential expression of α and β subunits in rat tissue membranes. Biochemistry 28:4531–4535
37. Bertorello A, Aperia A 1990 Inhibition of proximal tubule Na^+,K^+-ATPase activity requires the simultaneous activation of DA1 and DA2 receptors. Am J Physiol 259:924–928
38. Bertorello AM, Hopfield JF, Aperia A, Greengard P 1990 Inhibition by dopamine (Na^+K^+)ATPase activity in neostriatal neurons through D1 and D2 dopamine receptor synergism. Nature 347:386–388
39. Baum M, Hays SR 1988 Phorbol myristate acetate and dioctanoylglycerol inhibit transport in rabbit proximal convoluted tubule. Am J Physiol 254:F9–F14
40. Yanase M, Handler JS 1986 Activators of protein kinase inhibit sodium transport in A6 epithelia. AM J Physiol 250:C517–C522
41. Hannum YA, Loomis CR, Merrill Jr AH, Bell RM 1986 Sphingosine inhibition of protein kinase C activity and phorbol dibutyrate binding *in vitro* and in human platelets. J Biol Chem 261:12604–12609
42. Sposi NM, Bottero L, Cossu G, Russo G, Testa U, Peschle C 1989 Expression of protein kinase C genes during ontogenic development of the central nervous system. Mol Cell Biol 9:2284–2288