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The Effect of Dopamine on Adenylate Cyclase and Na^+,K^+-ATPase Activity in the Developing Rat Renal Cortical and Medullary Tubule Cells

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ABSTRACT. Dopamine has an age-dependent natriuretic and diuretic effect. We have investigated the ontogeny of the dopamine response on adenylate cyclase activity and Na^+,K^+-ATPase activity in two different cell populations in the infant (10-d-old) and the adult (40-d-old) rat kidney. Basal- and forskolin-stimulated adenylate cyclase activity in tubular suspensions of renal cortex was 5.4-fold ($p < 0.05$) higher in the 10-d-old rats than in the 40-d-old rats but unchanged between the ages in a suspension of medullary tubules. The dopamine-1-specific agonist fenoldopam did not stimulate adenylate cyclase activity in the cortical cells from 10-d-old rats but did stimulate activity 51 ± 16% ($p < 0.05$) in the 40-d-old rats. In the medullary suspension, fenoldopam stimulated adenylate cyclase activity by 43.5 ± 5% ($p < 0.001$) in the 10-d-old rats and by 32.0 ± 7% ($p < 0.01$) in the 40-d-old rats. In the isolated proximal convoluted tubule, dopamine inhibited Na^+,K^+-ATPase activity in both the 10-d-old (34 ± 3%, $p < 0.001$) and 40-d-old rats (44 ± 7%, $p < 0.001$). In contrast, in the medullary thick ascending limb of Henle, inhibition of Na^+,K^+-ATPase activity by fenoldopam was more pronounced in the 10-d-old (56 ± 6%, $p < 0.001$) than in the 40-d-old rat (33 ± 6%, $p < 0.001$). In summary, the renal tubular effects of dopamine on adenylate cyclase and Na^+,K^+-ATPase activity change during postnatal development in a cell-specific manner. (*Pediatr Res* 34: 308–311, 1993)

Abbreviations

DA_1, dopamine-1
PCT, proximal convoluted tubule
mTAL, medullary thick ascending limb of Henle
Na^+,K^+-ATPase, sodium, potassium adenosine triphosphatase
DMEM, Dulbecco's modified Eagle's medium

Intrarenally formed dopamine acts on tubular receptors to reduce sodium reabsorption (1–4). In the mTAL, dopamine inhibits Na^+,K^+-ATPase activity via activation of the DA_1 receptor, increase in adenylate cyclase activity, activation of cAMP-dependent protein kinase, and activation of the dopamine- and cAMP-regulated protein phosphatase inhibitor DARPP-32 (5–7). In the PCT, Na^+,K^+-ATPase activity is inhibited by a synergistic effect of DA_1 and DA_2 receptor agonists (8).

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The natriuretic effect of dopamine has been demonstrated in the neonate (9), but the sensitivity to the hormone may be different in the adult and infant kidneys. In the experimental situation, several studies have reported an absent or weak renal response to dopamine in young animals (10–13). In this study on infant and adult rats, we show that the renal effects of dopamine on adenylate cyclase and Na^+,K^+-ATPase activity change during postnatal development in a cell-specific manner.

MATERIALS AND METHODS

Male Sprague-Dawley rats aged 10–11 and 39–45 d were used. Body weights were 22–28 and 160–200 g, respectively. The adult rats were fed *ad libitum* with synthetic rat food and had free access to tap water. The pups were kept with their dams.

Preparation of tubular suspensions. For each experiment, material from two kidneys from 40-d-old and 12 kidneys from 10-d-old rats was used. Rats were anesthetized with Inactin (Byk-Gulden, Konstanz, Germany). Kidneys were rapidly removed and placed on ice. Medulla and outer cortex were dissected and minced separately. The tissue was incubated at 37°C for 1 h in DMEM (GIBCO Ltd., Paisley, Scotland) with collagenase 0.5%, butyrate 1 mM, and bicarbonate 24 mM during bubbling with O_2/CO_2 95%/5%. After cooling on ice, the cortical suspension was filtrated over nylon nets with mesh openings of 38, 53, 75, and 180 μm. The outer cortex contains approximately 90% PCT (14), and these are readily prepared into a tubular suspension as has been shown by previous investigators (15). By microscopic examination, we verified that this procedure yielded a suspension consisting mainly of single proximal tubules without any glomeruli. The medullary suspension was filtrated over the 180-μm nylon net to remove undigested tissue pieces. In the microscope, we estimated the suspension to contain approximately 70–80% mTAL tubules in accordance with previous studies (16, 17). Trypan blue coloring was used to evaluate the viability of the preparations, and only single cells (less than 1%) were colored in both preparations. Suspensions were washed twice in DMEM, and, after the second centrifugation at 500 rpm for 5 min, the pellets were resuspended in 1–2 mL of DMEM with butyrate and bicarbonate. Protein was measured by the method of BioRad (BioRad, Richmond, CA).

cAMP assay in tubular suspension. Drugs and cells were temperature-equilibrated for 2 min at 37°C before assay. Incubation was initiated by adding 100 μL of tubule suspension to 400 μL of incubation solution containing 0.5 mM isobutyl methylxanthine, 1 mM butyrate, and 24 mM bicarbonate in DMEM and incubated for 2 min at 37°C. Before the incubation, solutions were bubbled with O_2/CO_2. Pilot studies have shown the cAMP increment in our preparation to be linear for 1 to 5 min. The reaction was terminated by the addition of 500 μL of 12% trichloroacetic acid (BDH Chemicals Ltd., Poole, England) and rapid cooling to 4°C. After sonication, samples were centrifuged at 3600 × *g* at 4°C for 15 min on a Sorvall HS-4 rotor.

The supernatant was decanted into glass tubes and extracted four times with 3 mL of water-saturated ether (Casco Nobel, Malmö, Sweden). The water phase was then dried at 70°C under an air stream. Samples were frozen at −80°C until assay. cAMP assay (New England Nuclear, Boston, MA) was performed according to the manufacturer's instructions, and ^{125}I-cAMP was counted in a gamma counter. The effect of adenylate cyclase activation was calculated as pmol cAMP/mg protein/min. Values are expressed as means ± SEM. Statistical significance was calculated with the paired t test.

Preparation of single tubules. Rats were anesthetized with Mebumal vet (Nord Vacc, Stockholm, Sweden) or Inactin (Byk-Gulden). The left kidney was exposed and perfused with Ringer's solution, followed by a modified Hanks' solution having the following composition (in mM): NaCl 137, KCl 5, $MgSO_4$ 0.8, Na_2HPO_4 0.33, KH_2PO_4 0.44, $CaCl_2$ 2, $MgCl_2$ 1, Tris-HCl 10. Collagenase 0.05% (Sigma Chemical Co., St. Louis, MO) and BSA 0.1% (Behringwerke, Marburg, Germany) were added, and the pH was adjusted to 7.4 at 4°C. The kidney was removed and cut along the corticopapillary axis into small pyramids that were incubated for 20 min (PCT) or 5 min (mTAL) at 37°C in the perfusion solution bubbled with O_2. After incubation, the kidney pieces were rinsed in microdissection solution with the same composition as the perfusion solution, except that the $CaCl_2$ concentration was 0.25 mM. Microdissection was performed on ice. Individual tubule segments were photographed in an inverted microscope at ×100 magnification. Permeabilization was performed to allow ATP free access to the cell and largely followed the procedure described by Doucet *et al.* (18). It was optimized for each age group under stereomicroscopic observation, as the young tubules were more sensitive to permeabilization than the adult tubules. When 10-d-old PCT were permeabilized by the same procedure as the 40-d-old PCT, *i.e.* hypotonic shock and freeze-thaw, the Na^+,K^+-ATPase activity was slightly lower than the values with the optimized permeabilization procedure, *i.e.* only hypotonic shock (control value 818 ± 121 [$n = 6$] compared with 1049 ± 46 pmol phosphate/mm tubule/h [$n = 6$] and after dopamine incubation 587 ± 171 compared with 684 ± 23 pmol phosphate/mm tubule/h). There was no significant change in the inhibition of Na^+,K^+-ATPase activity by dopamine (28% compared with 34%), but the values with the milder permeabilization were more stable and less tubular disruption was observed in the microscope. Therefore, we chose to permeabilize by only hypotonic shock in the 10-d-old tubules. The tubules were preincubated for 30 min in room temperature in the absence or presence of drugs. Also, 1 mM butyrate was added to the preincubation solution.

Na^+,K^+-ATPase assay. Na^+,K^+-ATPase activity was assayed as previously described (18), with minor alterations. Tubules were incubated at 37°C for 15 min in a medium containing (in mM): NaCl 70, KCl 5, $MgCl_2$ 10, EGTA 1, Tris-HCl 100, Na_2ATP 10, and [γ-^{32}P]-ATP (New England Nuclear; 2–5 Ci/mmol) in tracer amounts (5 nCi/μL). For the determination of ouabain-insensitive ATPase activity, Tris-HCl concentration was 150 mM; NaCl and KCl were omitted; and ouabain 2 mM (USB Corp., Cleveland, OH) was added. The phosphate liberated by [^{32}P]-ATP hydrolysis was separated by filtration after absorption of the unhydrolyzed nucleotide on activated charcoal. The radioactivity was measured in a liquid scintillation spectrophotometer. For each animal, we determined the total ATPase and the ouabain-insensitive ATPase activity in five to eight tubular segments each. In each assay, we also determined ^{32}P release from [^{32}P]-ATP in samples of incubation solution without any tubular segment as a blank. Na^+,K^+-ATPase activity was calculated according to the following formula:

$$Na^+,K^+\text{-ATPase activity} = (\text{sample} - \text{blank}) \times 4/\text{specific radioactivity} \times \text{tubule length}$$

Values are given as means with SEM. Statistical analysis was performed with the unpaired t test.

Drugs. Fenoldopam (SKF 82526) is a selective agonist for the DA_1 receptor (19). It was used in a concentration of 10 μM because this gave a substantially larger increase in cAMP production than 0.1 μM in pilot studies (43% stimulation compared with 21% in cortical tubules and 32% compared with 14% in medullary tubules). In the Na^+,K^+-ATPase assay, fenoldopam was used in a concentration of 0.1 μM (5) and dopamine at 10 μM (20). Forskolin is an activator of adenylate cyclase, independent of G-protein and receptor activation (21). Isobutyl methylxanthine is an inhibitor of phosphodiesterase action (22) and was used in a concentration of 0.5 mM. Butyrate 1 mM was added to the preincubation solution in the Na^+,K^+-ATPase assay and to the DMEM in the cAMP assay to ensure ATP availability in the cells (23).

If not otherwise indicated, chemicals were purchased from Sigma Chemical Co. and E Merck (Darmstadt, Germany).

RESULTS

Basal and forskolin-stimulated cAMP levels in cortical and medullary cell suspensions in 10-d-old and 40-d-old rats are shown in Table 1. In the cortical cells, basal cAMP production was 5.4-fold higher ($p < 0.05$) in the 10-d-old than in the 40-d-old rats. The cAMP production was increased 24.6-fold ($p < 0.001$) by forskolin stimulation in the 10-d-old and 11.6-fold ($p < 0.001$) in the 40-d-old cortical suspension. The difference between the ages was statistically significant ($p < 0.001$). In the medullary cells, there was no difference in basal cAMP production between the ages. Forskolin incubation resulted in a large cAMP increase in both the 10-d-old (11.4-fold increase, $p < 0.001$) and the 40-d-old rats (8-fold, $p < 0.001$), but there was no difference between the ages.

In the cortical tubular suspension, cAMP production was not increased by fenoldopam in the young rats (1.8 ± 10%, $n = 3$, NS) but substantially increased in the adult rats (51 ± 16%, $n = 4$, $p < 0.05$) (Table 1). The difference between the ages was significant ($p < 0.05$). The percent values given are calculated as mean of percent of control within each experiment. Fenoldopam caused a significant increase in cAMP production in the medullary cells in both 10-d-old (43.5 ± 5%, $n = 4$, $p < 0.001$) and 40-d-old rats (32 ± 7%, $n = 6$, $p < 0.01$).

The basal Na^+,K^+-ATPase activity values (pmol phosphate/mm tubule/h) shown in Table 2 are similar in isolated PCT and mTAL in the 10-d-old rat. In the 40-d-old rat, mTAL values exceeded the 40-d-old PCT 1.5-fold ($p < 0.001$). In the PCT there was a 2.9-fold increase ($p < 0.001$), and in the mTAL there was a 4.1-fold increase ($p < 0.001$) in Na^+,K^+-ATPase activity from 10-d-old to 40-d-old rats. Ouabain-insensitive ATPase activity increased 1.7-fold in the PCT from 10-d-old to 40-d-old rats (1363 ± 47 pmol phosphate/mm tubule/h, $n = 6$, and 2340 ± 200 pmol phosphate/mm tubule/h, $n = 9$, $p < 0.01$), and in the mTAL there was a 1.5-fold increase (1297 ± 243 pmol phosphate/mm tubule/h, $n = 5$, and 2168 ± 47 pmol phosphate/mm tubule/h, $n = 6$, $p < 0.05$).

Dopamine inhibited Na^+,K^+-ATPase activity in both the 10-d-old (34 ± 3%, $n = 6$, $p < 0.001$) and the 40-d-old PCT (44 ± 7%, $n = 9$, $p < 0.001$) (Table 2). Ouabain-insensitive ATPase activity was not affected, and the data are therefore not included. Fenoldopam 0.1 μM inhibited Na^+,K^+-ATPase activity in both the 10-d-old (56 ± 6%, $n = 6$, $p < 0.001$) and the 40-d-old (33 ± 6%, $n = 6$, $p < 0.001$) mTAL. In the 10-d-old mTAL, inhibition was significantly higher ($p < 0.05$).

DISCUSSION

The rat nephron is still developing at the time of birth. Na^+,K^+-ATPase activity increases most rapidly around the weanling period (16- to 20-d-old rat) (24). This increase in enzyme activity coincides with an increase in the reabsorbtive capacity of the tubules and also by an increase in the urinary concentrating

Table 1. *cAMP production (pmol cAMP/mg protein/min) measured in cortical or medullary tubule suspension after incubation with vehicle, fenoldopam, or forskolin**

Tissue	Age	Control	Forskolin (10 μM)	Fenoldopam (10 μM)
Cortex	10-d-old	99.9 ± 35 ($n = 3$)	2458.7 ± 900 ($n = 3$) $p < 0.001$	94.2 ± 26 ($n = 3$) NS
	40-d-old	18.4 ± 4 ($n = 4$)	212.8 ± 15 ($n = 4$) $p < 0.001$	26.6 ± 5 ($n = 5$) $p < 0.05$
Medulla	10-d-old	118.3 ± 9 ($n = 4$)	1349.3 ± 75 ($n = 3$) $p < 0.001$	173.1 ± 10 ($n = 4$) $p < 0.001$
	40-d-old	124.8 ± 17 ($n = 6$)	1007.7 ± 105 ($n = 5$) $p < 0.001$	166.4 ± 22 ($n = 8$) $p < 0.01$

* Values are means of n experiments ± SEM. Statistical analysis was performed with paired t test. The p values depict differences from control value.

Table 2. *Na^+,K^+-ATPase activity (pmol phosphate/mm tubule/h) in single PCT or mTAL tubules measured after incubation with vehicle, dopamine, or fenoldopam**

Tissue	Age	Control	Dopamine (10 μM)
PCT	10-d-old	1049 ± 46 ($n = 6$)	684 ± 23 ($n = 6$) $p < 0.001$
	40-d-old	3052 ± 164 ($n = 9$)	1724 ± 258 ($n = 9$) $p < 0.001$
			Fenoldopam (0.1 μM)
mTAL	10-d-old	1148 ± 109 ($n = 6$)	508 ± 71 ($n = 7$) $p < 0.001$
	40-d-old	4704 ± 69 ($n = 6$)	3388 ± 58 ($n = 4$) $p < 0.001$

* Values are means of n experiments ± SEM. Statistical analysis was performed with t test. The p values depict differences from control value.

capacity (25). The time points we have chosen to study are well before and after this developmental surge.

In the cortical cells, basal and forskolin-stimulated cAMP production was much greater in the infant than in the adult. In medullary cells, on the other hand, basal and forskolin-stimulated cAMP production per mg protein were similar in the two age groups. Similar findings have been reported in renal membrane fractions from 2- to 48-d-old rats (26). The high adenylate cyclase activity may be involved in the rapid cell growth in the cortex at this time.

It is well documented that DA_1 receptors stimulate adenylate cyclase in the PCT as well as in other cells (27, 28). In this study, we report the response to dopamine at the receptor/adenylate cyclase level matures according to different time schedules in the cortical and medullary cells. Incubation of cortical cells with dopamine resulted in no significant increase in cAMP formation in the infant but a large increase in the adult. Kinoshita and Felder (12) reported an improved receptor/adenylate cyclase coupling in single PCT from 3- to 20-wk-old rats. In the medullary cells, fenoldopam caused a significant increase in cAMP production in both the infant and adult.

We found a 2.9-fold increase in basal Na^+,K^+-ATPase activity from 10-d-old to 40-d-old in the PCT and a 4.1-fold increase in mTAL, which is in accordance with previous investigators (13, 25, 29). Dopamine inhibited Na^+,K^+-ATPase activity in the PCT both in the infant and adult. In view of the complete lack of cAMP production by fenoldopam in the infant, some other mechanism must be responsible for this response. Possibly, the DA_2 receptor is of greater importance in the young. In the mTAL, fenoldopam inhibited Na^+,K^+-ATPase activity to a higher degree in the young. This possibly could be explained by the slightly larger cAMP production observed in the medullary cells.

The different patterns of response between cortical and medullary cells show that they mature in function in different ways. Imbert-Teboul *et al.* (30), who studied the effects of various hormones on adenylate cyclase in isolated mTAL and collecting tubules from 2- to 60-d-old rats, arrived at a similar conclusion. The difference in hormonal response may reflect ongoing developmental processes.

A natriuretic response to dopamine, measured as fractional sodium excretion, has been observed in infants of many species (9, 11, 31). This study shows that cellular mechanisms for hormonal control of natriuresis appear early in development and that they develop differently in different cell types.

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