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Regulation of fetal Na^+/K^+-ATPase in rat kidney by corticosteroids

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The enzymatic differentiation of various tissues is under hormonal control in the perinatal period. Since the regulation of Na^+/K^+-ATPase has not been explored prenatally, the aim of this study was to determine the corticosteroid sensitivity of sodium pump maturation in the fetal period. Na^+/K^+-ATPase activity was both measured in kidney homogenates of fetal rats and localized by in-situ histochemistry. Sodium pump activity was first quantifiable on day 18 of fetal development as 1.4 ± 0.17 μmol P_i/h per mg protein, and was increased 3.4-times by day 22 of gestation. While the Na^+/K^+-ATPase activity was the most intense in cortical tubules at an earlier fetal age (18th and 19th day), the reaction product in the medullary tubules increased with fetal age, becoming highly intense on the 21st and 22nd day of gestation. From the 18th to 21st day of fetal development homogenate Na^+/K^+-ATPase activity increased as a function of chronologic age. While mineralocorticoids were without any effect on Na^+/K^+-ATPase activity, on the last day of the fetal development, the glucocorticoid dexamethasone proved to be successful in stimulating enzyme activity in corticosteroid-suppressed animals. According to our results, glucocorticoid hormones seem to be operating as an endogenous driving force for sodium pump maturation at the end of fetal development.

Introduction

The biochemical equivalent of 'sodium pump' is the enzyme Na^+/K^+-adenosine triphosphatase which is responsible for active transport of sodium and potassium ions across the membrane. The regulation of Na^+/K^+-ATPase activity in the adult kidney has not yet been completely explained. Numerous studies, demonstrating a gradual decline in enzyme activity following adrenalectomy [1–3] point to the importance of corticosteroids in the regulation of active sodium transport in adult rats. Restoration of enzyme activity is both sensitive to mineralocorticoids [4–6] and glucocorticoids [1,7,8]. However, Na^+/K^+-ATPase activity is more sensitive to the influence of adrenocorticoid hormones in the immature rat kidney in the postnatal period [9] as compared with the activity in the adult rat kidney. Furthermore, stimulation of Na^+/K^+-ATPase activity by administration of aldosterone in young rats is probably mediated via glucocorticoid receptors [10]. This unresponsiveness of young rats to aldosterone may be explained by the immaturity of postreceptor processes [11].

Since the regulation of sodium pump development has not yet been explored prenatally, the aim of this investigation was to explain the mechanism of Na^+/K^+-ATPase maturation in the fetal period. We have demonstrated for the first time the appearance and the localization of Na^+/K^+-ATPase activity in the fetal rat kidney. Moreover,

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we showed the important role of glucocorticoids in the prenatal development of the active sodium transport system.

Materials and Methods

Pregnant females of Fischer strain rats weighing 200–250 g were kept on standard laboratory diet and water ad libitum in a dark-light (12 : 12 h) and temperature ($24 \pm 1°C$) controlled animal unit. Three-month-old females were caged with males overnight and examined each morning for the presence of vaginal spermatozoa. The day on which spermatozoa were found was designated as the 1st day of gestation. The delivery took place in the afternoon on the 22nd day. The fetuses in the age from the 18th–22nd day of intrauterine development were used.

Different experimental groups of fetuses were obtained by treatment of pregnant rats:

A, adrenalectomy of pregnant females was performed under ether anesthesia on the 15th day of gestation, the animals drank 0.9% saline.

AM, metyrapone (Metopirone, gift from Ciba-Geigy) was injected subcutaneously into a group of adrenalectomized females from the 15th–22nd day of gestation in a dose of 10 mg daily (in 0.3 ml 0.9% NaCl). Metopirone was injected once daily until the 19th day and twice daily from the 19th to the 22nd day of gestation. Metopirone inhibits 11β-hydroxylation of steroids.

AM + DOCA, deoxycorticosterone acetate was injected intramuscularly to an AM group of females from the 15th–22nd day of gestation in a dose of 5 mg/kg body wt.

NaCl, group of intact pregnant females drank 1.7% NaCl from the 12th to the 22nd day of gestation ad libitum.

AM + aldosterone, aldosterone (Merck), dissolved in ethanol and diluted with 0.9% NaCl, was injected intraperitoneally to an AM group of females in a dose of 40 μg/100 g body wt. twice daily from the 19th to the 22nd day of gestation.

AM + dexamethasone, dexamethasone (200 μg/100 g body wt. twice daily) was injected intraperitoneally to an AM group of females for 3 days (i.e. from the 19th to the 22nd day of gestation). In another experiment, dexamethasone (50, 110 or 200 μg/100 g body wt. twice daily) was injected for 2 days (on the 20th and 21st day of gestation).

Potassium canrenoate (Soldactone, Searle) was injected intramuscularly to intact pregnant females in a dose of 10 mg/100 g body wt. daily from the 17th to the 22nd day of gestation.

Aldosterone (0.4 μg per fetus, i.e. 20 μg/100 g body wt. in 25 μl 0.9% NaCl) or

Dexamethasone (2 μg per fetus, i.e. 100 μg/100 g body wt. in 25 μl 0.9% NaCl) was injected to the fetuses subcutaneously through the uterine wall on the 20th day at 12 a.m. and on the 21st day of gestation at 8 a.m., after a female's laparotomy performed under ether anesthesia. The fetuses were killed 4 h after the second injection.

Each homogenate contained the kidneys of 3–22 fetuses, depending on their age. The kidney weight of intact fetuses was 2.7 ± 0.16 mg on the 18th day ($n = 140$) and 31.4 ± 0.73 mg on the 22nd day of gestation ($n = 23$). A pregnant rat was laparotomized under ether anesthesia, the fetuses decapitated and their kidneys quickly removed and placed into cold STE buffer (0.25 mol/l sucrose, 10 mmol/l triethanolamine-HCl and 5 mmol/l EDTA-Na_2 (pH 7.6)). The kidneys were homogenized in 3 volumes of cold STE buffer (0–4°C) with a Potter-Elvehjem homogenizer (0.5 ml, Kontes Glass), using eight strokes of a Teflon pestle and 1300 rpm. The homogenate was mixed with water (1 : 10) and stored at −20°C. The enzyme activity was determined within 6 days after the isolation.

Prior to determination of Na^+/K^+-ATPase activity, the homogenate was incubated with detergent: 540 μl homogenate was incubated for 5 min at $22 \pm 1°C$ with 400 μl of a mixture containing: 1.645 g/l Lubrol WX (Sigma), 10 g/l bovine serum albumin (BSA, Cohn Fraction V, Armour) and 25 mmol/l imidazole (pH 7.5). The final concentration of Lubrol was 0.7 g/l and the proportion of the quantity of kidney proteins (μg)/quantity of detergents (μg) was in the range of 0.9–1.8 in all measurements of enzyme activity. The first incubation was stopped after placing the mixture on ice. Aliquots of 200 μl (200–300 μg proteins) were transfered for measurement of ATPases activities.

Na^+/K^+-ATPase activity was measured according to the modified method of Kinsolving

et al. [12]. One ml of the reaction mixture contained 5 mmol/l ATP-Na_2 (Sigma), 40 mmol/l D-histidine (Sigma), 40 mmol/l imidazole, 80 mmol/l NaCl, 16 mmol/l KCl, 5 mmol/l $MgCl_2$ and in pertinent cases 1 mmol/l ouabain. After temperature equilibrium was reached the reaction was started by the addition of aliquots of the detergent-treated homogenates. Samples were incubated for 15 min at 44° C. A blank contained an appropriate quantity of a medium for preincubation. The reaction was stopped by the addition of 1 ml ice-cold 20% (w/w) trichloroacetic acid. Na^+/K^+-ATPase activity was calculated from the difference between liberated μmoles of inorganic phosphate (P_i) per hour per mg protein in incubations without added ouabain (total, ($Na^+ + K^+ + Mg^{2+}$)-ATPase activity) and in the incubations with added ouabain (Mg^{2+}-ATPase activity). Inorganic phosphate was determined by the method of Fiske and SubbaRow [13] and proteins by the method of Lowry et al. [14].

Na^+/K^+-ATPase was localized histochemically in the fetal kidney [15] by means of a K^+-dependent *p*-nitrophenylphosphatase activity. The method was adapted to the fetal kidney. Fetal kidneys were fixed in 4% formaldehyde in 0.1 mol/l phosphate buffer (pH 7.2) for 1 h (on the 18th and 19th day) and for 4 h (from the 20th to the 22nd day of gestation), at 0–4° C. After fixation, the kidneys were washed overnight in phosphate buffer (80 ml 0.15 mol/l NA_2HPO_4 + 20 ml 0.15 mol/l KH_2PO_4) containing 15% (w/v) sucrose, at 0–4° C. K^+-dependent *p*-nitrophenylphosphatase activity was demonstrated in free floating frozen sections (8 μm). The incubation medium contained: 2.5 ml 1.0 mol/l glycine-KOH buffer (pH 9.0) (glycine: 250 mmol/l and K^+: 25 mmol/l in final concentrations); 4.0 ml 1% lead citrate dissolved in 50 mmol/l KOH (lead citrate: 4.0 mmol/l, K^+: 20 mmol/l); 2.5 ml DMSO (25% v/v); 1 ml 0.1 mol/l *p*-nitrophenylphosphate Mg^{2+} salt, Sigma: 10 mmol/l); 6.02 mg Levamisole (Sigma) (2.5 mmol/l) (final pH 8.8).

Statistical analysis. The difference between two means was evaluated by Student's *t*-test. After analysis of variance and the test of homogeneity of variance Student's *t*-test or Kramer's multiple range test [16] were used to calculate the significance of differences between means in experiments with more than two groups.

Results

Development of Na^+/K^+-ATPase activity in the fetal rat kidney

For unmasking of latent Na^+/K^+-ATPase activity, homogenates of fetal kidneys were incubated with different detergents (data not shown). After

TABLE I

DEVELOPMENT OF Na^+/K^+-ATPase ACTIVITY IN THE KIDNEY HOMOGENATES OF FETUSES FROM: INTACT FEMALES (INT); ADRENALECTOMIZED FEMALES (A); A + METOPIRONE (AM); AM + DEOXYCORTICOSTERONE ACETATE (AM + DOCA); AND 1.7% NaCl-LOADED FEMALES

Enzyme activity is expressed in μmol P_i/h per mg protein. See Materials and Methods for experimental details. The number of pregnant females/the number of preparations is shown in parentheses. The values are the means ± S.E. *, $P < 0.05$; **, $P < 0.01$ vs. INT; [a], $P < 0.01$ vs. day 18 if only the data from intact group are considered.

Fetal age (days)	INT	A	AM	AM + DOCA	NaCl
18	1.4 ± 0.17 (12/7)	1.0 ± 0.17 (10/5)	0.8 ± 0.12 (10/5)	1.0 ± 0.19 (6/3)	1.3 ± 0.16 (14/5)
19	1.4 ± 0.14 (5/5)	1.4 ± 0.33 (7/5)	1.6 ± 0.07 (4/4)	1.6 ± 0.04 (4/4)	1.1 ± 0.09 (5/4)
20	2.3 ± 0.24 [a] (7/7)	2.4 ± 0.39 (3/5)	1.6 ± 0.17 (3/5)	1.6 ± 0.16 (4/6)	1.7 ± 0.18 (4/5)
21	3.4 ± 0.32 [a] (3/6)	3.2 ± 0.19 (2/6)	3.2 ± 0.41 (1/4)	3.4 ± 0.16 (2/5)	3.0 ± 0.19 (3/5)
22	4.7 ± 0.20 [a] (4/7)	3.6 ± 0.22 * (2/5)	3.4 ± 0.24 ** (3/8)	3.7 ± 0.21 * (3/8)	4.4 ± 0.43 (4/7)

TABLE II

DEVELOPMENT OF Mg^{2+}-ATPase ACTIVITY IN THE KIDNEY HOMOGENATES OF FETUSES FROM: INTACT FEMALES (INT); ADRENALECTOMIZED FEMALES (A); A + METOPIRONE (AM); AM + DEOXYCORTICOSTERONE ACETATE (AM + DOCA); AND 1.7% NaCl-LOADED FEMALES

Enzyme activity is expressed in μmol P_i/h per mg protein. See Materials and Methods for experimental details. The number of pregnant females/the number of preparations is shown in parentheses. The values are the means ± S.E. *, $P < 0.05$; **, $P < 0.01$ vs. INT; [a], $P < 0.05$, [b], $P < 0.01$ vs. day 18 if only the data from intact group are considered.

Fetal age (days)	INT	A	AM	AM + DOCA	NaCl
18	4.5 ± 0.20 (12/7)	3.9 ± 0.29 (10/5)	4.7 ± 0.16 (10/5)	5.1 ± 0.53 (6/3)	5.1 ± 0.33 (14/5)
19	4.8 ± 0.24 (5/5)	5.5 ± 0.34 (7/5)	4.5 ± 0.11 (4/4)	4.6 ± 0.37 (4/4)	5.8 ± 0.36 (5/4)
20	6.0 ± 0.57 [a] (7/7)	5.5 ± 0.30 (3/5)	5.2 ± 0.63 (3/5)	5.7 ± 0.44 (4/6)	6.0 ± 0.68 (4/5)
21	7.0 ± 0.49 [b] (3/6)	7.2 ± 0.29 (2/6)	6.5 ± 0.11 (1/4)	7.3 ± 0.43 (2/5)	7.0 ± 0.59 (3/5)
22	8.3 ± 0.52 [b] (4/7)	6.8 ± 0.42 * (2/5)	6.7 ± 0.40 * (3/8)	6.0 ± 0.53 ** (3/8)	8.0 ± 0.69 (4/7)

the determination of optimal experimental conditions (concentration of detergents, pH, time of incubation), nonionic detergent Lubrol WX was shown as the most appropriate for fetal Na^+/K^+-ATPase activation.

The development of Na^+/K^+-ATPase and Mg^{2+}-ATPase specific activity in the fetal kidney homogenates in the period from the 18th to the 22nd day of intrauterine development is shown in Tables I and II. The Na^+/K^+-ATPase activity was first demonstrable on the 18th day of gestation. The largest rise in enzyme activity was noticed in the period between the 21st and 22nd day of fetal development. Na^+/K^+-ATPase activities were the same on the 18th and 19th day of gestation, while the activity increased significantly ev-

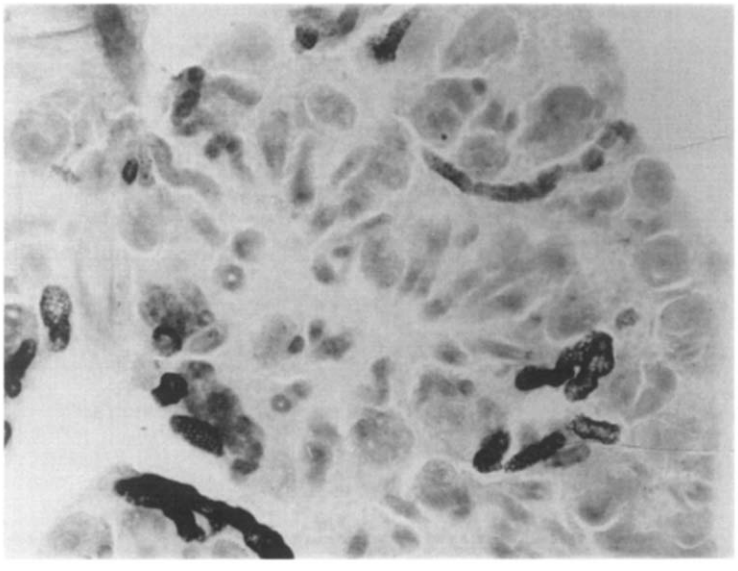

Fig. 1. K^+-dependent *p*-nitrophenylphosphatase activity in the kidney cortex on the 19th day of fetal development. Strong reaction first appeared in cortical tubules. Magnification: 50×.

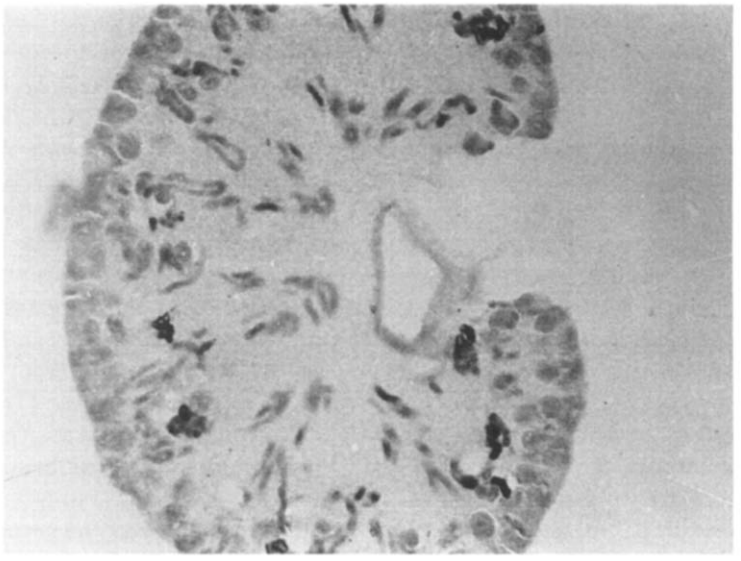

Fig. 2. K^+-dependent *p*-nitrophenylphosphatase activity on the 20th day of fetal development. Strong reaction is observed in the rare cortical tubules. Magnification: 20×.

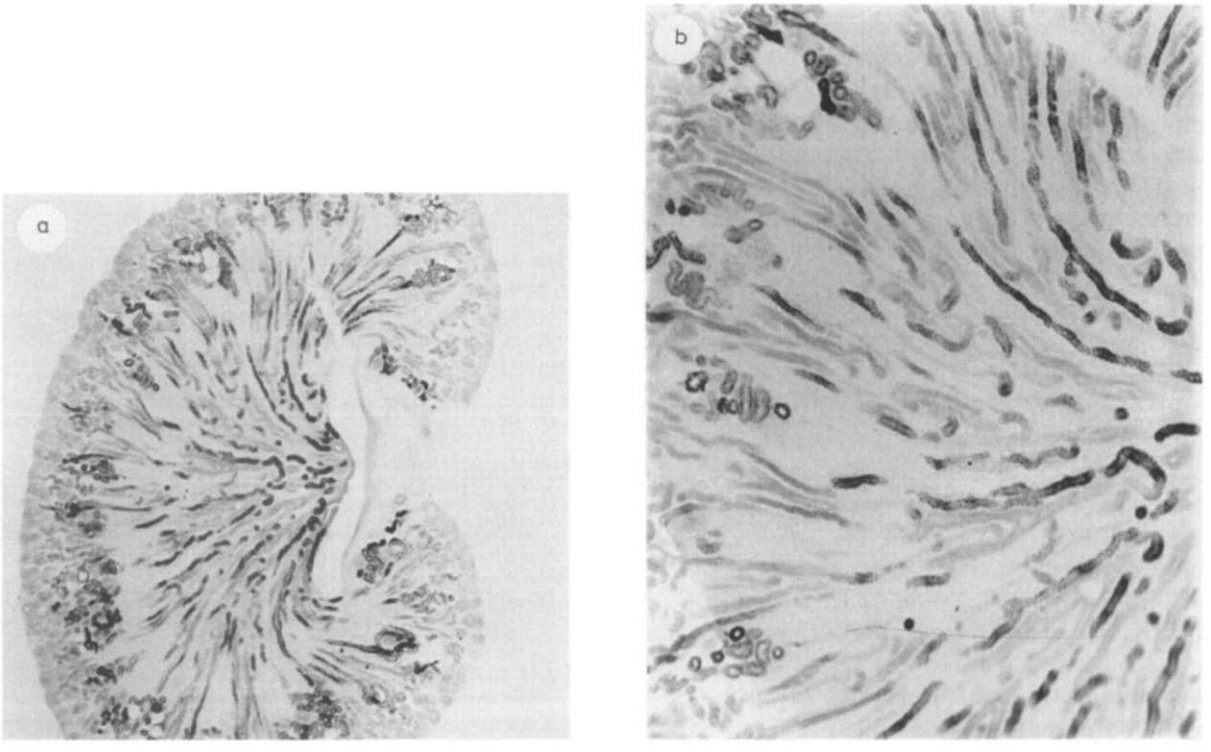

Fig. 3. K^+-dependent *p*-nitrophenylphosphatase activity on the 22nd day of fetal development. Strong reaction occurs in proliferative tubules of cortex and medulla. Magnification: (a) 20×; (b) 50×.

ery next day in relation to the previous day. The rise of Mg^{2+}-activity, however, was only 1.8-fold between day 18 and day 22.

Histochemical localization of Na^+/K^+-ATPase in fetal kidney

The distribution of Na^+/K^+-ATPase activity in the fetal rat kidney was shown by histochemical localization of a K^+-dependent *p*-nitrophenylphosphatase activity, sensitive to ouabain [15]. The activity of K^+-dependent *p*-nitrophenylphosphatase, which represents the second (dephosphorylation) step of the Na^+/K^+-ATPase system was first demonstrable on the 18th day of gestation, in a few cortical tubules. The intensity and distribution of K^+-dependent *p*-nitrophenylphosphatase activity on the 19th day of gestation (Fig. 1) remained unchanged in comparison with the 18th day. On the 20th day of fetal development the K^+-dependent *p*-nitrophenylphosphatase reaction was more pronounced in a few isolated cortical tubules below the nephrogenic zone, and found in moderate amounts in medullary tubules (Fig. 2). On the 21st and 22nd day of fetal development, the number of tubules with positive K^+-dependent *p*-nitrophenylphosphatase reaction increased (Fig. 3a). The cortical tubules below the nephrogenic zone (proximal and distal) were the sites of the most intense K^+-dependent *p*-nitrophenylphosphatase reaction product. However, the enzyme activity in the numerous medullary tubules increased, becoming moderate to strong (Fig. 3b).

Regulation of Na^+/K^+-ATPase development in the fetal kidney

The adrenalectomy of pregnant rats did not influence the development of Na^+/K^+-ATPase and Mg^{2+}-ATPase activity in the period from the 18th to the 21st day of fetal development (Tables I and II). The Na^+/K^+-ATPase activity in the fetuses of intact females increased 38% ($P < 0.01$) on the 22nd day of gestation in comparison with the 21st day, while the activities of these enzymes in the fetuses from adrenalectomized females were nonsignificantly different on days 21 versus 22.

In the absence of endogenous corticosteroids (maternal adrenalectomy + metopirone treatment) the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase were similar to those activities in fetuses from adrenalectomized mothers (Tables I and II). The activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase in a group of fetuses from adrenalectomized rats treated with metopirone and the mineralocorticoid deoxycorticosterone acetate (AM + DOCA) did not differ from the activities in the control (AM) group from the 18th to the 22nd day of gestation (Tables I and II). Furthermore, the loading of pregnant rats with 1.7% NaCl did not cause any change in Na^+/K^+-ATPase and Mg^{2+}-ATPase activity from the 18th to the 22nd day of fetal development (Tables I and II).

Potassium canrenoate, a competitive antagonist of aldosterone was administered to intact females in a dose of 10 mg/100 g body wt. daily from the 17th to the 22nd day of gestation. The blocking of mineralocorticoid receptors did not cause any change in Na^+/K^+-ATPase activity in the fetal kidney on the 22nd day of gestation (4.0 ± 0.34 in treated vs. 4.1 ± 0.37 in intact group; $n = 7$).

TABLE III

SPECIFIC ACTIVITIES OF Na^+/K^+-ATPase AND Mg^{2+}-ATPase IN KIDNEY HOMOGENATES ON THE 22nd DAY OF GESTATION AFTER ADMINISTRATION OF ALDOSTERONE OR DEXAMETHASONE

Dexamethasone (200 μg/100 g body wt. twice daily) or aldosterone (40 μg/100 g body wt. twice daily) was injected intraperitoneally for 3 days beginning with the 19th day of gestation to adrenalectomized females previously treated with metopirone (AM). Pregnant rats were adrenalectomized on the 15th day of gestation and metopirone was injected subcutaneously (10 mg daily) from the 15th to the 22nd day of gestation. The number of pregnant females/the number of preparations is shown in parentheses. For each preparation 3 fetuses were used. The values are the means ± S.E.

	Enzyme activity (μmol P_i/h per mg protein)	
	Na^+/K^+-ATPase	Mg^{2+}-ATPase
Intact (5/13)	4.4 ± 0.18	7.3 ± 0.29
AM (5/14)	3.1 ± 0.19	6.1 ± 0.39
AM + aldosterone (3/9)	3.3 ± 0.23	6.2 ± 0.30
AM + dexamethasone (5/16)	4.8 ± 0.26	7.8 ± 0.36
P AM vs. AM + aldosterone	n.s.	n.s.
P AM vs. AM + dexamethasone	< 0.001	< 0.01
P intact vs. AM	< 0.001	< 0.05

TABLE IV

SPECIFIC ACTIVITIES OF Na^+/K^+-ATPase AND Mg^{2+}-ATPase IN KIDNEY HOMOGENATES ON THE 21st DAY OF GESTATION AFTER ALDOSTERONE OR DEXAMETHASONE ADMINISTRATION TO THE FETUSES

Aldosterone (0.4 μg per fetus, i.e. 20 μg/100 g body wt.) or dexamethasone (2 μg per fetus, i.e. 100 μg/100 g body wt.) in 25 μl 0.9% NaCl was injected subcutaneously to the fetuses through the uterine wall on the 20th and 21st day of gestation, after the laparotomy of a female. The kidneys were taken 4 h after the second injection. The number of pregnant females/the number of preparations is shown in parentheses. For each preparation three fetuses were used. The values are the means ± S.E.

	Enzyme activity (μmol P_i/h per mg protein)	
	Na^+/K^+-ATPase	Mg^{2+}-ATPase
Intact (7/16)	2.9 ± 0.14	6.5 ± 0.54
Aldosterone (7/15)	3.3 ± 0.23	6.4 ± 0.58
	n.s.	n.s.
Intact (2/4)	3.5 ± 0.49	7.3 ± 0.57
Dexamethasone (2/6)	3.3 ± 0.29	8.8 ± 0.34
	n.s.	n.s.

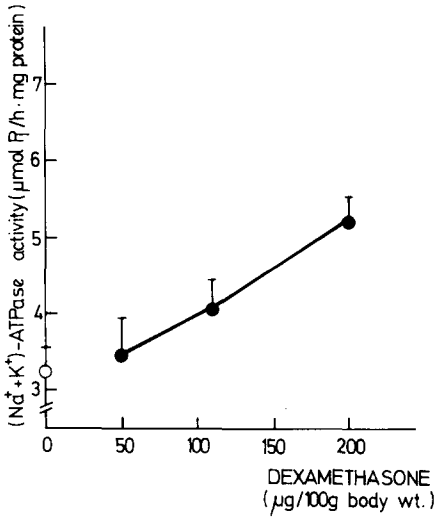

Fig. 4. Dose dependence of Na^+/K^+-ATPase specific activity on dexamethasone administered for 2 days in fetal kidney homogenates on the 22nd day of gestation. Dexamethasone was injected intraperitoneally on the 20th and 21st day of gestation twice daily (●) to adrenalectomized mothers previously treated with metopirone from the 15th to the 22nd day of gestation (○). The values plotted are the means ± S.E. of 5–7 observations.

Aldosterone (40 μg/100 g body wt. twice daily) which was given for 3 days to adrenalectomized females injected with metopirone (AM), did not have any effect on the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase on the 22nd day of gestation (Table III). However, injecting dexamethasone (200 μg/100 g body wt. twice daily) for 3 days completely restored depressed Na^+/K^+-ATPase activity resulting from a corticosteroid insufficiency on the 22nd day of gestation (Table III).

TABLE V

BODY AND KIDNEY WEIGHTS OF FETUSES FROM: INTACT FEMALES (INT); ADRENALECTOMIZED FEMALES (A); A + METOPIRONE (AM); AM + DEOXYCORTICOSTERONE ACETATE (AM + DOCA); AND 1.7% NaCl-LOADED FEMALES

[a] Fetal weight (g); [b] kidney weight (mg). The kidney weight is the average weight of the two kidneys. The number of pregnant females ranged from 4 to 9 and the number of fetuses from 15 to 50. The values are the means ± S.E. * $P < 0.05$; ** $P < 0.01$ vs. INT.

Fetal age (days)	INT	A	AM	AM + DOCA	NaCl
18	0.7 ± 0.01 [a]	0.6 ± 0.04	0.6 ± 0.04	0.6 ± 0.06	0.7 ± 0.02
	2.7 ± 0.16 [b]	2.3 ± 0.19	2.4 ± 0.20	2.5 ± 0.01	2.6 ± 0.14
19	1.3 ± 0.03	1.1 ± 0.04 *	1.0 ± 0.01 *	1.1 ± 0.03 *	1.2 ± 0.04 *
	6.9 ± 0.44	5.7 ± 0.19 *	4.6 ± 0.30 *	6.1 ± 0.27	5.7 ± 0.40 *
20	1.9 ± 0.04	1.7 ± 0.07 *	1.6 ± 0.07 *	1.7 ± 0.09 *	1.9 ± 0.06
	11.7 ± 0.29	10.1 ± 0.59 *	9.4 ± 0.31 *	10.5 ± 0.27	11.0 ± 0.59
21	3.0 ± 0.02	2.8 ± 0.19	2.6 ± 0.07	2.9 ± 0.10	2.8 ± 0.06
	20.4 ± 1.52	18.6 ± 0.80	17.6 ± 0.43	20.8 ± 0.48	18.2 ± 0.46
22	4.4 ± 0.06	3.6 ± 0.13 *	3.3 ± 0.27 *	3.7 ± 0.31 *	4.1 ± 0.04
	31.4 ± 0.73	25.0 ± 1.63 **	24.9 ± 1.52 **	27.3 ± 0.91 *	32.2 ± 1.94

The administration of 2 injections of aldosterone or dexamethasone to the fetuses from intact mothers did not stimulate the sodium pump activity on the 21st day of gestation (Table IV). However, the administration of dexamethasone for 2 days enhanced the sodium pump activity on the 22nd day in the fetuses of adrenalectomized females treated with metopirone (Fig. 4). The Na^+/K^+-ATPase activity increased linearly with an increase of dexamethasone dose in the range from 50 to 200 μg dexamethasone/100 g body wt.

Fetal and kidney weights in experimental groups where existed the insufficiency of corticosteroids were less than in the intact group of fetuses on days 19, 20 and 22 of gestation (Table V). The weights of fetuses from adrenalectomized females injected with metopirone were diminished 15% and 24% on days 20 and 22. At the same time, kidney weights were decreased 20% in comparison with the intact group.

Discussion

The onset of the prenatal development of Na^+/K^+-ATPase activity measured in rat kidney homogenates (Table I) agreed with the results of a histochemical analysis of ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity (Figs. 1–3). The Na^+/K^+-ATPase activity was very low on the 18th and 19th day of gestation (1.4 μmol P_i/h per mg protein) and was located in the rare cortical tubules. The enzyme activity was 3.4-times greater on the 22nd day relative to day 18 of gestation, and was expressed in numerous medullary as well as cortical tubules (Fig. 3). The Na^+/K^+-ATPase activity increased gradually from the 19th through 22nd day of gestation.

The appearance of Na^+/K^+-ATPase activity in the fetal rat kidney, on the 18th day of gestation, seemed to correspond to the beginning of the morphologic differentiation of tubules. Whereas the medullary ascending, thick loop of Henle and distal cortical tubules were described as the sites of the most intense K^+-dependent *p*-nitrophenylphosphatase activity in the adult rat kidney [15,17], the cortical tubules were the most reactive in the earlier fetal age – on the 18th and 19th day (Fig. 1). The reaction in the medullary tubule was moderate on the 20th day (Fig. 2) and strong on the 22nd day (Fig. 3). This difference in the distribution pattern of fetal K^+-dependent *p*-nitrophenylphosphatase activity as compared with that in adults could be assigned to the close relationship between the appearance of K^+-dependent *p*-nitrophenylphosphatase and the maturation of tubules.

It is known that pregnant rats have an elevated plasma concentration of corticosteroids [18] and that the fetal adrenal gland is capable of synthesizing corticosteroids in the last days of intrauterine development. The steroids of fetal origin cross the placenta maintaining the normal concentration of corticosteroids in plasma of adrenalectomized mothers [19]. Several experimental models were used including a partial insufficiency of adrenal steroids (mother's adrenalectomy) or the absence of endogenous corticosteroids in female and fetus (mother's adrenalectomy + metopirone treatment). Metopirone is an inhibitor of 11β-hydroxylation of steroids that crosses the placenta [20]. In the period from the 18th to the 21st day of fetal growth, the insufficiency of corticosteroids did not hinder the development of the sodium pump (Table I). Accordingly, the development of Na^+/K^+-ATPase activity in the period from the 18th to the 21st day proceeds along with the fetal maturation as a function of chronologic age (Table I). However, in the period from the 21st to the 22nd day, the increase of the enzyme activity was not noticed in the absence of the mother's adrenal glands or in the absence of endogenous corticosteroids in female and fetus. Thus the rise in the enzyme activity which was found on the 22nd day could be assigned to the sudden rise of corticosteroid concentration in the female on the last day of gestation [18]. It could be, therefore, concluded that corticosteroids are necessary for the development of the sodium pump only at the end of fetal life. Mg^{2+}-ATPase activity was also diminished in the conditions of corticosteroid deficiency on the 22nd day of fetal development (Table II). This is opposite to the results obtained in adult rats, where the adrenalectomy did not influence Mg^{2+}-ATPase activity [2,21].

It is known that chronic application of deoxycorticosterone acetate increases the renal Na^+/K^+-ATPase activity in adult rats [3], sodium pump activity and protein content in cortical and

medullary distal and collecting tubules in adult rabbits [6,22], as well as the surface area of the basolateral membranes of the rabbit cortical collecting tubules [23]. However, a diminished enzyme activity was noticed in the corticosteroid-deficient state on the 22nd day of gestation that could not be restored by DOCA treatment (Table I).

The administration of aldosterone for 3 days to the adrenalectomized females previously treated with metopirone had no effect on the Na^+/K^+-ATPase activity on the 22nd day of gestation (Table III), nor did the blocking of mineralocorticoid receptors by potassium canrenoate change the activity. Furthermore, the application of aldosterone to the fetuses did not stimulate the enzyme activity on the 21st day of gestation (Table IV).

On the basis of presented results (Tables I, III and IV) it may be concluded that the process of sodium pump maturation at the end of fetal development is independent of mineralocorticoids. However, a glucocorticoid, dexamethasone, restored the diminished Na^+/K^+-ATPase activity, resulting from a corticosteroid insufficiency on the last day of fetal development (Table III and Fig. 4). When dexamethasone was injected to corticosteroid-replete fetuses on the 20th and 21st day of intrauterine development, Na^+/K^+-ATPase was not further stimulated on the 21st day (Table IV). On the contrary, postnatally, one dose of dexamethasone stimulated Na^+/K^+-ATPase activity in the proximal tubules of 10-day-old rats after 16 h and maximally after 24–30 h [9]. The glucocorticoids accelerate renal maturation also in other species. Thus betamethasone accelerated the differentiation of kidney in fetal rhesus monkey so that the nephrogenic zone became considerably narrower [24]. Glucocorticoids also accelerated the maturation of glomerules and tubules in fetal sheep kidney [25].

According to our results, glucocorticoid hormones seem to be operating as an endogenous driving force in the sodium pump maturation at the end of the fetal development.

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