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Functional renal maturation in rat neonates after prenatal exposure to furosemide

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Abstract. We investigated the pattern of functional renal maturation and electrolyte handling on postnatal day 1 (PD1), day 5 (PD5), and day 12 (PD12) in rat neonates, after mothers were given furosemide during pregnancy. The drug was administered (75 mg/kg per day i.p.) on day 7–11 (organogenesis) and 14–18 (nephrogenesis) of gestation. On PD1 and PD5, there was a disturbance of the urinary concentrating ability with a hyperdiuresis and an overstimulated ionic exchange, mainly the distal sodium reabsorption. From PD1 to PD12, a progressive functional recovery in electrolyte handling appeared. However, on PD12, when nephrogenesis was achieved, the renal concentrating defect remained. We discuss the possibility of a drug-induced delay in the development of the loop of Henle, leading to functional and morphological adaptations of already developed parts of the nephron.

Key words: Pregnancy – Furosemide – Prenatal furosemide administration – Renal functional maturation – Neonatal kidney

Introduction

The effects of drugs on the developing kidney are commonly studied in infants or neonates who are given the product directly. Little attention, however, has been paid to possible damage in neonates who are exposed to toxicants through the placenta [1–5].

Furosemide, a "loop diuretic," is frequently prescribed for hypertension and edema of pregnancy, sometimes for the whole period of gestation [6–9]. A delay of renal maturation following in utero exposure to furosemide has been reported in rats [10]. The present study was undertaken to investigate whether these morphological changes are accompanied by functional modifications.

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We focused on the postnatal renal functions of neonates after exposure to furosemide given to the mother during two critical periods of gestation (normal duration in rats = 21 days): organogenesis (7th–11th day of pregnancy) and nephrogenesis (14th–18th day). Since renal maturation in rats continues after birth for 11–12 days [11, 12], the functional aspects were studied on posnatal day 1 (PD1), day 5 (PD5), and day 12 (PD12).

Materials and methods

Groups of animals. Female Wistar rats were allocated to one control and one treated group. They were mated with males. The day when sperm were found in the vaginal smear was considered the 1st day of pregnancy. The control group (15 females) did not receive any drug. The second group (15 females) was given furosemide (Lasilix, Hoechst) 75 mg/kg per day, at 5 p.m., on days 7–11 and 14–18 of gestation. In this strain, delivery occurs on the evening of the 22nd day of gestation. The day following birth was considered PD1. Whatever their age, control and in utero-exposed neonates were individually weighed; blood and urine samples were taken from each animal.

Sample collections. Since neonates cannot micturate spontaneously, bladders were emptied using the external stimulus of gently stroking the belly in the perineal region, as described by Kavlock and Gray [13]. Because pups are poikilothermic in early development, they were kept for 3 h (clearance period) in beakers placed in a water bath with a bedding surface kept at 32° C–34° C.

At the start of the test, bladders were emptied and urine discarded. At the end of the clearance period, the urine was quantitatively collected in calibrated capillary tubes. Blood was obtained after decapitation from PD1 and PD5 neonates and by a small cut of the tail under slight ether anesthesia for PD12 rats. Blood was collected into serum separator tubes (Becton Dickson) and centrifuged.

Biochemistry. Aliquots of serum and urine were analyzed for sodium (Na^+), potassium (K^+), inorganic phosphate (Pi), and creatinine. Samples, diluted when necessary, were analyzed in order to obtain individual data. Creatinine concentration was determined using an automatic analyzer (ABA 100, Laboratoire Abbott, Paris, France), applying the kinetic method of Jaffe. A Corning 902 Na/K analyzer was used for Na^+ and K^+ and a Corning 925 Chloride analyzer for Chloride. P_i was determined by a colorimetric method. Diuresis was given in microliters per gram per hour.

Table 1. Body weight of control and furosemide-treated rat mothers during pregnancy and of neonates on postnatal day 1 (PD1), day 5 (PD5), and day 12 (PD12)[a, b]

	Control	Furosemide
Mothers		
Body weight (g)		
during pregnancy start	318.2±16.3 (15)	333.3±16.3 (15)
end	393.8±21.0 (15)	403.1±13.1 (15)
Weight gain (%)	24.7±6.2	22.1±3.3
(Weight gain/*n* of neonates) ratio	1.7±0.4	1.9±0.2
Neonates		
Body weight (g) on PD1	7.12±0.07 (79)	6.51± 0.08 (84)***
Body weight (g) on PD5	13.73±0.3 (54)	13.48±0.2 (35)
Body weight (g) on PD12	26.7±0.9 (39)	26.8±0.4 (37)

Comparison (*t*-test) between controls and furosemide ***$P < 0.001$

[a] Numbers of animals are in parentheses

[b] Results are presented as mean ± SEM

Creatinine clearance (C_{Cr}, used as an index of glomerular filtration rate) was calculated using a standard formula and fractional excretions of water (FE_{H_2O}), Na (FE_{Na}), and K (FE_K) as:
$FE_x = [(U_x \times V)/C_{Cr} \times P_x]100$; U_x being urinary concentration of x, P_x the plasma concentration, and V the diuresis.

Urinary electrolyte excretions were calculated as ($U_{electrolyte} \times V$) and given as nanomoles per gram per hour.

Results

The body weight gain during pregnancy was similar in both groups (Table 1). Deliveries occurred at term. The number of neonates was 11.2 ± 0.5 in control litters and 12.9 ± 0.6 in the furosemide-exposed group. The ratio of weight gain of mothers during pregnancy (as a percentage) to the number of neonates was 1.91 ± 0.18 in the furosemide group and 1.73 ± 0.36 in the control group (Table 1).

Plasma electrolyte, creatinine, and protein concentrations, diuresis, C_{Cr}, electrolyte excretions, and FE_{Na} on PD1, PD5, and PD12 are reported in Table 2. On PD1, furosemide-exposed neonates had a lower body weight than controls ($P < 0.001$). Later on PD5 and PD12, the body weight was not significantly different between treated and control animals (Table 1).

Plasma Na^+ and K^+ concentrations were higher in furosemide-exposed neonates than in controls on PD1 and PD5 (Table 2). The diuresis decreased regularly in the control group from PD1 to PD12. In the furosemide-exposed group, it decreased dramatically between PD1 and PD5 and then remained at the same level on PD12. Therefore, the diuresis, higher at birth in the furosemide-exposed neonates, was still higher than in controls on PD12. The FE_{Na} decreased regularly from PD1 to PD12 in controls; it was lower in the furosemide group at the three ages, but the decrease was less dramatic.

Plasma creatinine concentration did not change between groups whatever the age (Table 2). The C_{Cr}, higher in the furosemide group on PD1 and PD5, was similar on PD12 to that of the control group.

Discussion

In utero-treated rats had a lower body weight than controls. A decrease in placenta perfusion owing to a furosemide-induced contracted blood volume is unlikely, since, in both groups of mothers, the weight gain throughout pregnancy was similar and the serum electrolytes, creatinine, and urea were not significantly different after this protocol [10]. This

Table 2. Plasma sodium (PNa), potassium (PK), plasma creatinine (PCr), diuresis, creatinine clearance (C_{Cr}), urinary electrolyte excretions (E), serum proteins, and fractional excretion of Na (FENa) on PD1, PD5, and PD12 of rat neonates born to control mothers or to mothers treated with furosemide during pregnancy

	PNa (mmol/l)	PK (mmol/l)	PCr (mg/l)	Diuresis (µl/g per hour)	C_{Cr} (µl/g per hour)	ENa (nmol/g per hour)	EK (nmol/g per hour)	ECl (nmol/g per hour)	EPi (nmol/g per hour)	Proteins (g/l)	FENa
PD1											
Control	134.7±1.8	6.3±0.1	4.8±0.07	3.2±0.1	63.1±1.8	177.9±18.4	102.5±5.7	198.8±8.3	ND	22.2±0.4	2.1±0.2
n	75	79	79	79	79	79	76	78	–	79	75
Furosemide	179.1± 3.4***	11.8± 1.9***	5.0±0.08	3.6± 0.1**	80.8± 3.34***	116.2± 11.5***	148.8± 6.9***	140.0± 8.6***	ND	21.2±0.2	1.3± 0.1*
n	84	84	84	83	82	79	77	81	–	79	79
PD5											
Control	139.9±3.2	5.9±0.2	4.5±0.06	2.7±0.2	78.9±6.5	179.4±21.4	183.8±19.2	51.7±5.3	37.1±6.7	28.6±0.7	2 ±0.6
n	52	54	54	54	54	54	54	54	43	51	52
Furosemide	166.8± .45***	6.6± 0.2**	4.2±0.1	2.5±0.1	85.8±3.5	217.2±31.1	178.2±40.0	75.3±10.7	74.4± 3.9**	29.4±0.4	1.3±0.2
n	35	35	35	35	35	31	35	35	30	34	31
PD12											
Control	140.1±3.5	6.6±0.2	4.6±0.1	1.9±1.2	92.4±5.9	127.1±16.1	175.9±17.7	35.2±4.6	47.5±4.7	40.8±0.7	1 ±0.01
n	29	36	36	38	36	38	39	38	32	38	29
Furosemide	138.8±3.6	6 ±0.1	5.0±0.1	2.6± 0.2**	92.8±3.1	102.3±7.5	186.5±14.3	31.2±2.5	60.6± 5.3	40.9±0.6	0.8±0.1
n	35	30	32	37	32	35	35	35	30	27	35

ND, Not determined; Cl, chloride; Pi, phosphate

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ furosemide vs. control

lower body weight could be partly accounted for by the higher number of neonates in furosemide-exposed litters and partly by a volume depletion of neonates induced by the observed increase in diuresis.

Of particular interest are the hypernatremia and hyperkalemia observed in the furosemide-exposed group on PD1 and, to a lesser extent, on PD5. A water depletion is possible. However, after such a hypernatremia, the expected kidney response would be to excrete a minimal urinary volume and a large natriuresis. Conversely, the diuresis and the C_{Cr} were high, while Na^+ and chloride excretions and FE_{Na} were low and the plasma protein concentration was not elevated. Therefore, dehydration seems unlikely to be the only explanation.

It is known that chronic exposure to furosemide results in an increase in size and function of the cortical distal tubule in adults [14], which reduces the drug's effect. A similar adaptation of the nephron can be seen when Doca (desoxycorticosterone acetate) is chronically administered: the hormone-induced alteration in Na^+ excretion in the cortical distal tubule is hidden by the "escape phenomenon." Thus, in our series, the renal electrolyte handling could have been modified in the same fashion. We suggest that furosemide-exposed neonates had a delay in the maturation of the loop of Henle at birth, due to a direct chronic drug effect, together with secondary stimulation of proximal and distal tubular functions over a long period covering organogenesis and the nephrogenesis [14–19]. Accordingly, the FE_{Na} was low at birth. This, along with the continual absorption of amniotic fluid, close in composition to saline, could explain an electrolyte gain.

Morphometric investigations in our laboratory have shown a decrease in the number of glomeruli per volume unit of renal cortex after in utero furosemide treatment [10, 20]. This is in accordance with the hypothesis of a tubule adaptation to long exposure to furosemide: the resulting increase in tubular volume would decrease the number of glomeruli per unit volume of cortex, while the absolute number of glomeruli in the kidney could remain unmodified. This could result in altered rates of maturation for glomerular and tubular functions, causing an abnormal glomerulo-tubular balance [15, 18, 21], and, thus, an electrolyte imbalance.

In conclusion, after in utero exposure to furosemide, rat neonates had impaired renal functional development. A delay in the maturation of Henle's loop is likely; the ensuing proximal and distal tubular adaptations could account for the apparent decrease in the number of glomeruli reported earlier and the modifications of body weight, diuresis, and electrolyte handling reported here. Twelve days later, after nephrogenesis was achieved, these functional alterations were partly corrected.

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