Development of Renal Potassium Excretion Capacity in the Neonatal Rat

Naohiko ANZAI, Yoshiro SUZUKI, Mariko NISHIKITANI, Ibuki IZUMIDA-MORIGUCHI, Asako KÔKUBO, and Katsumasa KAWAHARA

Department of Physiology, Kitasato University School of Medicine, Sagamihara, 228-8555 Japan

Abstract: We investigated the capacity of newborn rats to excrete an acute potassium load to understand the development of a renal potassium excretion system. Three groups of the rats (7–14 d) were used to collect urine periodically over 6 h after oral infusion of potassium: control (no potassium loading) and low- and high-potassium-loaded rats. In the low-potassium-loaded group, infused with about 0.6 μEq of potassium chloride/g body wt., the rate of renal potassium excretion increased from 0.08±0.02 (7 d) to 0.13±0.02 (10 d) and 0.21±0.03 (14 d) μEq/h/g body wt. The high-potassium-loaded rats (1.5–2.8 μEq/g body wt. potassium load) excreted potassium at a higher rate of 0.18±0.05 (7 d), 0.30±0.02 (10 d), and 0.45±0.10 (14 d) μEq/h/g body wt. They excreted 77% (7 d), 76% (10 d), and 95% (14 d) of the potassium load. These values were much larger than the rate of 0.026 μEq/h/g body wt. of the control rats and of 0.08 μEq/h/g body wt., a mean potassium excretion rate during development from 7 to 14 d calculated from the data in the previous study (Kanno T et al.: J. Pediatr. Gastr. Nutr. 24: 242–252, 1997). In the same period, serum potassium concentration in the newborn rats decreased significantly (p<0.01) from 7.2±0.1 (7 d) to 6.7±0.1 mEq/l (14 d). All these results suggest that a renal potassium excretion system in the rat develops at least in the second week of life, and its capacity is high enough to excrete the daily potassium intake. [Japanese Journal of Physiology, 51, 745–752, 2001]

Key words: newborn rats, urine flow, renal sodium excretion, renal potassium excretion, serum potassium concentration.

Potassium is the principal cation in the intracellular fluid (ICF) and comprises a small fraction, about 2%, of the extracellular fluid (ECF), including blood plasma [1]. Plasma potassium concentration (PK) is normally regulated in narrow ranges (3.5–5 mEq/l) by the kidney [2]. However, PK is higher in the newborn animals [3, 4] and infants [5, 6]. High PK in the newborns is probably the result of potassium imbalance between intake and output and/or inappropriate potassium distribution between ICF and ECF. In healthy newborn animals, potassium imbalance is primarily due to the immaturity of renal potassium excretion [7, 8]. Satlin [7] demonstrated that in the rabbit CCDs, net potassium secretion was absent in the first 3 weeks of life and became evident at 4 weeks of age. It is also reported that the renal potassium excretion capacity is lower in the newborn dog (0.87±0.09 μEq/min/g kidney wt.) than in the adult (3.06±0.24 μEq/min/g kidney wt.) [9]. On the other hand, according to a study of extremely low-birth-weight infants (ELBW) [10], hyperkalemia was not associated with the renal dysfunction, but with a shift of potassium from the ICF to the ECF, probably resulting from low activity of Na⁺/K⁺ ATPase in the cell [11, 12]. This was because the extraordinarily high concentration of plasma potassium in ELBW infants decreased toward the nor-
nal level after the onset of physiologic diuresis in the first 2–3 d of life [10]. Moreover, numerous papers have reported that newborn animals, such as infants [3], piglets [13], and dogs [9] can excrete the exogenous potassium load. Thus high PK in the newborn animals may not be abnormal, but it may be the result of a physiological state of positive potassium balance for growth. In a study of 1-week-old human neonates with a gestational period of 30–41 weeks [14], PK decreased from 6.5±0.5 mEq/l (30–32 weeks) to 5.1±0.2 mEq/l (39–41 weeks), whereas the potassium balance did not change significantly. Furthermore, the fetus plasma potassium is known to be higher than the maternal plasma potassium concentration, which is maintained by an active transport of potassium across the placenta [15, 16].

The purpose of this study, therefore, was to quantify and to characterize the renal responses of the newborn rats to an exogenous potassium load. In adult animals, the continuous intravenous infusion of KCl solution can be used to study the renal potassium excretion rate. However, a much less invasive oral infusion was also reliable and useful, particularly for pediatric studies. We examined time-dependent changes of the renal potassium excretion after an oral infusion of potassium. We found that the rate of renal potassium excretion was very low at the condition of no-extra potassium loading, but it quickly increased in response to the exogenous potassium load. We also investigated potassium balance at the condition of low- and high-potassium loading. The renal potassium excretion capacity of the newborn rat was high enough to excrete daily potassium intake.

**METHODS**

**General procedure.** According to Guiding Principles of Animal Experiments in Kitasato University School of Medicine (1998), studies were performed on newborn Wistar rats of both sexes at 7, 10, and 14 d of life. The newborn rats were isolated from their mothers at about noon on the day of the experiments (4 h before the beginning of potassium loading; time −4). They were placed singly into small plastic boxes lined with paper, which were warmed to 30–31°C [17]. Three groups at each age were used for a determination of the renal potassium excretion capacity. The renal response to excrete potassium may be affected by the developmental change in the intestinal absorption of potassium, but it was not considered in the present study.

**No-potassium-loaded rats.** The purpose of this protocol was to determine the basal potassium excretion rate by the kidney under no-extra-potassium loading conditions. Newborn rats were infused only with some amounts of a 5% glucose solution at time 0 and after every urine collection during the period of experiments. To avoid the effects of breast milk in the stomach, we examined the potassium excretion rate in the two phases: early phase (between time −4 and 0, n = 7–8 in each age) and late phase (between time 0 and 6, n = 2–4).

**Low-potassium-loaded rats.** The purpose of this protocol was to examine the renal responses of the newborn rats to the exogenous potassium load. The newborn rats in each age (n=4×3) were orally infused with a 5% glucose solution containing 0.1 M potassium chloride (KCl) 4 h after removal from their mother (time 0). The total potassium intake in each rat was determined by the product of increase in body weight (=KCl fluid intake) and the concentration of KCl (0.1 M). An infusion of Group 1 contained about a 1/10th part potassium in the daily milk [18]. The rats were also infused with some amounts of a 5% glucose solution (no potassium) after every urine collection not to be dehydrated.

**High-potassium-loaded rats (n=4 in each age).** The purpose of this protocol was to determine the maximum potassium excretion capacity by the newborn rat kidney. The oral infusion of potassium was 2–5 times higher in this group than in the low-potassium-loaded group for the same age. These rats were also infused with a 5% glucose solution after every urine collection.

**Collection of urine.** The method of urine collection was identical to the previous study [17]. Briefly, urine was collected from the external urethra by prodding the suprapubic area with a cotton stick. The weighed values (mg) of urine were used for analysis as volume (μl) without modification as described previously [17].

**Collection of blood.** Separately, 12 individuals for the control neonates were lightly anesthetized with ether and decapitated. The blood (0.3–0.8 ml) was directly collected from the neck stumps without heparin. This method of collection minimized the problem of hemolysis in the case of small newborn rats. Blood samples were centrifuged for 3 min at 6,000 rpm within 20 min after collection. The supernatant (serum) was stored in a refrigerator (4°C) until measurement.

**Electrolytes.** The electrolyte concentrations of serum and urine were measured with an automated electrolyte (Na+, K+, and Cl−) analyzer (Radiometer ABL505, Copenhagen). The renal potassium excre-
Development of Renal K⁺ Excretion System

RESULTS

Our first set of studies examined the serum potassium concentration in newborn rats. As was expected, it was higher than that of the adult [2], but more important, it decreased significantly from 7.2±0.1 (7 d, n=6) to 6.7±0.1 mEq/l (14 d, n=6) (p<0.01). Next, the renal potassium excretion rate was examined in the control rats (no-potassium loading). The potassium excretion rate between time −4 and 0 was significantly (p<0.001) higher in the 14-d-old rat than in the younger rats; however, that of between time 0 and 6 revealed no significant differences (p>0.05) (Fig. 1). Furthermore, the potassium excretion rate in the 14-d-old rat was significantly (p<0.05) higher in the early phase (between time −4 and 0) compared with the late phase (between time 0 and 6). Thus the potassium excretion rates in the late phase were adopted as the basal potassium excretion rate during the no-extra-potassium loading.

We examined the renal potassium excretion capacity in response to an acute potassium load. The 7- to 14-d-old newborn rats were orally infused with 5% glucose solutions containing either 0.1 (low-potassium loading) or 0.5 M KCl (high-potassium loading) at time 0. The mean values of potassium intake were 0.67±0.012 (7 d), 0.52±0.02 (10 d), and 0.61±0.02 μEq/g body wt. (14 d) for the low-potassium loading, whereas they were 1.47±0.21 (7 d), 2.39±0.14 (10 d), and 2.81±0.27 μEq/g body wt. (14 d) for the high-potassium loading. After potassium loading, the mean potassium excretion rate increased significantly at the first collection in all newborn rats (top in Fig. 2). Subsequently, the rate of renal potassium excretion gradually decreased after the peak. But, exceptionally, it slowly increased during the period of 6 h in the low-potassium-loaded rats were 10 d old. The mean potassium excretion was significantly higher in the high-potassium-loaded rats than in the low-potassium-loaded rats of the same age, and the rise was steeper in the eldest of the high-potassium-loaded rats. On the other hand, the mean sodium excretion was low and did not increase significantly through the experiments (middle in Fig. 2). It was not intended, but the mean urine flow rates significantly changed during the period of 6 h in the low-potassium-loaded rats (low- and high-potassium-loaded groups) and in the 14-d-old rats (high-potassium-loaded group) (bottom in Fig. 2). The effects of changes in urine flow on the renal potassium excretion will be discussed.

Potassium balance during the entire 6-h experiment was examined in the newborn rats of 7, 10, and 14 d. At time 0, they were acutely infused with either about 0.6 μEq of potassium chloride/g body wt. (low-potassium loaded) or 2–5 times more potassium (high-potassium loaded). In the low-potassium-loaded group, the 14-d-old rats unexpectedly excreted more potassium than they took in (negative potassium balance) (Fig. 3). On the other hand, the high-potassium-loaded rats, infused with 1.5–2.8 μEq/g body wt., excreted less potassium than their intake (positive potas-
They excreted 77% (7 d), 76% (10 d), and 95% (14 d) of the potassium load.

Fig. 2. Time-dependent changes in the renal potassium and sodium excretion and urine flow in the newborn rats of 7, 10, and 14 d (low- and high-K\(^+\) loading). * and ** indicate that the potassium excretion was significantly (p<0.05 and 0.01, respectively) higher than that of the control (at time 0) in the same group of each age. † and †† indicate significant differences between low- and high-K\(^+\)-loaded rats (p<0.05 and 0.01, respectively).

Fig. 3. Potassium balance in the low- and high-potassium-loaded rats of 7–14 d. Left (open) and right (hatched) columns indicate the potassium intake and sum of the urinary potassium excretion (6 h), respectively. Positive and negative potassium balances are indicated as upward and downward, respectively, by the filled boxes. The low-potassium-loaded rats excreted potassium of 0.53±0.07 (7 d), 0.78±0.11 (10 d), and 1.27±0.10 μEq/g body wt. (14 d) in the first 6 h, whereas the high-potassium-loaded rats excreted 1.08±0.13 (7 d), 1.80±0.08 (10 d), and 2.67±0.35 μEq/g body wt. (14 d). * and ** p<0.05 and 0.01, respectively; indicate a significant difference between the potassium intake and potassium excretion in the same age groups.

Fig. 4. The total potassium excretion by kidney during the period of 6 h in relation to the potassium load for the same age. Circles, triangles, and squares indicate 7-, 10-, and 14-d-old rats, respectively. Data from the low- and high-potassium-loaded rats are expressed as open and filled symbols, respectively. Regression lines are y=0.54x+0.24 (r=0.79, p=0.017; 7 d); y=0.53x+0.53 (r=0.94, p<0.001; 10 d); and y=0.69x+0.81 (r=0.94, p<0.001; 14 d).
renal potassium excretion capacity (6 h) and the potassium load of the individual newborn rats of 7–14 d. Regression lines calculated by using the least square method in each age group indicate that (1) the renal potassium excretion rate increased linearly in proportion to the increase in the potassium load (Pearson’s correlation analysis). (2) The value of renal potassium excretion of the 14-d-old rat was significantly higher than those of the 7- and 10-d-old rats (p<0.01). (3) Extrapolated values of the total potassium excretion during the period of 6 h at “zero potassium loading” were 0.04 (7 d), 0.09 (10 d), and 0.14 (14 d) μEq/g body wt., which were larger than those of the basal potassium excretion rate of 0.023–0.031 μEq/h/g body wt. in the 7- to 14-d-old rats.

**DISCUSSION**

**Renal potassium excretion.** The present study was designed to characterize the renal responses of the newborn rats to the exogenous potassium load and to evaluate the postnatal development of a potassium excretion system. The renal potassium excretion rate in the newborn rats of 7–14 d was low in the late phase (between time 0 and 6) of no-extra-potassium-loaded rats and did not significantly change with age (0.023–0.031 μEq/h/g body wt.; ANOVA, p>0.05). These values are comparable with those of newborn dogs (0.015 μEq/h/g body wt.) [9] and of low-birthweight infants (0.038 μEq/h/g body wt.) [10]. This further indicates that kidney tubules in newborn rats reabsorb almost all filtrated potassium in spite of high PK, when potassium supplement was absent in stomach and intestine. On the other hand, the renal potassium excretion rates increased quickly in response to an oral infusion of potassium and showed significant increases with age. As expected, the potassium excretion rates in the potassium-loaded groups were much higher than those of the minimum potassium excretion for the same age determined in the no-potassium-loaded group. Our findings are consistent with previous studies presenting that under certain conditions, i.e., after dietary potassium loading, the healthy premature infants can excrete potassium at a higher rate than it is filtered [3] and that piglets in the first day of life could excrete most potassium chloride (KCl) given by stomach tube [13]. In the present study, the renal potassium excretion rate quickly changed in response to the oral potassium load and the elder rats could excrete more potassium within a shorter period. The observed renal K+ excretion rate may be affected by the developmental change in potassium absorption across the intestine of the newborn rats.

In the present study we attempted to maintain consistent urine output through the experiments by giving appropriate amounts of a 5% glucose solution to the rats; however, the mean urine flow rates unexpectedly changed in several groups, i.e., the potassium-loaded rats 10 d old and the high-potassium-loaded rats 14 d old (bottom in Fig. 2). This suggests that glomerular filtration rate (GFR) could not be maintained constant. However, GFR is not a major factor affecting the renal potassium secretion because 90% of the filtered potassium is absorbed by both proximal tubule and Henle’s loop in adult animals [21]. Although flow-dependent potassium excretion has not yet been demonstrated in newborn animals [22], a part of potassium excretion in the 10- and 14-d-old rats may be stimulated by the increase in urine flow or the converse may be true. It is possible that a sudden and large excretion of potassium will result in increased urine flow because of osmotic diuresis.

**Potassium balance.** Lorenz et al. [10] showed that hyperkalemia in ELBW was not associated with the renal dysfunction, but with a shift of potassium from the ICF to the ECF. This idea is good to explain a transient increase in PK without potassium intake, but it cannot explain the continuous high PK during the newborn period. In the present study serum potassium concentration in the newborn rats significantly decreased from 7.2±0.1 (7 d) to 6.7±0.1 (14 d) mEq/l (p<0.01), but it was still higher than the control level of the adults [2]. Thus the renal potassium excretion capacity was directly compared with an obligatory potassium excretion (OPE), which is defined as the difference between daily potassium intake and estimated potassium accumulation in the ICF (APPENDIX). This is because in the younger rats, most potassium in milk was accumulated in the intracellular fluid (ICF), which may be undergoing rapid growth [13]. The changes in potassium content in the ECF were assumed to be negligible because more than 98% of body potassium is accumulated in the cells (ICF) [1]. The values, such as daily potassium intake of 5.9–6.2 μEq/g body wt., were cited from the data of Table 1 and Fig. 1 presented by Kanno et al. [18]. Furthermore, we assumed that kidneys in the newborn rats also excrete 90% of potassium intake [21] and that the intracellular potassium concentration of newborn rats is similar to that of newborn rabbits (120 mEq/l) [23]. After we considered the positive potassium balance in this period, the estimated OPE was 0.08 μEq/h/g body wt. (0.9×300 μEq/7 d/20 g body wt.). Consequently, only a small part of the potassium load is excreted into the urine during the newborn period. We realized that the renal potassium excretion rate of the 7- to 14-
d rats in the present study (0.2–0.45 μEq/h/g body wt.) was much higher than the estimated OPE in the same period. Thus we conclude that the continuous high PK in the newborns is not due to the low renal potassium excretion capacity.

It is strange, but negative potassium balance was observed in the low-potassium-loaded 10- and 14-d-old rats (Fig. 3, left panel). We speculate that the rate of renal potassium excretion may be overstimulated after an oral infusion of potassium. It is known that in the hours following meals or ingestion or infusion of supplementary potassium salts, the rate of urinary excretion of potassium can increase greatly to approach or even exceed the rate at which it is filtered [21].

**Tubular potassium excretion.** Because of the generalization that filtered potassium is largely reabsorbed by both proximal tubule segments and Henle’s loop and that excreted potassium is secreted by distal nephron segments, including collecting ducts [21], renal dysfunction to excrete potassium can be attributed to the immaturity of distal tubule functions. The kidney in mammalian newborns is immature in anatomy [23, 24] and function [7]. Satlin [7] showed in vitro no active potassium transport in rabbit cortical-collecting ducts (CCD) before 4 weeks of life. She also reported that there is little chance of single K+ (ROMK) channels in the CCD preparation from 1-week-old rabbit kidney [8]. Her results can account for low renal potassium excretion ability in the neonates. In contrast, principal cells of the CCD have been identified in the midcortex and inner cortex of 2-d-old rabbits [24]. Furthermore, a clearance study in potassium-loaded dogs provided evidence that amiloride-sensitive potassium secretion (equal to distal potassium secretion) was identified in the newborn of 6–20 d of age (0.87 μEq/min/g kidney wt.), though the ability was much smaller than that of the adult (3.1 μEq/min/g kidney wt.; \( p<0.05 \)) [9]. These results, inconsistent with Satlin [7], suggest that newborn rats may develop an alternative potassium excretion system to maintain PK. Our observations are consistent with the previous studies of Evan et al. [24] and Lorenz et al. [9]. First, we have shown that renal potassium excretion rates increased and decreased immediately in response to the exogenous potassium load (top in Fig. 2). The increase and decrease in potassium excretion was not simply related to the change in urine flow rate. Furthermore, increases in potassium excretion were much larger than those in urine flow rate. Second, the relationships between potassium excretion and potassium load in each age showed no saturation kinetics, but they were linear (Fig. 4). This suggests that potassium transport through kidney tubules is mediated by an ionic channel-like process, at least in our experimental conditions. Third, it is unlikely that potassium was transported through a paracellular shunt pathway against high potassium concentrations in lumen. In the high-potassium-loaded rats, potassium concentrations in urine increased from 20.7±6.2 to 71.0±12.9 (7 d), from 13.5±1.4 to 132.6±20.2 (10 d), and from 88.3±13.2 to 177±7.5 mEq/l (14 d). All these results are consistent with the characteristics of a tubular (cellular) potassium secretion system and support the idea that an alternative transport system develops early on the neonate rat kidney, prior to the observed active potassium transport system [7].

**Development of potassium excretion system.** Although the transcellular shifting of potassium is an efficient mechanism to reduce serum potassium even in newborns because the number and total volume of the cells increase during development, potassium must be excreted by the kidney to maintain external balance [25]. Thus a decrease in serum potassium during the period of 2-week-old rats indicates that the kidney may function to reduce serum potassium. When does the newborn rat start to excrete the surplus potassium? Figure 5 illustrates the development of the minimum and regulatory excretion of potassium by newborn rat kidneys. When the plots are linearly extrapolated, the minimum potassium excretion is positive at day 0 and the regulatory potassium excretion capacity crosses the x-axis at 1.7 d. This suggests that the onset of the extra potassium excretion ability in the immature kidney may be as early as...
the second day of life. The result is consistent with the observation [10] that the mean plasma potassium concentration in ELBW infants decreased during the 2–3 d after birth when physiological diuresis begins.

In summary, the maximum renal potassium excretion capacity determined in newborn rats of 7–14 d suggests that they can excrete the daily potassium intake contained in milk. The newly developing potassium excretion system in the early newborn periods has a channel-like property based on the relationship between renal potassium excretion and potassium load. Further research will serve to corroborate this hypothesis.

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REFERENCES


APPENDIX

According to the results [18], mean body weights of the 7- and 14-d rats are 14.8 and 25.8 g, respectively. An estimated accumulation in intracellular fluid be-
between the two age groups is 4.62 ml (11 g of body weight change × 0.7 × 0.6), where water content is assumed to be 70% of body weight and intracellular fluid is assumed to be 60% of the body fluid. Increased potassium in the cell is calculated from an equation of 120 (mEq/l) × 0.00462 (l) = 554 (µEq). On the other hand, potassium intake for 7 d is 6.1 (µEq/g body weight) × 20 (g) × 7 (d) = 854 (µEq). Thus obligatory potassium excretion (OPE) for 7 d = 854 − 554 = 300 (µEq). This is translated into 0.09 µEq/h/g body wt. Since the kidney excretes 90% of dietary potassium intake [21], it excretes potassium at 0.08 µEq/h/g body wt.