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Effects of birth on energy metabolism in the rat kidney

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The oxygen-consumption rates and the activities of fumarase and β-hydroxyacyl-CoA dehydrogenase were compared in mitochondria isolated from fetal- and neonatal-rat kidney. Whole-organ ATP, phosphocreatine and creatine contents were determined in parallel. Kidney mitochondrial respiratory rates in the presence of succinate, glutamate/malate and palmitoyl-L-carnitine increased between 21 days *post coitum* and 1 day *post partum*, together with activities of oxidative enzymes. However, this postnatal maturation of oxidative metabolism was not yet initiated in mitochondria isolated from kidney 1 h *post partum*. An increase in ATP and phosphocreatine was observed immediately after delivery; newborn-rat kidney ATP content then remained high, whereas phosphocreatine reserves decreased considerably between 6 h and 1 day *post partum*. It is concluded that the increase in high-energy phosphate compounds observed at birth is not initially related to an activation of oxidative phosphorylation, and probably involves a transient stimulation of anaerobic glycolysis, while a progressive mitochondrial maturation takes place in the rat kidney during the first day of newborn life.

INTRODUCTION

In the developing rat kidney, some proximal functions such as glomerular filtration [1] or phlorrhizin-inhibited glucose reabsorption [2] develop between the fetal stages of 18 and 20 days *post coitum*. During the same prenatal period, biochemical markers of renal differentiation (Na^+/K^+-dependent ATPase, enzymes of gluconeogenesis and ammoniagenesis etc.) become detectable [3,4]. By the end of gestation, however, the rat kidney is still structurally very immature: only the juxtamedullary nephrons are well developed, and peripheral to these lies an active nephrogenic zone in which new tubules differentiate up to 1 week after birth. Because of this immaturity, it is generally admitted that the kidney of the rat fetus plays a minor role in the control of acid/base and hydromineral homoeostasis.

Birth therefore represents a critical period of development, since, after the interruption of the maternal relation, the kidneys must take charge of these homoeostatic functions. Such an activation of kidney functions is likely to require an enhanced energy supply, and, consequently, an adaptation of energy metabolism. In addition, changes in kidney metabolism can also be expected in response to the increase in pO_2, and to the modifications of nutritional status after birth. Nevertheless, little information is available on the adaptation of kidney energy metabolism to postnatal life. In previous studies [5,6], we have shown that birth induces a stimulation of ATP production in the rat kidney: within the first hour of extra-uterine life, the whole-organ ATP content, ATP/ADP ratio and energy charge (ATP+0.5ADP)/(ATP+ADP+AMP) values increase significantly. Using adrenoreceptor agonists and antagonists, we have demonstrated [6] the role of catecholamines as a triggering factor of these modifications. In the present work, we studied the nature of metabolic pathways involved in this neonatal increase of kidney ATP production.

Preliminary data obtained in our laboratory [7] suggested an increase in kidney oxidative phosphorylation during the early postnatal period. To determine whether this could be involved in the quick rise in energy charge observed at birth, we have studied the evolution of respiratory capacities in isolated kidney mitochondria between the stages of 21 days *post coitum* and 1 h *post partum*, and compared them with values 1 day *post partum*. In addition, the activity of two oxidative enzymes, fumarase and β-hydroxyacyl-CoA dehydrogenase, was measured in the purified mitochondria at the same stages.

On the other hand, we have studied the evolution of kidney phosphocreatine and creatine contents during the perinatal period: the mobilization of phosphocreatine reserves is known to be a quick way to produce ATP in the kidney and other tissues during critical situations [8]; it was then conceivable that such a mobilization was involved in the increase in ATP level and energy charge observed at birth.

EXPERIMENTAL

Chemicals

All reagents used were of analytical grade. Respiratory substrates were from Sigma (St. Louis, MO, U.S.A.). Reagents used in fluorimetric assays were from Sigma or Boehringer–Mannheim.

Animals

Pregnant Wistar rats were bred and mated in our laboratory as previously described [4]. They had free access to food (commercial diet UAR 113; UAR, Villemoisson sur Orge, France) and water. Fetuses were delivered by caesarian section, after cervical dislocation of the mother, in the morning of day 21 *post coitum*. The 1 h-*post-partum* newborns were obtained as described in [6]; 6 h- and 1-day-*post-partum* newborns were spontaneously delivered animals; they were killed by cervical dislocation. Developmental stages were monitored by

Table 1. Comparison of respiratory functions in mitochondria isolated from fetal- or neonatal-rat kidney, incubated in the presence of succinate or glutamate/malate

Respiratory rates were measured polarographically and are expressed as ng-atoms of O/min per mg of protein, as described in the Experimental section. Succinate and glutamate/malate concentrations were 5 mM and 5 mM + 5 mM respectively. State-3 respiration was determined after addition of 310 nmol of ADP. ΔO_2 = State 3 − State 4′. For uncoupled respiration 2,4-dinitrophenol was added (final concn. 35 μM). Each value represents the mean ± S.E.M. for n experiments. Significance of difference from 21-days-*post-coitum* fetus was calculated by Student's t test: $*P \leqslant 0.05$; $**P \leqslant 0.01$; $***P \leqslant 0.001$.

		Stage of development		
		Fetus 21 days *post coitum* (n = 8)	Newborn 1 h *post partum* (n = 9)	Newborn 1 day *post partum* (n = 11)
State-4′ respiration	Succinate	60.4 ± 8.1	63.9 ± 7.9	87.5 ± 6.0
	Glutamate/malate	29.9 ± 3.1	39.8 ± 8.5	44.2 ± 4.5
State-3 respiration	Succinate	208.3 ± 19.6	215.4 ± 20.0	286.7 ± 19.1*
	Glutamate/malate	134.2 ± 15.9	155.1 ± 8.9	217.7 ± 22.1*
ΔO_2	Succinate	147.9 ± 13.6	146.0 ± 15.9	199.6 ± 14.6*
	Glutamate/malate	104.8 ± 13.7	115.3 ± 21.6	173.0 ± 18.2**
Uncoupled respiration	Succinate	181.6 ± 18.5	200.9 ± 27.0	279.0 ± 21.2*
	Glutamate/malate	141.5 ± 18.8	151.4 ± 31.9	168.5 ± 17.0
Respiratory control ratio	Succinate	3.6 ± 0.1	3.3 ± 0.2	3.5 ± 0.2
	Glutamate/malate	4.6 ± 0.4	3.8 ± 0.5	5.0 ± 0.2
ADP/O	Succinate	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
	Glutamate/malate	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1

weighing the fetuses. Kidneys were quickly removed and either transferred to ice-cold 0.9% NaCl for further isolation of mitochondria, or immediately plunged into liquid N_2 and stored at −80 °C until enzyme determinations.

Isolation of mitochondria

The procedure was adapted from that of Aprille & Asimakis [9]: 1.5–2 g wet wt. of tissue (40–60 kidneys) was homogenized in 20 ml of a medium containing 70 mM-sucrose, 220 mM-mannitol, 0.1 mM-EDTA and 2 mM-Hepes, adjusted at pH 7.4. The supernatant obtained after low-speed centrifugation (600 ***g***, 10 min, 0 °C) of this homogenate was centrifuged at 8000 ***g*** (10 min, 0 °C). The pellet obtained was washed twice in 20 ml of homogenizing medium and finally suspended in the same medium to about 20 mg/ml. Protein was determined by the Lowry [10] method, with crystalline bovine serum albumin fraction V (Sigma) as a standard.

Measurement of mitochondrial respiration

O_2 consumption was measured using a Clark O_2 electrode (Gilson Oxygraph; Middleton, WI, U.S.A.) in a 2 ml chamber thermostatically maintained at 30 °C. The mitochondrial O_2 consumption in the presence of succinate or glutamate/malate was determined as follows: the respiratory medium contained 20 mM-K_2HPO_4/KH_2PO_4, 225 mM-sucrose, 10 mM-KCl, 1 mM-EDTA, 5 mM-$MgCl_2$, 10 mM-Tris/HCl, pH 7.4, and a 10–50 μl portion of purified mitochondria. State-4 respiration was measured after addition of succinate (final concn. 5 mM) or glutamate (5 mM) + malate (5 mM). Then 310 nmol of ADP was added, and State-3 respiration was recorded. Finally, the respiratory rate after depletion of the added ADP, i.e. State-4′ respiration, was also measured. These O_2-consumption rates were expressed in ng-atoms of O/min per mg of mitochondrial protein. The respiratory control ratio, ADP/O and net rate of O_2 consumption (ΔO_2 = State 3 − State 4′) were calculated. The addition of 2,4-dinitrophenol (final concn. 35 μM) was used to determine the uncoupled respiration rate.

Experiments with palmitoyl-L-carnitine as respiratory substrate were performed in the medium described by Osmundsen & Sherratt [11], which includes 1 mM-ADP and 2.5 mM-malate. After addition of 10–50 μl of purified mitochondria, O_2 consumption was first recorded in the absence of palmitoyl-L-carnitine (termed the 'resting state'); this was then added in limited amount (10 nmol), and the rapid rate of O_2 consumption that ensued ('active state') was recorded. Finally, after exhaustion of added palmitoyl-L-carnitine, mitochondrial O_2 consumption returned to resting-state values. The difference between the active and the resting state (ΔO_2) was calculated. Under these conditions, the mitochondrial rate of palmitate utilization (Δpalmitate, expressed in nmol/min per mg of protein) and the amount of O_2 consumed per mol of fatty acid oxidized (ΔO_2/Δpalmitate) could be calculated.

Tissue homogenates and extracts

Homogenates were prepared from two kidneys of the same animal as 4% (w/v) suspension in ice-cold 6.8 M-glycerol/60 mM-Na_2HPO_4/NaH_2PO_4, pH 7.4, with a motor-driven Teflon/glass homogenizer. $HClO_4$ tissue extracts were made as described in [6]. Fumarase (EC 4.2.1.2) and β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) activities were assayed fluorimetrically at 20–22 °C by the Burch *et al.* [12] method. Phosphocreatine, creatine and ATP were determined in neutralized tissue extracts as described by Lowry & Passonneau [13].

Table 2. Comparison of respiratory functions of mitochondria isolated from fetal- or neonatal-rat kidney, incubated in the presence of palmitoyl-L-carnitine

Respiratory rates were measured polarographically and are expressed as ng-atoms of O/min per mg of protein, as described in the Experimental section. For the 'active' state, O_2-consumption rate was measured in the presence of 1 mM-ADP + 5 μM-palmitoyl-L-carnitine; for the 'resting' state, palmitoyl-L-carnitine was omitted. ΔO_2 = active state − resting state. ΔPalmitate is the rate of palmitate oxidation under active-state conditions, expressed in nmol/min per mg of protein. 35 μM). Each value represents the mean ± S.E.M. for n experiments. Significance of difference from 21-days-*post-coitum* fetus was calculated by Student's t test: $*P \leqslant 0.05$; $**P \leqslant 0.01$; $***P \leqslant 0.001$.

	Stage of development		
	Fetus 21 days *post coitum* ($n = 9$)	Newborn 1 h *post partum* ($n = 4$)	Newborn 1 day *post partum* ($n = 5$)
Active state	103.2 ± 5.6	90.2 ± 10.4	144.8 ± 10.00**
Resting state	40.2 ± 2.4	37.4 ± 4.6	55.9 ± 6.3
ΔO_2	63.0 ± 3.9	52.8 ± 6.2	90.0 ± 5.4***
ΔPalmitate	2.8 ± 0.2	2.3 ± 0.3	4.0 ± 0.2***
ΔO_2/Δpalmitate	22.3 ± 0.8	22.6 ± 0.5	22.8 ± 1.0

RESULTS

Evolution of mitochondrial respiration

No changes in kidney mitochondrial respiratory functions were noted between 21 days *post coitum* and 1 h *post partum*, whatever the respiratory substrate used (Tables 1 and 2). By contrast, between 1 h and 1 day *post partum*, the maximum rates of O_2 consumption (State 3, Table 1; active state, Table 2) increased significantly. The relative increase in State-3 respiration between the two newborn stages was 33% in the presence of succinate as respiratory substrate, and 40% in the presence of glutamate/malate (Table 1). When palmitoyl-L-carnitine was used, a 60% rise in active-state respiration was observed during the same developmental period (Table 2). The basal rate of O_2 consumption (State 4', Table 1; resting state, Table 2) was higher in mitochondria 1 day *post partum* incubated in the presence of succinate or palmitoyl-L-carnitine: the increase was proportionally comparable with that observed for maximum respiration-rate values. Therefore, with all substrates tested, a significant increase in net O_2-consumption rates (ΔO_2; Tables 1 and 2) was observed in kidney mitochondria between 1 h and 1 day *post partum*.

These developmental changes in mitochondrial respiration occurred without variation in the ADP/O ratio, which showed the expected values for succinate and glutamate (Table 1). Neither did respiratory control ratios change significantly (Table 1). The rate of O_2 consumption in the presence of 2,4-dinitrophenol was comparable with State-3 respiration at all stages considered (Table 1). The oxygen/palmitate ratio (ΔO_2/Δpalmitate) was close to 22 during all the perinatal period (Table 2), reflecting a complete β-oxidation of palmitate to acetyl-CoA, followed by oxidation of this product to CO_2.

Table 3. Evolution of fumarase and β-hydroxyacyl-CoA dehydrogenase activities in kidney mitochondria during the perinatal period

Activities were assayed fluorimetrically at 20–22 °C as described in the Experimental section. They are expressed in μmol/h per mg of protein. Each value represents the mean ± S.E.M. for n experiments. Significance of difference from 21-days-*post-coitum* fetus was calculated by Student's t test: $**P \leqslant 0.01$.

	Stage of development		
	Fetus 21 days *post coitum* ($n = 5$)	Newborn 1 h *post partum* ($n = 5$)	Newborn 1 day *post partum* ($n = 4$)
Fumarase	13.5 ± 1.1	17.5 ± 1.6	24.0 ± 2.4**
β-Hydroxyacyl-CoA dehydrogenase	46.6 ± 7.2	49.5 ± 6.8	76.6 ± 5.9**

Fumarase and β-hydroxyacyl-CoA dehydrogenase activities

Fumarase activity in kidney mitochondria increased steadily during the perinatal period (Table 3). The overall difference between the fetus 21 days *post coitum* and the 1 newborn day *post partum* (+78%) was highly significant ($P < 0.01$). The differences between 21 days *post coitum* and 1 h *post partum*, or between 1 h and 1 day *post partum*, were not ($0.1 > P > 0.05$ and $P = 0.05$ respectively). β-Hydroxyacyl-CoA dehydrogenase activity was unchanged between 21 days *post coitum* and 1 h *post partum*, and increased markedly (+45%; $P < 0.05$) at 1 day *post partum*.

Phosphocreatine, creatine and ATP (Table 4)

Kidney phosphocreatine concentration was very low in the fetus 21 days *post coitum* (1.0 ± 0.4 μmol/g of protein), a value below the AMP content at this stage [5]. Birth clearly induced a quick increase in phosphocreatine content, which reached 6.1 ± 1.1 μmol/g of protein in the newborn (1 h *post partum*) kidney. As previously reported [5,6], kidney ATP content increased by 38% during the same period. There was a slight decrease in creatine content between the fetal and 1 h-*post-partum* stages (from 59.2 to 50.5 μmol/mg of protein; $P = 0.05$). Between 1 h and 6 h *post partum*, the phosphocreatine concentration remained constant, whereas that of creatine fell considerably (by 43%; $P < 0.001$); ATP concentration continued to increase during this period. At 1 day *post partum*, a significant decrease in kidney phosphocreatine was observed (1.6 ± 0.2 μmol/g of protein, versus 6.1 ± 0.4 at 6 h *post partum*), without change in creatine and ATP contents, compared with the earlier newborn stages. The pool of phosphocreatine per kidney, expressed in nmol, was 2.0 ± 0.5 at 21 days *post coitum*, 12.7 ± 0.5 at 1 h, 17.8 ± 1.4 at 6 h and 6.1 ± 0.9 at 1 day *post partum*.

Table 4. Evolution of rat kidney ATP, phosphocreatine and creatine contents during the perinatal period

ATP, phosphocreatine and creatine were determined fluorimetrically in kidney extracts, as described in the Experimental section. They are expressed in μmol/g of protein. Each value represents the mean $\pm$ S.E.M. for n experiments. Significance of difference from the preceding stage of development was calculated by Student's t test: $*P \leqslant 0.05$; $**P \leqslant 0.01$; $***P \leqslant 0.001$.

	Stage of development			
	Fetus 21 days *post coitum*	Newborn 1 h *post partum*	Newborn 6 h *post partum*	Newborn 1 day *post partum*
ATP	21.5±0.5 ($n = 12$)	29.4±0.5*** ($n = 15$)	32.6±0.9** ($n = 11$)	32.0±0.7 ($n = 12$)
Phosphocreatine	1.1±0.3 ($n = 12$)	6.1±0.3*** ($n = 15$)	6.0±0.4 ($n = 11$)	1.6±0.2*** ($n = 12$)
Creatine	59.2±3.7 ($n = 8$)	50.5±3.1* ($n = 8$)	28.4±1.2*** ($n = 8$)	26.2±1.5 ($n = 8$)

DISCUSSION

The results obtained from polarographic studies and enzyme determinations on isolated mitochondria are consistent with an increase in kidney oxidative metabolism during the first day of extrauterine life: the mitochondrial respiratory functions, and the activity of both oxidative enzymes studied, were significantly higher at 1 day *post partum* than at the fetal stage. Since all our data were obtained from purified mitochondria, and are expressed per mg of mitochondrial protein, the observed changes cannot be attributed to an increase in the kidney mitochondrial number during the perinatal period.

Our results clearly indicate that the postnatal increase in mitochondrial oxidative metabolism is not yet achieved at 1 h *post partum*, and therefore cannot be responsible for the increase in ATP and phosphocreatine observed between the fetal stage and 1 h. This adaptation of rat kidney energy metabolism to newborn life is, to some extent, comparable with that described in the liver [9,14–16], where an increase in ATP content and mitochondrial respiration is also known to occur soon after birth. However, compared with the liver, our results differ in several points, mainly: (*a*) there is no change in kidney mitochondrial respiratory control ratios between the fetal and newborn stages; (*b*) at the fetal stage, the uncoupled respiration rate of kidney mitochondria is not higher than the State-3 respiration; (*c*) the postnatal maturation of respiratory functions is not initiated immediately after delivery. These observations lead us to conclude that, in the newborn kidney, as opposed to the liver, the mitochondrial maturation demonstrated here cannot be attributed to major modifications in oxidative-phosphorylation coupling, or inner-membrane structure, induced by parturition. In support of this, electron-microscopic studies of the developing rat kidney [1] do not show changes in the structure of mitochondria during the perinatal period studied: at 21 days *post coitum*, the mitochondria of differentiated proximal cells are already morphologically comparable with the adult cells' organelles and, as in the mature cell, are arranged perpendicular to the tubule axis; at the newborn stage, little change occurs in the ultrastructure of the most differentiated nephrons.

We therefore suggest that a progressive increase in respiratory functions would take place in kidney mitochondria during the first day of newborn life. Comparison of palmitoyl-L-carnitine oxidation rates and β-hydroxyacyl-CoA dehydrogenase activity during the perinatal period shows a simultaneous increase in their values between 1 h and 1 day *post partum*. This enhanced enzyme activity is not related to changes in kinetics in our assay system, and could reflect a synthesis of β-hydroxyacyl-CoA dehydrogenase. Therefore, in addition to carnitine palmitoyltransferase, whose activity is very low at 21 days *post coitum* [17], the activity of other β-oxidation-pathway enzymes could limit palmitate oxidation capacities in the developing rat kidney.

The evolution of fumarase activity suggests a significant increase in the tricarboxylic acid-cycle activity between the fetal stage and 1 day *post partum*: this could, in part, be responsible for the maturation of mitochondrial respiratory functions. However, the postnatal development of mitochondrial respiration in the presence of succinate, a tricarboxylic acid-cycle intermediate, does not coincide with a significant rise in fumarase activity. Other determinations of tricarboxylic acid-cycle enzymes would be necessary to clarify the relation between development of respiratory functions and cycle maturation. It must also be noted that this postnatal development of kidney oxidative metabolism probably involves factors other than oxidative enzymes and, in particular, the activities of various mitochondrial metabolite transporters.

Our results indicate important variations of kidney phosphocreatine and creatine contents during the perinatal period, and especially during the first 1 h of newborn life, when phosphocreatine increases while creatine decreases. Both variations, when expressed in absolute values, are quite comparable, which strongly suggests that part of the creatine pool present at the time of delivery is immediately phosphorylated. In any case, between the fetal stage and 1 h *post partum*, the kidney phosphocreatine/creatine ratio changes from 0.02 to 0.12; a true charge in high-energy phosphate groups therefore occurs during the immediate postnatal period. Between 1 and 6 h *post partum* the phosphocreatine concentration does not change, and a simultaneous decrease in creatine is observed; consequently, the phosphocreatine/creatine ratio reaches its highest value

(0.21) at 6 h *post partum*. Afterwards, the phosphocreatine reserves are utilized, as indicated by the decrease in content and the fall of the phosphocreatine/creatine ratio, leading to a very low value at 1 day *post partum*.

In all adult tissues, the equilibrium constant for the interconversion of phosphocreatine (+ADP) into creatine (+ATP) strongly favours ATP formation. Consequently, it is admitted that accumulation of phosphocreatine can only occur when ATP production exceeds ATP utilization. Assuming that this is also true in the developing kidney, the simultaneous increase in kidney ATP and phosphocreatine during the first 1 h of newborn life therefore emphasizes the stimulation of energy metabolism triggered by parturition.

Mitochondrial oxidative metabolism is not initially involved in this increased production of high-energy phosphate compounds, as indicated by the results of polarographic studies on 1 h-*post-partum* mitochondria. As glycolytic activity is high in the fetal kidney [18,19], a stimulation of anaerobic glycolysis might then be responsible for those immediate changes in ATP and phosphocreatine. The fact that phosphocreatine synthesis slows down between 1 and 6 h *post partum*, and then stops completely, might reflect a progressive decrease of ATP production through anaerobic pathway(s), after birth. It can be speculated that the simultaneous development of mitochondrial oxidative metabolism during the first day of neonatal life would then be a necessary adaptation of kidney energy metabolism, to avoid a possible depletion of tissue ATP.

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