Perinatal maturation of rat kidney mitochondria

Béatrice PRIEUR,* Laurence CORDEAU-LOSSOUARN,† Agnès ROTIG,‡ Jeanne BISMUTH,* Jean-Pierre GELOSO* and Evelyne DELAVAL*§

*Laboratoire de Différenciation Fonctionnelle, INSERM U 319, Université Paris 7, tour 33-23, 1er étage, 2 Place Jussieu, 75251 Paris cedex 05, France, †Biochimie des Antigènes, Département immunologique, Institut Pasteur, Paris, France, and ‡INSERM U 12, Hôpital Necker, Paris, France

In the rat kidney, NaK-ATPase activity increased between days 19 and 20 of gestation (+50%) and between 1 and 24 h after birth (+20%), requiring an increased energy supply. In order to determine whether mitochondrial changes were involved, renal mitochondrial development was investigated from day 19 of gestation to 1 day after birth. Slot-blot analyses of mitochondrial-DNA/nuclear-DNA ratio and determination of citrate synthase activity showed a doubling in the mitochondrial pool between days 19 and 20 of gestation. In isolated mitochondria, oxygen consumption remained unchanged between days 19 and 20 of gestation, and then it was enhanced between days 20 and 21 of gestation (+70%) and between 1 and 24 h after birth (+50%). We also focused on one of the respiratory-chain complexes, ATP synthase, and measured its activity and content during the perinatal period. We demonstrated increases in both activity and content of ATP synthase between days 20 and 21 of gestation and between 1 and 24 h after birth, thus suggesting that changes in ATP synthase activity are ascribed to an increase in the mitochondrial density of ATP synthase complexes. Moreover, the mitochondrial ATP/ADP ratio only increased between 1 and 24 h (+90%), indicating a critical step in the renal respiratory-chain maturation at that time. We therefore conclude that the postnatal enhancement of renal mitochondrial oxidative capacity might depend on protein synthesis *de novo* and on changes in the adenine nucleotide concentrations.

INTRODUCTION

The late fetal period is particularly important for the development of the rat kidney, since this is the time when several major energy-requiring functions start. Glucose reabsorption initiates its development on day 19 of gestation [1], NaK-ATPase activity becomes detectable at the same stage [2], and renal gluconeogenesis and ammoniogenesis are first detected on day 20 of gestation [3]. Another critical period for energy metabolism demand takes place at birth, when the kidneys must quickly assume most of their regulatory functions.

In previous studies we demonstrated that the total nucleotide concentration in the rat kidney decreases during the late fetal period and that energy balance falls on the last day of gestation [4]. Birth is accompanied by increased ATP production, and the mitochondrial oxidative capacity expands during day 1 of extrauterine life [5]. However, little information is available on kidney energy metabolism during fetal life. On the one hand, high levels of glycolytic-enzyme activity have been reported, as well as high production of lactate from glucose [6,7]. On the other hand, towards the end of fetal life, there is very little oxidation of long-chain fatty acids, which are the main energy substrates in the adult renal cortex, suggesting that there is a rate-limiting step in mitochondrial oxidative capacity [8].

This work was carried out to obtain further information on the fetal and postnatal development of rat kidney mitochondrial functions and the changes that take place during mitochondrial maturation. We measured mitochondrial oxidative capacities and the activities of two enzymes : ATP synthase, an enzyme of the respiratory chain, and citrate synthase, an enzyme of the tricarboxylic acid cycle, during the fetal and postnatal periods. Since ATP synthase plays a key role in the coupling between respiration and phosphorylation, we also studied the development of its content, as estimated by semi-quantitative blotting of the protein during the fetal and postnatal periods. The physiological significance of these results was evaluated with reference to the ATP concentrations of isolated mitochondria and to the NaK-ATPase activity of renal homogenates. Changes in the mitochondrial pool were also investigated by monitoring the amount of mitochondrial genome in the organ, compared with the amount of nuclear genome.

MATERIALS AND METHODS

Animals

Wistar rats were bred and mated in our laboratory. Pregnant rats were kept in individual cages and fed on a standard laboratory chow. In this strain, delivery occurs on day 22 of gestation. The mother was killed by cervical dislocation, and fetuses were delivered by caesarian section on days 19, 20 or 21 *post coitum* (d.p.c.). The newborns 1 h *post partum* (h.p.p.) were obtained as previously described [4]. The 1-day newborns were killed by cervical dislocation 24 h after spontaneous delivery. The kidneys were quickly removed and placed in ice-cold 0.15 M NaCl solution for mitochondrial isolation or measurement of NaK-ATPase activity. Some kidneys were immediately plunged into liquid N_2 and stored at −80 °C for Western-blot and slot-blot analyses. For adult-rat kidneys, the medulla was discarded and only the cortex was used.

Isolation of mitochondria

The kidneys were homogenized in an ice-cold solution of 220 mM mannitol, 70 mM sucrose, 2 mM Hepes, 0.1 mM EDTA and 0.5% BSA, pH 7.4. Nuclei and unbroken cells were pelleted by centrifugation at 800 *g* for 10 min at 0 °C. Mitochondria were separated from the supernatant by centrifugation at 8000 *g* for

Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; OSCP, oligomycin-sensitivity-conferring protein; mtDNA, mitochondrial DNA.

§ To whom correspondence should be addressed.

10 min at 0 °C. The mitochondrial pellet was washed twice with homogenization medium and suspended at 15–30 mg of protein/ml, as determined by the method of Lowry et al. [9].

Measurements of mitochondrial respiration

Oxygen-consumption rates were measured polarographically with a Clark electrode (Gilson Oxygraph: Middleton, WI, U.S.A.) in the medium described by Aprille and Asimakis [10] in a thermostatically controlled (30 °C) 2 ml closed chamber. The rate of oxygen consumption was measured in the presence of 300 nmol of ADP and 10 mM succinate (active State 3), and when all the ADP had been consumed (resting State 4). Oxygen-consumption rates are expressed as ng-atoms of oxygen consumed/min per mg of mitochondrial protein. The respiratory control ratio, corresponding to the ratio of oxygen consumptions in States 3 and 4, the ADP/O ratio and the net rate of oxygen consumption (state 3 – state 4) were calculated. Oxygen consumption was also measured in the presence of 40 μM 2,4-dinitrophenol (uncoupled state).

Enzyme activities

ATP synthase

The hydrolytic activity of the ATP synthase (or F_1 ATPase activity) was measured on isolated mitochondria in the presence of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The specificity of the reaction was checked with two inhibitors, oligomycin and efrapeptin. Oligomycin binds to the oligomycin-sensitivity-conferring protein (OSCP) of F_0 and specifically inhibits membrane complexes of ATP synthase. Efrapeptin binds to F_1 and inhibits membrane ATP synthase complexes, as well as F_1 sectors which could be detached from the membrane [11]. Since these free F_1 sectors have greater ATPase activity than complete ATP synthase complexes, comparison of the residual activities in the presence of oligomycin and efrapeptin provides a check on the quality of the mitochondrial preparations at all developmental stages.

The residual ATPase activity obtained after oligomycin inhibition was about 25%, and about 15% after efrapeptin. These values indicate that a constant fraction of F_1 was solubilized during mitochondrial isolation and that the change in total ATPase activity paralleled the specific F_1 ATPase activity. In these conditions, total mitochondrial ATPase activity may be used as an index of the activity of ATP synthase complexes.

Mitochondria were incubated in a buffer containing 50 mM Tris/HCl, 5 mM $MgCl_2$ and 6 μM FCCP, pH 8, at 37 °C for 10 min. The reaction was started by adding 5 mM ATP, continued for 10 min, and was stopped by adding 10% (w/v) SDS and placing the mixture on ice for 5 min. Oligomycin was used at 20 μg/ml and efrapeptin at 4 μg/ml (final concns.). Released P_i was measured by the colorimetric method of Chen et al. at 700 nm [12]. Preliminary studies showed that P_i production in these experimental conditions is linear with incubation time until 20 min. The activity is expressed as nmol of P_i released/min per mg of mitochondrial protein.

Citrate synthase

The activity of citrate synthase was determined on renal homogenates and on isolated mitochondria by fluorimetry as previously described [5]. The activity is expressed as nmol of NAD^+ formed/min per mg of protein.

NaK-ATPase and Mg-ATPase

Total ATPase activity was determined in deoxycholate-treated renal homogenates as described by Katz and Epstein in the presence of 6 mM Na_2ATP [13]. For determination of Mg-ATPase activity, NaCl was omitted and 2 mM ouabain was added. A 1 h incubation was performed at 37 °C in 100 mM NaCl/20 mM KCl/6 mM $MgCl_2$/50 mM Tris buffer, with or without ouabain, before the addition of ATP. The reaction was carried out for 10 min and stopped by addition of ice-cold 10% (w/v) trichloroacetic acid. In these conditions, we checked that the assay is linear over 15 min. P_i released was determined as described above [12]. NaK-ATPase activity was calculated as the difference between the total ATPase activity and the Mg-ATPase activity. Results are expressed as μmol of P_i released/h per mg of renal protein.

Mitochondrial adenine nucleotide concentrations

Adenine nucleotides in isolated mitochondria in State 4 were extracted with $HClO_4$ as previously described [4]. Concentrations of ADP and ATP were determined by fluorimetric assay using a Hitachi (F2000) spectrofluorimeter [14]. Results are expressed as nmol of nucleotide produced/mg of mitochondrial protein.

Semi-quantification of ATP synthase

Western-blot analysis of OSCP and F_1 ATPase subunits

About 100 of mg renal tissue was homogenized in 1.8 ml of buffer containing 0.32 mM sucrose, 1 mM Tris/HCl, pH 7.4, plus the protease inhibitors, 1 mM phenylmethanesulphonyl fluoride, 2 mg/ml leupeptin and 10 mg/ml aprotinin. Protein concentration was measured by the method of Bradford, with crystalline BSA as standard [15].

Renal homogenate or mitochondria (40 mg of protein) was denatured by heating at 100 °C for 5 min in 25 mM Tris/25% (w/v) glycerol/50 mM dithiothreitol/50% (w/v) SDS and 1 mg/ml Bromophenol Blue. Proteins were separated on a 0.1%-SDS/15%-polyacrylamide gel for 16 h at 12 mA and electrophoretically transferred to nitrocellulose (Schleicher and Schuell, 0.1 μm pore) for 1 h at 0.8 mA/cm^2 of gel with a 2117 Multiphor II instrument (LKB) [16].

Immunoblotting of OSCP and F_1 ATPase subunits

The filters were immersed in a blocking solution [20 mM Tris/HCl, pH 7.6, and 137 mM NaCl, containing 5% (w/v) skimmed milk], for 1 h. Polyclonal antibodies to OSCP and F_1 ATPase subunits were gifts from Professor P. V. Vignais (CEN, Grenoble, France) and were raised against bovine heart mitochondrial ATP synthase [17]. They were incubated for 2 h with the filters, and the filters were washed six times in 20 mM Tris/HCl (pH 7.6)/137 mM NaCl/0.01% Tween 20. Then they were incubated for 45 min with goat anti-(rabbit IgG)–peroxidase conjugate [BIOSYS, anti-rabbit IgG(H + L)/peroxidase]. The filters were washed again six times, and peroxidase was detected with an Amersham ECL kit.

The intensity of the 60 kDa immunoreactive band, corresponding to the α and β subunits of the F_1 ATPase, and the intensity of the 20 kDa immunoreactive band, corresponding to the OSCP subunit, were analysed by scanning densitometry (Bio-Image, Millipore). For each film, the results are expressed as percentages of the adult value.

Semi-quantification of mitochondrial DNA (mtDNA)

Total DNA was extracted from about 20 mg of renal homogenates for slot-blot analysis. The DNA was denatured by heating at 100 °C for 4 min in 1.4 mM NaOH/25 mM EDTA and blotted directly on to a nylon filter (HYBOND N^+) soaked with 20 × SSEP (1 × SSEP is 150 mM NaCl/10 mM NaH_2PO_4/1 mM Na_2EDTA, pH 7.4) in a slot-blot apparatus (Minifold II, Schleicher and Schuell), and washed with 400 μl of 20 × SSEP. For each sample, three amounts of total DNA (2, 1 and 0.5 μg) were blotted on to two nylon filters. One nylon filter was hybridized to a [^{32}P]dCTP-labelled mtDNA probe [18], and the second one was hybridized to a [^{32}P]dCTP-labelled 18 S DNA probe [19]. The mtDNA and nuclear DNA were quantified by scanning densitometry (Bio-Image, Millipore). On each film, values are expressed as percentages of the adult value, and the ratio mtDNA/nuclear DNA was calculated for each amount of total DNA initially blotted.

Statistics

Results are means ± S.E.M. Significance of difference from the preceding stage was calculated by ANOVA test : $*P \leqslant 0.05$; $**P \leqslant 0.01$; $***P \leqslant 0.001$. Numbers reported in parentheses indicate the numbers of independent experiments.

RESULTS

Change in mitochondrial pool (Figure 1)

The high viscosity of the DNA prevented accurate spotting on the filter for direct assay of mtDNA. We therefore used the mtDNA/nuclear DNA ratio. As total DNA is mainly nuclear DNA (mtDNA is less than 1% of total DNA), changes in the mtDNA/nuclear DNA ratio mainly reflect changes in the mtDNA content.

Figure 1(a) shows a film from one slot-blot experiment, where Figure 1(a)A shows the spots obtained after hybridization with the labelled nuclear probe, and Figure 1(a)B shows the spots obtained after hybridization with the labelled mtDNA probe. In Figure 1(b)A, mtDNA/nuclear DNA ratios are reported from day 19 of gestation (19 d.p.c.) to 1 day after birth. Renal mtDNA doubled between 19 and 20 d.p.c., then remained fairly constant until 24 h.p.p., when it reached 60% of the adult value.

Citrate synthase activity in renal homogenates (Figure 1bB) markedly increased between 19 and 20 d.p.c. (+55%), and progressively increased between 21 d.p.c. and 24 h.p.p. (+40%).

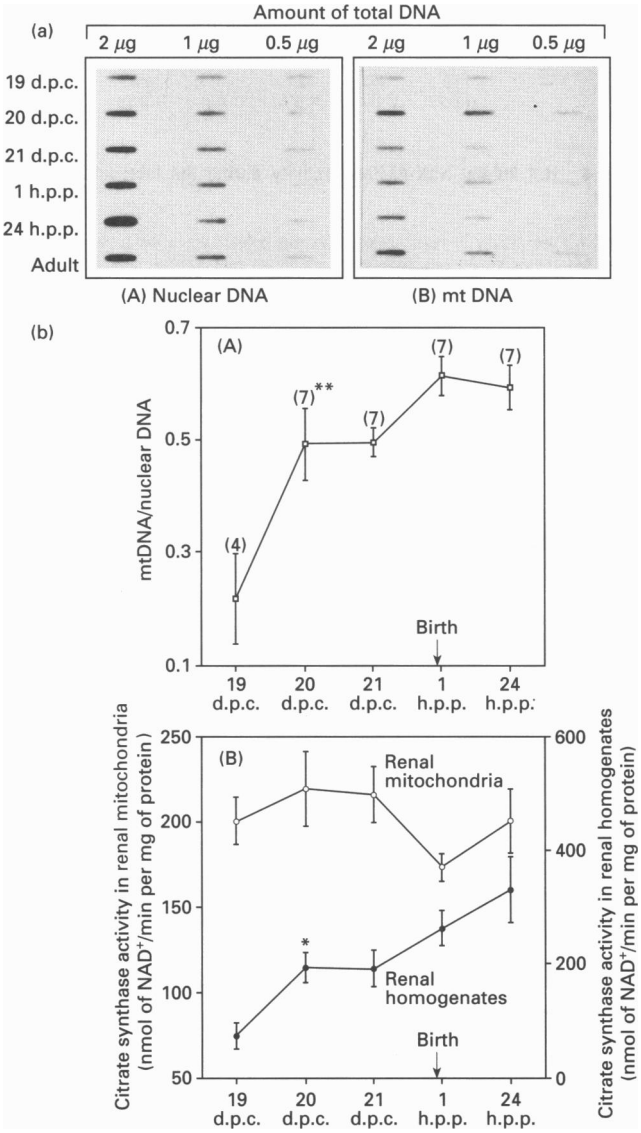

Figure 1 Mitochondrial proliferation in the rat kidney during the fetal and perinatal periods

(**a**) Slot-blot quantification of rat kidney DNA during the fetal and perinatal periods. Total DNA was isolated from renal homogenates, denatured and spotted on to nylon filters (as described in the Materials and methods section): (A) hybridization with an 18 S rDNA probe; (B) hybridization with an mtDNA probe. (**b**) (A) mtDNA/nuclear DNA ratio obtained after analyses of autoradiograms by scanning densitometry. Numbers of measurements are given in parentheses. (B) Citrate synthase activity determined by fluorimetry on renal homogenates and on isolated mitochondria and expressed as nmol of NAD^+ formed/min per mg of protein. Results are means ± S.E.M. of 6 experiments. Values significantly different from preceding age at: * $P \leqslant 0.05$; ** $P \leqslant 0.01$.

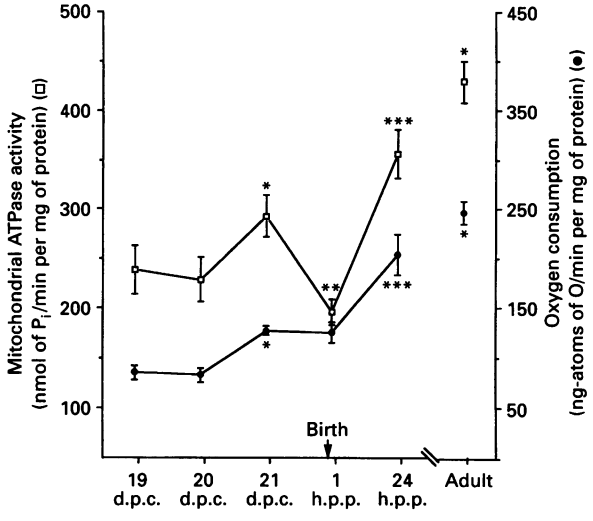

Figure 2 Oxygen consumption and ATPase activity in rat kidney mitochondria during fetal and perinatal development

Net oxygen consumption was measured polarographically at 30 °C, in the presence of 10 mM succinate and 300 nmol of ADP. Results are expressed as ng-atoms of oxygen consumed/min per mg of mitochondrial protein. ATPase activity was measured in the presence of 5 mM ATP and 6 μM FCCP and expressed as nmol of P_i released/min per mg of mitochondrial protein. Results are means ± S.E.M. of 6–20 experiments. Values significantly different from preceding age at: * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$.

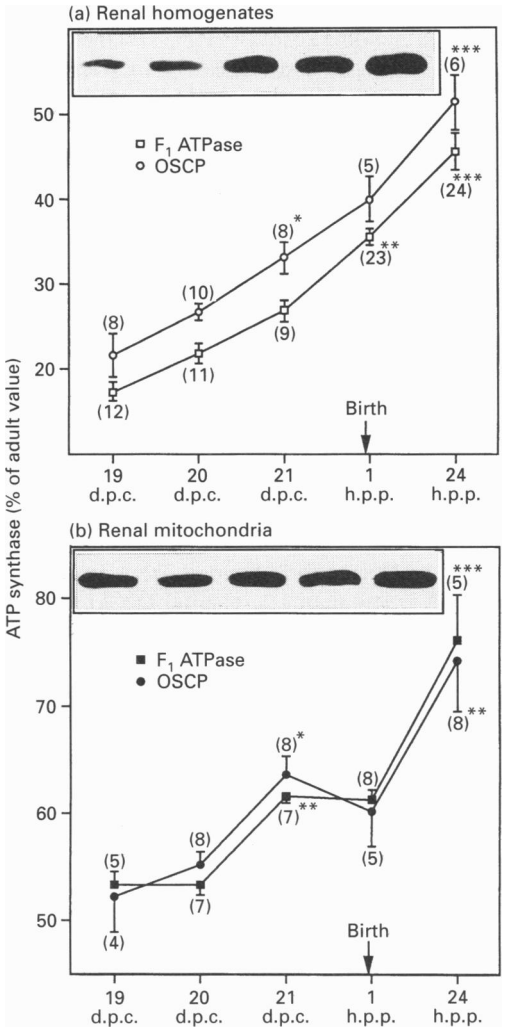

Figure 3 ATP synthase content in renal homogenates and in isolated mitochondria during the fetal and perinatal periods

Samples (40 μg of protein) of renal homogenate or isolated mitochondria, at each developmental age, were separated by SDS/PAGE, transferred to nitrocellulose and immunoblotted with antibodies against F_1 ATPase or OSCP subunits. The bands obtained were analysed by scanning densitometry (photos show α- and β-subunit spots) : (**a**) in renal homogenates; (**b**) in isolated mitochondria. Results are expressed as percentages of the adult value. Values significantly different from preceding age at: * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$.

At this time, the activity was about 50% of the adult value. In isolated mitochondria (Figure 1bB), citrate synthase activity did not change significantly between 19 d.p.c. and 24 h.p.p. (about 450 nmol of NAD^+/min per mg of protein), but remained lower than the adult value (673 ± 65 nmol of NAD^+/min per mg of protein; $n = 11$).

Oxygen consumption

The quality of isolated mitochondria was checked by polarographic measurements. The respiratory control ratio (RCR = 3) and ADP/O ratio (2 in the presence of succinate) remained unchanged throughout the period studied. The oxygen consumption in uncoupled conditions was not different from that in active State 3 at all stages of development. The net oxygen consumptions of mitochondria isolated from day 19 of gestation to day 1 after birth are shown in Figure 2. The respiration rates

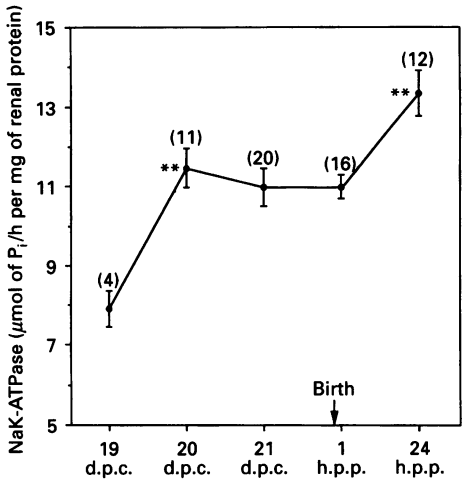

Figure 4 Rat kidney NaK-ATPase activity during the fetal and perinatal periods

The total ATPase activity in renal homogenates was determined in the presence of 6 mM Na_2ATP; Mg-ATPase activity was measured after adding 2 mM ouabain ; NaK-ATPase activity was calculated as the difference between the total and Mg-ATPase activities, and is expressed as μmol of P_i released/h per mg of mitochondrial protein. Values significantly different from preceding age at: ** $P \leqslant 0.01$.

were similar on 19 and 20 d.p.c., then increased between 20 and 21 d.p.c. (+55%). A second increase occurred 1 day after birth (+70%), when oxygen consumption reached a level lower than the adult value.

Mitochondrial ATP synthase

Enzyme activity

Total ATPase activity (Figure 2) did not change between 19 and 20 d.p.c., but increased significantly between 20 and 21 d.p.c. During the first 1 h of extrauterine life, this activity decreased; it subsequently increased 1 day after birth to reach a level higher than that measured on 21 d.p.c. At 1 day after birth, it was then 85% of the adult value.

Enzyme content

Figure 3 shows the immunoreactive bands of 60 kDa obtained after immunoblotting with antibodies against F_1 ATPase corresponding to α and β subunits of F_1 ATPase.

In renal homogenates, the relative contents of OSCP and F_1 ATPase subunits (expressed as percentages of the adult content) were similar at all developmental stages (Figure 3a). They gradually doubled between 19 d.p.c. and 1 h.p.p. and increased significantly between 1 and 24 h.p.p., when the values reached 50% of the adult level.

In isolated mitochondria, the relative contents of OSCP and F_1 ATPase subunits were also similar, regardless of the developmental age (Figure 3b). They did not change between 19 and 20 d.p.c., and increased significantly between 20 and 21 d.p.c. and between 1 and 24 h.p.p., when values reached 75% of the adult level.

Mitochondrial ATP and ADP concentrations

The ATP concentration, measured under State-4 conditions, in isolated mitochondria remained unchanged throughout the fetal period (about 16 nmol/mg of protein), but increased 1 day after birth to reach 28 ± 2.5 nmol/mg of protein ($n = 7$).

The mitochondrial ADP concentration, also measured under State-4 conditions, did not change between 21 d.p.c. and 24 h.p.p. [10.6±0.9 nmol/mg of protein ($n = 5$) and 8.4±0.1 nmol/mg of protein ($n = 4$) respectively].

Renal NaK-ATPase activity (Figure 4)

The renal NaK-ATPase activity exhibited two increases during the period studied : the first between days 19 and 20 d.p.c. (+45%) and the second between 1 and 24 h.p.p. (+30%).

DISCUSSION

The present study examines mitochondrial evolution during renal development from day 19 of gestation to day 1 after birth. At each developmental stage, we observed that the rate of oxygen consumption under State-3 conditions is equivalent to that measured in the presence of an uncoupler. This result indicates that neither the ATP synthase nor the nucleotide adenine translocase limits respiratory-chain capacities during this period. Hence, rat renal mitochondrial phosphorylation and oxidation were efficiently coupled throughout the perinatal period, in contrast with the situation in rat liver [20]. Nevertheless, the rates of oxygen consumption by the renal mitochondria increased during this period, indicating changes in the mitochondria at the end of fetal life, presumably by changes in respiratory-chain components.

Fetal period

The mtDNA/nuclear DNA ratio markedly increased between days 19 and 20 of gestation, reflecting considerable replication of the mtDNA during this period. This suggests an increase in the number of mitochondria, although the number of genomic copies in mitochondria is not clearly established. An increase in mtDNA content might not be necessarily accompanied by an increase in the number of mitochondrial units [21,22]. However, the fact that citrate synthase activity also markedly increased in renal homogenates between days 19 and 20 of gestation, although it did not change in isolated mitochondria, argues for mitochondrial proliferation. In this case, the increase in mitochondrial ATP supply could be used for the increase in NaK-ATPase activity.

The mitochondrial proliferation seems to be followed 1 day later by mitochondrial differentiation, as indicated by synthesis of specific mitochondrial protein, such as ATP synthase complexes. The quantitative changes observed in the content of this respiratory-chain enzyme correlate well with changes in ATP synthase activity as well as oxygen consumption, suggesting that this fetal enhancement of mitochondrial oxidative metabolism is due to synthesis *de novo* of respiratory-chain complexes.

Effect of birth

We have previously reported an increase in renal ATP content between day 21 of gestation and 1 day after birth (+30%) and postulated an increase in glycolysis during this period [23]. In the present study, we did not find any change in oxygen consumption or in mitochondrial ATP concentration during this period, thus confirming the efficiency of glycolysis in renal ATP production. An additional evidence for a high level of anaerobic glycolysis is provided by the marked decrease in F_1 ATPase activity between 21 d.p.c. and 1 h.p.p., without any change in its content. This result suggests a specific inhibition of this enzyme activity. Such an inhibition is possible in the presence of IF_1, an inhibitor of F_1 ATPase activity, found in several organs of some mammals and particularly active in ischaemic conditions [24,25].

Postnatal increase in mitochondrial function

The period between 1 and 24 h.p.p. appears to be important for the development of mitochondrial oxidative metabolism.

During this period we observed an increase in mitochondrial ATP concentration, without any change in ADP concentration; AMP concentration was not measured, since its value in renal homogenates is much lower than those of ADP and ATP [4]. These results suggest a mitochondrial ATP influx through the ATP-Mg/P_i carrier, a new anion carrier described in liver by Aprille and co-workers [26,27]. As a consequence, the ATP/ADP translocase activity could enhance, leading to an increase in the respiratory-chain activity (+70%) and probably to an increase in the protonmotive force [28]. At this stage, the increase in F_1 ATPase activity could be due, on the one hand, to a high mitochondrial ATP concentration and, on the other hand, to a fall in anaerobic glycolysis. Indeed, in bovine heart, IF_1 becomes displaced from the enzyme when energy is generated by substrate oxidation [29]. Moreover, Lippe et al. have shown that a rise in the protonmotive force could increase F_1 ATPase activity through a release of the IF_1 inhibitor [24]. Hence, 1 day after birth, the mitochondrial activity seems to become the major source of ATP production. These data are consistent with a physiological switch from anaerobic glycolysis to aerobic respiration that depends on mitochondrial maturation. At 1 day after birth, we also observed a marked increase in renal NaK-ATPase activity, so that NaK-ATPase maturation could depend on the maturation of mitochondrial oxidative metabolism.

Quantitative changes in OSCP and F_1 ATPase subunits are well correlated with changes in ATP synthase activity and oxygen consumption, indicating that the postnatal maturation of mitochondrial oxidative capacities is due to protein synthesis and not to activation of pre-existing complexes. Moreover, such results imply that all the components of ATP synthase are present at this time (the F_1 sector and OSCP, encoded by the nuclear genome, as well as subunits of the F_0 sector, including those encoded by the mitochondrial genome).

Hence, the synthesis of ATP synthase complexes could be implicated in the enhancement of oxidative capacities, during the fetal period as well as 24 h after birth. In rat liver, the postnatal increase in mitochondrial enzyme activities (succinate dehydrogenase, cytochrome *c* oxidase and F_1 ATPase) parallels that in respiratory rates and in the proton electrochemical gradient. Moreover, Valcarce et al. have demonstrated that liver mitochondrial maturation depends on postnatal synthesis of respiratory-chain proteins [30]. Recently it has been accepted that the genes which encode for the respiratory-chain components are expressed by a co-ordinated system [31,32]. Indeed, synthesis *de novo* of specific mitochondrial proteins seems to be important for the acquisition of functional mitochondria in the kidney, as has been demonstrated in the liver.

Thus the maturation of renal mitochondria at the end of gestation leads first to a mitochondrial proliferation stage, and then to an increase in the mitochondrial density of complexes involved in oxidative phosphorylation, leading to mitochondrial differentiation. A second mitochondrial period of differentiation occurs 24 h after birth, when mitochondria take charge of the renal energy supply. This postnatal mitochondrial maturation seems to depend on protein synthesis as well as on changes in the adenine nucleotide pool size, and might be determinant for the development of the renal NaK-ATPase activity.

We are grateful to Dr. J. Lunardi for his helpful suggestions on this work and to Professor P. V. Vignais for supply of the antibodies.

REFERENCES

1 Lelièvre-Pégorier, M. and Geloso, J. P. (1980) Biol. Neonate **38**, 16–24
2 Geloso, J. P. and Basset, J. C. (1974) Pflügers Arch. **348**, 105–113
3 Delaval, E., Moreau, E. and Geloso, J. P. (1979) Pflügers Arch. **379**, 95–100
4 Bastin, J., Sofack, F., Boulekbache, H., Bismuth, J. and Geloso, J. P. (1986) J. Dev. Physiol. **8**, 97–103
5 Delaval, E., Razanoelina, M., Bastin, J., Freund, N., Bismuth, J. and Geloso, J. P. (1990) J. Dev. Physiol. **14**, 1–5
6 Burch, H. B., Kuhlman, A. M., Skerjance, J. and Lowry, O. H. (1971) Pediatrics **47**, 199–206
7 Freund, N., Sedraoui, M. and Geloso, J. P. (1982) J. Dev. Physiol. **4**, 215–226
8 Razanoelina, M., Delaval, E., Bastin, J., Bismuth, J., Freund, N. and Geloso, J. P. (1987) C. R. Acad. Sci. Ser. 3 **304**, 251–254
9 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
10 Aprille, J. R. and Asimakis, G. K. (1980) Arch. Biochem. Biophys. **201**, 564–575
11 Boyer, P. D. (1993) Biochim. Biophys. Acta **1140**, 215–250
12 Chen, P. S., Toribara, T. Y. and Warner, H. (1956) Anal. Chem. **28**, 1756–1758
13 Katz, A. I. and Epstein, F. H. (1976) J. Clin. Invest. **46**, 1999–2011
14 Lowry, O. H. and Passonneau, J. V. (1972) in A Flexible System of Enzymatic Analysis: A Collection of Metabolite Assays (Lowry, O. H., ed.), pp. 146–193, Academic Press, New York
15 Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
16 Kyshe-Andersen, J. (1984) J. Biochem. Biophys. Methods **10**, 203–209
17 Dupuis, A., Lunardi, J. and Vignais, P. V. (1984) Biochemistry **24**, 734–739
18 Lutfalla, G., Blanc, H. and Bertolotti, R. (1985) Somatic Cell Mol. Genet. **11**, 223–238
19 Raynal, F., Michot, B. and Bachellerie, J. P. (1984) FEBS Lett. **167**, 263–268
20 Pollack, J. K. (1975) Biochem. J. **150**, 477–488
21 Robin, E. D. and Wong, R. (1988) J. Cell. Physiol. **136**, 507–513
22 Veltri, K. L., Espiritu, M. and Singh, G. (1990) J. Cell. Physiol. **143**, 160–164
23 Bastin, J., Delaval, E., Freund, N., Razanoelina, M., Djouadi, F., Bismuth, J. and Geloso, J. P. (1988) Biochem. J. **252**, 337–341
24 Lippe, G., Sorgato, M. C. and Harris, D. A. (1988) Biochim. Biophys. Acta **933**, 1–11
25 Rouslin, W. (1991) J. Bioenerg. Biomembr. **23**, 873–888
26 Aprille, J. R. (1988) FASEB J. **2**, 2547–2556
27 Dransfield, D. T. and Aprille, J. R. (1993) Am. J. Physiol. **264**, C663–C670
28 Valcarce, C. and Cuezva, J. M. (1991) FEBS Lett. **294**, 225–228
29 Van De Stadt, R. J. and Van Dam, K. (1974) Biochim. Biophys. Acta **347**, 240–252
30 Valcarce, C., Navarrete, R. M., Encabo, P., Loeches, E., Satrùstegui, J. and Cuezva, J. M. (1988) J. Biol. Chem. **263**, 7767–7775
31 Suzuki, H., Hosokawa, Y., Nishikimi, M. and Ozawa, T. (1991) J. Biol. Chem. **266**, 2333–2338
32 Tomura, H., Endo, H., Kagawa, Y. and Ohta, S. (1990) J. Biol. Chem. **265**, 6525–6527

Received 1 August 1994/15 September 1994; accepted 21 September 1994