Transcriptional regulation by glucocorticoids of mitochondrial oxidative enzyme genes in the developing rat kidney

Fatima DJOUADI*, Jean BASTIN*‡, Daniel P. KELLY† and Claudie MERLET-BENICHOU*

*INSERM U319, Université Paris 7, 2 place Jussieu, 75251 Paris Cedex 05, France, and †Departments of Medicine and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO, U.S.A.

Mitochondrial fatty acid β-oxidation plays a major role in providing the ATP required for reabsorptive processes in the adult rat kidney. However, the molecular mechanisms and signals involved in induction of the enzymes of fatty acid oxidation during development in this and other organs are unknown. We therefore studied the changes in the steady-state levels of mRNA encoding medium-chain acyl-CoA dehydrogenase (MCAD), which catalyses the initial step in mitochondrial fatty acid β-oxidation, in the rat kidney cortex and medulla between postnatal days 10 and 30. Furthermore, we investigated whether the expression of MCAD and of mitochondrial malate dehydrogenase (mMDH), a key enzyme in the tricarboxylic acid cycle, might be co-ordinately regulated by circulating glucocorticoids in the immature kidney during development. In the cortex, the levels of MCAD mRNA rose 4-fold between day 10 and day 21, and then decreased from day 21 to day 30. In the medulla a postnatal increase in the concentration of MCAD mRNA (8-fold) was observed during the same period. Adrenalectomy prevented the 16–21-day developmental increases in MCAD and mMDH mRNA levels in the cortex and medulla; these could be restored by dexamethasone treatment. A single injection of dexamethasone into 10-day-old rats led to a rise in MCAD and mMDH mRNA levels in the renal cortex due to stimulation of gene transcription, as shown by nuclear run-on assays. Therefore MCAD and mMDH gene expression is tightly regulated at the transcriptional level by developmental changes in circulating glucocorticoid levels. These hormones might thus represent a good candidate as a co-ordinating factor in the expression of nuclear genes encoding mitochondrial enzymes in the kidney during postnatal development.

INTRODUCTION

The mechanisms involved in the regulation of expression of nuclear genes encoding mitochondrial oxidative enzymes during postnatal development are poorly understood. Medium-chain acyl-CoA dehydrogenase (MCAD; 2,3-oxidoreductase; EC 1.3.99.3) is a nuclear-encoded mitochondrial enzyme that catalyses the initial step of medium-chain fatty acid β-oxidation. The importance of MCAD in cellular energy metabolism is underscored by the severe and often fatal clinical manifestations of inherited MCAD deficiency [1]. The expression of MCAD mRNA and protein varies markedly between tissues, in parallel with fatty acid oxidation rates and energy demands [2–4]. The highest levels of MCAD mRNA and protein are found in a number of organs with high ATP turnover rates, such as the kidney, heart, liver and brown adipose tissue. Studies of MCAD expression during perinatal mammalian development have demonstrated that MCAD mRNA levels are low in most rat tissues until the end of gestation. At birth and during the postnatal period, expression of MCAD is induced in tissue-specific patterns [2–4]. Following birth, marked changes occur in the plasma levels of several hormones, including glucocorticoids, thyroid hormones, insulin and glucagon [5,6]. However, relatively little is known about the role played by these hormones in the developmental regulation of MCAD gene expression [4].

The developing kidney is an excellent model in which to test the hypothesis that endocrine factors play an essential role in triggering the gene regulatory programme involved in the induction of mitochondrial oxidative enzyme capacity during postnatal development. In fact, our previous studies have demonstrated that the known rise in circulating glucocorticoids during the weaning period provides a necessary signal for induction of the activities of enzymes involved in multiple mitochondrial oxidative pathways including the tricarboxylic acid cycle and ketone body oxidation [7]. We also demonstrated that this induction correlates temporally with a marked postnatal proliferation of mitochondria in the developing kidney [8]. In the cortex and medulla of the adult rat kidney, fatty acid oxidation provides a large part of the ATP required for Na^+ reabsorption [9]. Until the end of fetal life, the capacity of the rat kidney to oxidize fatty acids remains comparatively very low [10,11]. After birth, the use of fatty acids as energy fuel by the immature kidney depends on the development of key enzymes of the mitochondrial β-oxidation pathway. Indeed, marked increases in the activities of carnitine palmitoyltransferase [12] and β-hydroxyacyl-CoA dehydrogenase [13] occur in the rat kidney during the postnatal period. However, the molecular mechanisms and signals involved in the postnatal induction of β-oxidation enzyme expression in the kidney and in other organs have not yet been delineated.

The present study was performed in order to evaluate whether changes in the circulating levels of glucocorticoids might account for changes in MCAD gene expression in the developing kidney. A second objective was to investigate whether glucocorticoids might be involved in the co-ordinate induction of MCAD and other mitochondrial oxidative enzymes. Indeed, the development of the fatty acid oxidation pathway is likely to require the enhanced expression of numerous genes encoding mitochondrial proteins, particularly enzymes of the tricarboxylic acid cycle. We

Abbreviations used: MCAD, medium-chain acyl-CoA dehydrogenase; mMDH, mitochondrial malate dehydrogenase.

‡ To whom correspondence should be addressed.

therefore studied a possible regulation by glucocorticoids of the postnatal expression of the mRNA encoding mitochondrial malate dehydrogenase (mMDH; L-malate:NAD^+ oxidoreductase; EC 1.1.1.37), which catalyses the dehydrogenation of L-malate to oxaloacetate in the final step of the tricarboxylic acid cycle. Finally, the molecular mechanisms underlying the effects of glucocorticoids on MCAD and mMDH gene expression were investigated. We demonstrate that circulating glucocorticoids are necessary for the induction of MCAD and mMDH gene expression during postnatal development in the rat kidney. Furthermore, this regulation is at the level of transcription, depends on developmental stage and occurs via renal cortex- and medulla-specific mechanisms.

MATERIALS AND METHODS

Animals

Rat pups were obtained from pregnant Wistar rats bred and mated in our laboratory as previously described [13]. Litters were reduced to 8 pups 1 day after birth. On day 21, litters were weaned by removing the dam. Control studies were carried out on groups of male and female rats aged 10, 16, 21 and 30 days. Standard solid food (UAR 113; UAR, Villemoisson-sur-Orge, France) and tap water were provided *ad libitum*.

Dexamethasone treatment of 10-day-old rats

Rats of 10 days old, kept with their mothers, were given a subcutaneous injection of dexamethasone (10 μg/100 g body weight). Kidneys were removed 6 h after injection.

Adrenalectomy and dexamethasone administration

Bilateral adrenalectomy was performed via a dorsal incision in 16- and 30-day-old rats under light anaesthesia. In each litter, two pups were sham-operated and six were adrenalectomized. The 16-day-old adrenalectomized rats were given daily subcutaneous injection of 0.8 ml of 0.9 % NaCl after surgery up to day 21. For 30-day-old adrenalectomized rats, 0.9 % NaCl was provided in the drinking water. Half of the adrenalectomized rats were given a 5-day treatment with glucocorticoid, either from day 16 to day 21 or from day 30 to day 35. Glucocorticoids were administered as daily injections of dexamethasone (10 μg/100 g body weight).

The adequacy of adrenalectomy was checked by measuring plasma corticosterone according to the method of Nahoul et al. [14]. As expected, the plasma level of corticosterone was undetectable in adrenalectomized rats.

Kidneys were rapidly removed, immediately frozen in liquid nitrogen and stored at -80 °C. The dissection of kidney cortex and medulla was performed freehand at -20 °C.

Isolation of total RNA and Northern-blot analysis

Total cellular RNA was isolated from the kidney cortex and medulla of developing rats using the RNAzol B technique [15]. The concentration of RNA was estimated from the A_{260}. Northern-blot analysis of total RNA (15 μg) was performed after formaldehyde/agarose gel electrophoresis. Measurement of mRNA levels was accomplished by hybridizing with ^{32}P-labelled rat MCAD (*Eco*RI fragment of 871 bp) [16] or rat mMDH cDNA (*Eco*RI fragment of 1151 bp) [17]. Radioactive MCAD or mMDH cDNA probes were prepared by labelling with [α-^{32}P]dCTP using the random-primer technique. Prehybridization and hybridization were performed in 5 × SSPE, 50 % deionized formamide, 5 × Denhardt's solution, 1 % SDS and 100 μg/ml salmon sperm DNA. Prehybridization was carried out in a shaking water bath at 42 °C for 4 h, and hybridization was carried out for 18–24 h under the same conditions with labelled cDNA probe. The membranes were then washed twice with 2 × SSC for 10 min at room temperature, once with 2 × SSC and 1 % SDS for 10 min at room temperature, and twice with 1 × SSC and 1 % SDS for 20 min at 42 °C. Autoradiographs were obtained by exposing the membranes to Kodak X-OMAT film with two intensifying screens at -80 °C. Multiple exposures were performed to ensure that the signals were within the linear range of the film sensitivity. Quantification of signal intensity was performed by densitometric analysis of the autoradiograms using a computerized video densitometer (Biocom, Les Ulis, France).

As a control for RNA loading and transfer, the blots were also hybridized with an 18 S rRNA cDNA probe to allow correction for variations in the amount of RNA loaded.

Measurement of MCAD activity

MCAD activity was determined spectrophotometrically by following the decrease in ferricenium ion absorbance at 300 nm, as described by Lehman et al. [18]. Briefly, tissue samples (30–70 mg) were weighed frozen and the homogenates were immediately prepared as 20 % (w/v) suspensions in ice-cold 100 mM Hepes, pH 7.6/0.1 mM EDTA using a motor-driven Teflon/glass homogenizer. The homogenates were then centrifuged briefly and MCAD activity was determined immediately at 37 °C by adding 5 μl of supernatant to 500 μl of reaction mixture containing 100 mM Hepes, pH 7.6, 0.1 mM EDTA, 200 μM ferricenium hexafluorophosphate, 0.5 mM sodium tetrathionate and 50 μM octanoyl-CoA. The decrease in ferricenium hexafluorophosphate absorbance in the presence of homogenate was found to be stable for at least 3 min, and the results were calculated from the decrease observed over the initial 60 s period. The results were corrected by subtracting the absorbance of a tissue blank, measured in the absence of octanoyl-CoA in the reaction mixture.

In vitro nuclear run-on assay

Isolation of nuclei

Nuclei were isolated from the renal cortex, 6 h after dexamethasone injection, by the method of Sierra et al. [19]. Cortices from four to six young rats were quickly dissected and homogenized on ice in approx. 10 vol. of homogenization buffer containing 10 mM Hepes, pH 7.6, 15 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 2.4 M sucrose (RNase-free), 0.5 mM dithiothreitol and 0.5 mM PMSF. The homogenate was filtered through nylon cloth, layered carefully on top of a 10 ml sucrose cushion and centrifuged at 75000 ***g*** for 60 min at 4 °C. In this procedure, the homogenization buffer is used both for homogenization and for preparation of the cushions. The clean nuclei were resuspended in nuclei storage buffer [50 mM Tris/HCl, pH 8.3, 40 % (v/v) glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA] and stored at -80 °C.

RNA synthesis *in vitro*

The transcription rate was determined by the method described by Greenberg [20]. Aliquots of 10×10^6 nuclei were incubated for 30 min at 30 °C in 200 μl of reaction mixture containing 10 mM Tris/HCl, pH 8.0, 5 mM $MgCl_2$, 0.3 mM KCl, 1 mM each of ATP, CTP and GTP, 5 mM dithiothreitol, 500 units/ml RNasin and 200 μCi of [α-^{32}P]UTP (3000 Ci/mmol). The reaction mixture was then treated with RNase-free DNase I (20 μg/ml) for 5 min at 30 °C, and deproteinized by digestion with 100 μg/ml pro-

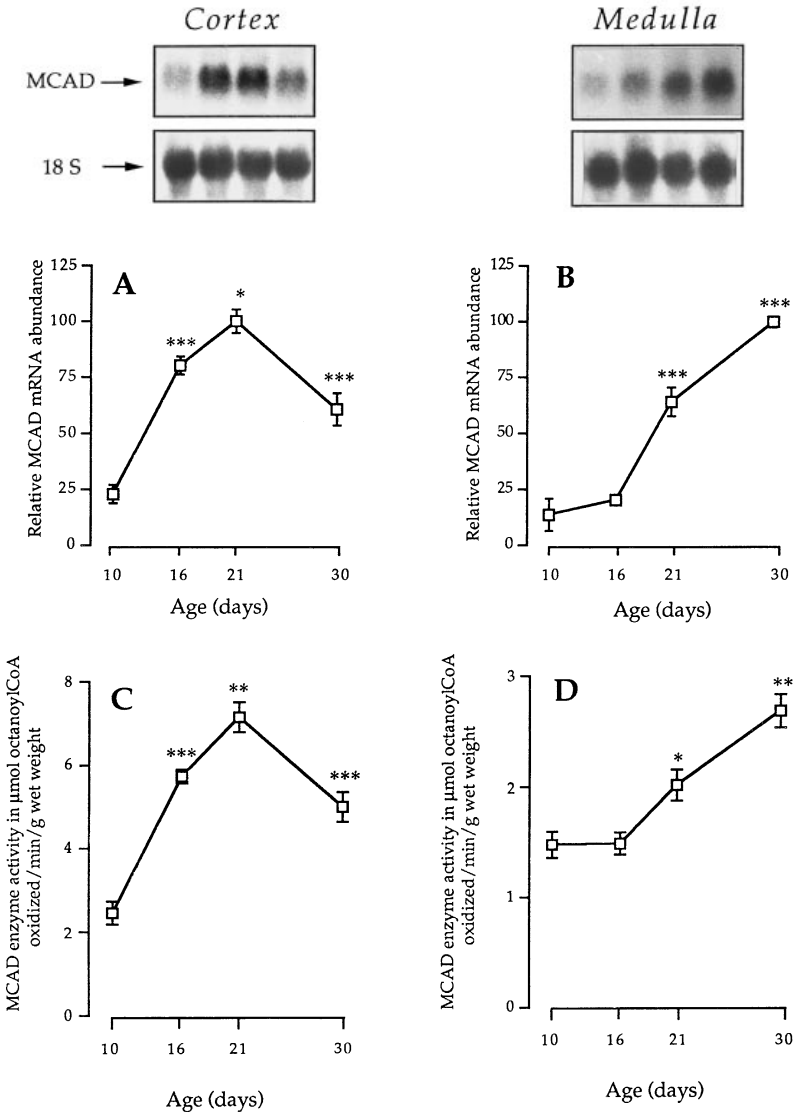

Figure 1 Postnatal changes in the steady-state levels of MCAD mRNA and in MCAD enzyme activity in rat kidney cortex and medulla

Typical Northern blot experiments and quantification of MCAD mRNA are shown (**A** and **B**). An arbitrary 100% value was given to the highest absorbance value (day 21 for the cortex and day 30 for the medulla). Results are means ± S.E.M. The number of animals ranged from five to ten. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the preceding developmental stage. The absence of error bars indicates that the S.E.M. is smaller than the symbol.

teinase K at 42 °C for 30 min. After extraction with phenol/chloroform (1:1, v/v), the aqueous phase was precipitated for 30 min on ice with cold 10% trichloracetic acid in the presence of 60 mM $Na_4P_2O_7$ and 10 μg/ml *Escherichia coli* tRNA carrier to remove unincorporated ^{32}P-labelled nucleotides. The precipitate was filtered on to Whatman GF/A glass-fibre filters, and the filters were washed three times with 5% trichloroacetic acid/30 mM $Na_4P_2O_7$. The filters were then incubated for 30 min at 37 °C in a mixture containing 20 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 25 μg/ml RNase-free DNase I. The reaction was quenched by 15 mM EDTA and 1% SDS. The samples were heated for 10 min at 65 °C to elute the RNA, and the supernatants were then removed and saved. This procedure removes more than 95% of the radioactivity from the filters. The supernatants containing ^{32}P-labelled RNA were again digested with proteinase K (30 μg/ml) for 30 min at 37 °C and extracted with phenol/chloroform. The aqueous phase was precipitated overnight at −20 °C with 0.1 vol. of 3 M sodium acetate and 2.5 vol. of ethanol. After washing the precipitate with 75% (v/v) ethanol, the radiolabelled RNA was resuspended in 200 μl of hybridization mixture.

Hybridization to immobilized DNA

^{32}P-labelled RNA was hybridized to cDNA plasmids previously immobilized on nylon filters. Samples of 5 μg each of MCAD plasmid, mMDH plasmid, β-actin plasmid and PBR322 and PGEM3Z control plasmids were denaturated and blotted under vaccum on to a nylon membrane. Prehybridization, hybridization, washing, autoradiography and image analysis were performed as described for Northern-blot analysis.

Expression of results and statistical analysis

mRNA abundance is expressed on a relative percentage basis. MCAD enzyme activity is expressed as μmol of octanoyl-CoA oxidized/min per g wet weight. We confirmed that the protein content of the kidney per unit wet weight did not change during the postnatal period studied or following adrenalectomy. The data are expressed as means $\pm$ S.E.M. The means from 3–7 rats in each experimental group were subjected to one-way analysis of variance (ANOVA) and the Fisher test; $P < 0.05$ was considered significant. Statistical differences between 10-day-old dexamethasone-treated rats and age-matched control rats were determined using the two-tailed unpaired Student's t test.

RESULTS

Ontogeny of MCAD mRNA levels and enzyme activity in kidney cortex and medulla

To delineate the pattern of postnatal expression of MCAD mRNA, RNA blot analyses were performed with total RNA isolated from the cortex and medulla of kidneys from 10–30-day-old rats. In the cortex (Figure 1A), MCAD mRNA levels markedly increased (4.4-fold) between days 10 and 21. Thereafter, a 40 % decrease occurred between days 21 and 30. In contrast, in the medulla a steady increase in the MCAD mRNA level occurred between days 16 and 30; during this period levels rose 5-fold (Figure 1B). Thus MCAD mRNA levels increased simultaneously in the kidney medulla and cortex during postnatal days 10–21, with different patterns thereafter. The changes observed for MCAD activity in the cortex (Figure 1C) and medulla (Figure 1D) closely paralleled those found for MCAD mRNA, suggesting that the developmental regulation of gene expression occurs mainly at a pretranslational level.

Role of glucocorticoids in the regulation of steady-state MCAD and mMDH mRNA levels in the developing kidney

In order to investigate the role of circulating glucocorticoids in the regulation of the steady-state MCAD mRNA level, we studied the effects of depriving young rats of adrenal steroids during a period of development when the plasma level of these hormones normally rises. We also investigated the effects of treating adrenalectomized rats with glucocorticoids. In control rats the serum corticosterone concentration increased significantly from day 16 (44 ± 3 ng/ml) to day 21 (147 ± 18 ng/ml) and day 30 (305 ± 36 ng/ml). As can be seen in Figure 2, adren-

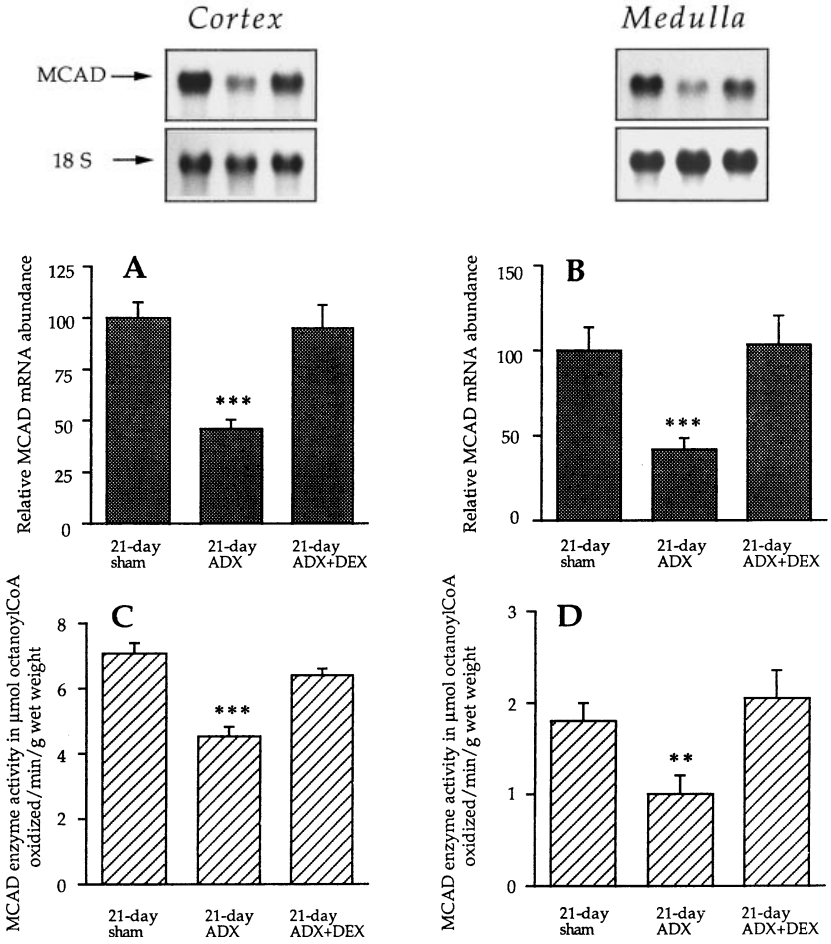

Figure 2 Effect of adrenalectomy on MCAD mRNA steady-state levels and on MCAD enzyme activity in the kidney cortex and medulla of 21-day-old rats

Northern-blot analysis of total RNA and enzyme assays were performed on 21-day-old rats sham-operated on day 16 (21-day sham) and on 21-day-old rats adrenalectomized on day 16 and receiving either no hormone (21-day ADX) or dexamethasone (21-day ADX + DEX) from day 16 to day 21. Protocols for adrenalectomy and dexamethasone treatment are given in the Materials and methods section. Results are means $\pm$ S.E.M. from four to six animals. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the 21-day sham value (100%).

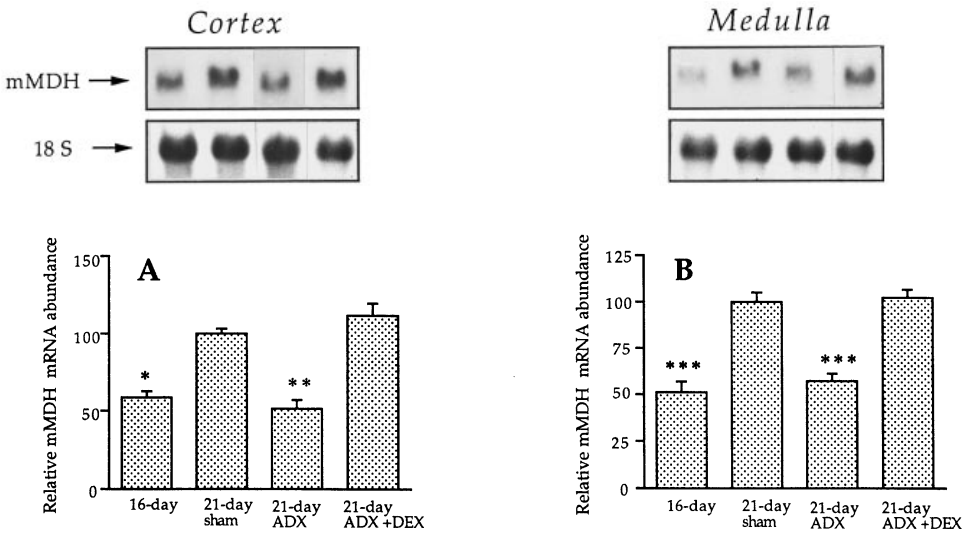

Figure 3 Effect of adrenalectomy on the mMDH mRNA steady-state level in the kidney cortex and medulla of 21-day-old rats

Abbreviations are as in Figure 2. Representative Northern blots of mMDH mRNA are shown. Results are means ± S.E.M. of four to six animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the 21-day sham value (100%).

alectomy led to significant changes in MCAD mRNA levels in both cortex and medulla. Compared with 21-day-old sham-operated animals, adrenalectomized rats receiving no hormone supplementation (21-day ADX) showed a 2.2-fold decrease ($P < 0.001$) in the steady-state level of MCAD mRNA in the cortex (Figure 2A) and a 2.4-fold decrease ($P < 0.001$) in the medulla (Figure 2B). Moreover, treatment of adrenalectomized animals with dexamethasone for 5 days restored the MCAD mRNA to normal levels in both regions of the kidney. Figures 2(C) and 2(D) show that the changes observed in MCAD mRNA after adrenalectomy led to parallel changes in MCAD activity in both regions of the kidney.

To determine whether glucocorticoids can play a role in controlling the expression of other mitochondrial oxidative enzyme mRNAs, the effect of adrenalectomy on mMDH mRNA abundance was studied in the kidney cortex and medulla. In 21-day-old rats adrenalectomized on day 16 (21-day ADX), the mMDH RNA steady-state level was significantly decreased (by 50%) compared with that observed in 21-day-old sham-operated rats, in both the kidney cortex ($P < 0.01$) and medulla ($P < 0.001$) (Figure 3). Treatment of adrenalectomized rats with dexamethasone (21-day ADX + DEX) restored the normal levels of mMDH mRNA in both regions of the kidney.

Adrenalectomy performed on 30-day-old animals produced no change in the steady-state levels of MCAD (Figure 4A) and mMDH (Figure 4B) mRNAs in the renal cortex, but led to significant decreases in MCAD and mMDH mRNA abundances in the renal medulla (Figures 4C and 4D). In 30-day-old adrenalectomized rats treated with dexamethasone until day 35 (35-day ADX + DEX), the levels of MCAD and mMDH mRNAs were significantly higher than in 35-day adrenalectomized rats, in both the cortex and medulla.

To determine whether MCAD and mMDH mRNAs could be precociously induced by glucocorticoids in the developing kidney, we studied the effect of dexamethasone injection on the expression of both genes in 10-day-old rats, i.e. before the natural surge of adrenal steroid hormones has occurred and when the plasma level of glucocorticoids is still very low. At this postnatal stage, a single injection of dexamethasone was able to produce, within 6 h, increases in the steady-state levels of MCAD (Figure 5A) and mMDH (Figure 5B) mRNAs in the cortex (1.9- and 1.8-fold respectively; $P < 0.01$) and in the medulla (1.6- and 1.5-fold respectively; $P < 0.05$; results not shown).

Effect of glucocorticoids on the *in vitro* transcription of the MCAD and mMDH genes

In order to investigate whether the effect of glucocorticoids on the levels of MCAD and mMDH mRNAs is due to stimulation of gene transcription, we studied the effects of these hormones on the *in vitro* transcription of the MCAD and mMDH genes in nuclei isolated from the kidney cortex of 10-day-old rats. Four sets of experiments were performed; a typical experiment is shown in Figure 5(C). Figure 5(D) shows that a single injection of dexamethasone had increased MCAD and mMDH gene transcription (1.97 ± 0.18- and 2.03 ± 0.15-fold respectively) 6 h after administration. In contrast, the transcription rate of the β-actin gene was not modified by dexamethasone treatment.

DISCUSSION

The present data demonstrate a marked postnatal increase in MCAD gene expression in the renal cortex and medulla, in keeping with the preferred use of fatty acids as an energy substrate in the adult kidney [9]. We also show that glucocorticoids control MCAD gene transcription in the postnatal developing kidney. These results suggest a mechanism whereby MCAD gene expression is induced in the kidney cortex and medulla during the 10–21-day period following birth. In the rat, previous studies have shown that the patterns of MCAD mRNA accumulation during the suckling and weaning periods are distinct in different tissues, suggesting that tissue-specific and developmental-stage-specific regulatory signals exist [2–4]. We have shown recently that the human MCAD gene promoter contains a series of novel nuclear receptor response elements in

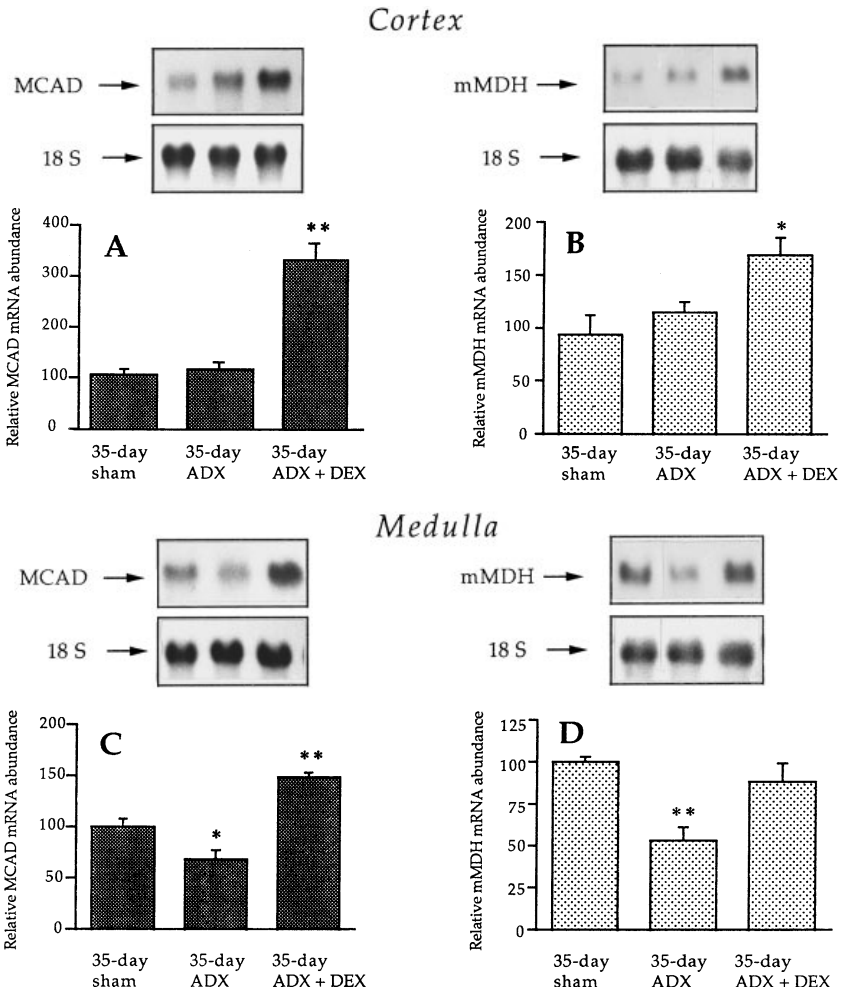

Figure 4 Comparison of MCAD and mMDH mRNA steady-state levels in the kidney cortex and medulla of control and adrenalectomized 35-day-old rats

Rats were sham-operated on day 30 (35-day sham) or adrenalectomized on day 30 and treated daily with dexamethasone (35-day ADX + DEX) or with vehicle only (35-day ADX). Protocols for adrenalectomy and dexamethasone treatment are given in the Materials and methods section. Results are means ± S.E.M. from three to six animals. $^{*}P < 0.05$; $^{**}P < 0.001$ compared with the 35-day sham value (100%).

close proximity to Sp1 binding sites [21]. Co-transfection studies performed *in vitro* have revealed that the nuclear receptor response elements confer tissue-specific modulation of MCAD promotor activity by interacting with diverse tissue-enriched orphan nuclear receptors, including COUP-TF, HNF4 and SF1 [21–23]. It is possible that glucocorticoids confer developmental regulation of MCAD gene transcription in a tissue-specific manner by interacting directly or indirectly with these orphan-nuclear-receptor-mediated regulatory pathways.

The up-regulation of MCAD gene expression by glucocorticoids in the renal medulla was demonstrated not only during the 10–21-day suckling period but also between days 21 and 35 after birth, i.e. following weaning. In keeping with the rise in circulating glucocorticoids during this period, MCAD mRNA increases in the medulla and, accordingly, adrenalectomy of 30–35-day-old rats had similar effects to those observed in 16–21-day-old rats. In contrast, a decrease in MCAD mRNA abundance occurs in the renal cortex between days 21 and 30 despite a high plasma level of glucocorticoids, and adrenalectomy of 30–35-day-old rats produced no change in cortex MCAD mRNA abundance. It can be noted, however, that administration of dexamethasone to adrenalectomized 30–35-day-old rats resulted in increased MCAD mRNA levels in the cortex; this rules out the hypothesis that the responsivness of the MCAD gene to glucocorticoids was lost, as might occur for other genes in the course of postnatal development [24,25]. Thus MCAD gene expression is tightly controlled by glucocorticoids in the immature cortex (10–21 days after birth), but later in development the stimulatory effect of glucocorticoids on MCAD gene expression is probably influenced by other factors. This developmental-stage-specific regulation of MCAD gene expression coincides with the weaning period, and might therefore involve changes in the concentrations of other hormones such as insulin, glucagon or tri-iodothyronine, as well as dietary factors [5,6]. In fact, it has been shown that administration of glucagon or changes in nutritional status can lead to changes in MCAD gene expression in rat heart and liver [4]. In agreement with this, we previously demonstrated that the transition from a high- to a low-fat diet, characteristic of the weaning period, was responsible for the decrease in the activity of another fatty acid β-oxidation marker, β-hydroxyacyl-CoA dehydrogenase, in the proximal tubule of the developing nephron [13].

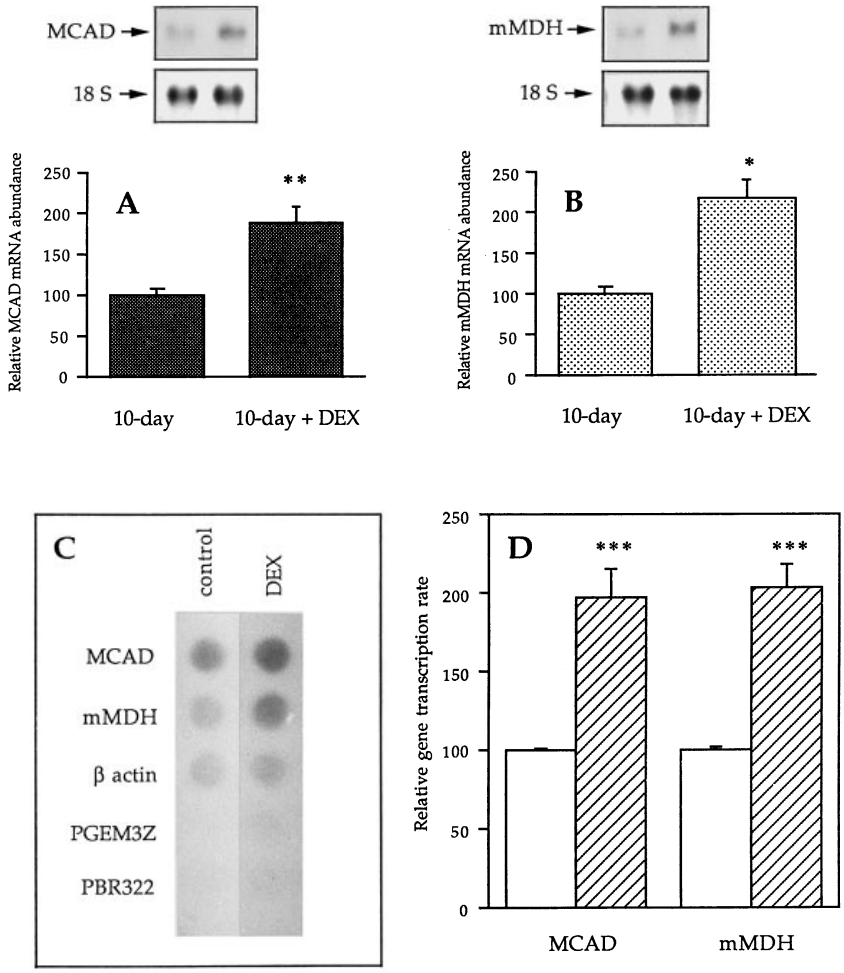

Figure 5 Effect of dexamethasone injection on MCAD and mMDH mRNA abundance (A, B) and on the transcription rate of MCAD, mMDH and β-actin genes (C, D) in the renal cortex of 10-day-old rats

(**A**, **B**) Rats were injected with 10 μg/100 g body weight dexamethasone (10-day + DEX) or saline (10-day), and the kidneys were sampled 6 h later. Results are means $\pm$ S.E.M. of three or four rats. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with the 10-day value (100%). (**C**, **D**) *In vitro* transcription in isolated nuclei from the renal cortex of 10-day-old rats receiving NaCl (open bars) or dexamethasone injection (hatched bars); the kidneys were removed 6 h after treatment. A representative nuclear run-on assay is shown in (**C**). A 100% value was assigned to the control transcription rate in (**D**). Results are means $\pm$ S.E.M. from four experiments (in each experiment the cortices from four to six rats were pooled). $^{***}P < 0.001$ compared with control. No hybridization to the MCAD, mMDH or β-actin plasmids was observed with RNA elongated in the presence of 1 μg/ml α-amanitin (results not shown). In each experiment ^{32}P-labelled transcripts were also hybridized to control PBR322 and PGEM3Z plasmids, which led to undetectable signals.

The results obtained in the present study clearly support the hypothesis that glucocorticoids could act as a common transcription stimulatory factor for MCAD and mMDH gene expression in the kidney since, with each experimental protocol tested, changes in glucocorticoid status led to parallel changes in MCAD and mMDH mRNA levels. Moreover, nuclear run-on assays demonstrated a similar stimulatory effect of glucocorticoids on MCAD and mMDH gene transcription. In renal cells, therefore, glucocorticoids might represent a major coordinating factor for the expression of nuclear genes encoding mitochondrial enzymes during postnatal development.

In the kidney and other organs with high energy demands, the progressive development of ATP-requiring functions during the postnatal period is accompanied by profound alterations in cellular energy production which are linked to the biogenesis of mitochondria [8,26]. The biogenesis of mitochondria is well documented in the developing liver, where it results from proliferation and differentiation of the organelles [26–29]. Recent studies of β-F_1-ATPase gene expression suggest that proliferation of mitochondria might essentially result from enhanced transcription of nuclear-encoded mitochondrial proteins, while mitochondrial differentiation most likely involves post-transcriptional regulatory mechanisms [30]. From a regulatory point of view, a role for thyroid hormones in the differentiation and proliferation of rat liver mitochondria has been demonstrated [30–32]. In particular, it has been shown that the expression of the nuclear-encoded β-F_1-ATPase gene is regulated by thyroid hormones at the transcriptional level [32]. In the kidney we have previously demonstrated that biogenesis of mitochondria occurs during the 16–30-day postnatal period in the main oxidative cell type of the renal medulla, namely the thick ascending limb of Henle loop, and is characterized by: (i) a doubling in mitochondrial density and amount of mitochondrial DNA, (ii) a marked increase in the mitochondrial inner membrane surface density, and (iii) co-ordinate increases in the activities of mitochondrial inner membrane and matrix oxidative enzymes [8]. Consequently, the

enhanced MCAD and mMDH gene expression demonstrated here in the kidney medulla of 16–30-day-old rats parallels mitochondrial biogenesis. The present data clearly demonstrate that glucocorticoids are essential to trigger the expression of the MCAD and mMDH genes in the kidney during the postnatal development period. However, previous studies have shown that these hormones do not regulate the proliferation of mitochondria or the accompanying replication of mitochondrial DNA during development in the immature medulla [8]. Thus, in contrast to the developing liver (in which the proliferation and differentiation of mitochondria are controlled by a common regulatory factor), mitochondrial differentiation and proliferation in developing kidney cells, though efficiently coupled, appear to be controlled by different regulatory factors.

In conclusion, the present study provides additional evidence to support the recent hypothesis that the expression of ubiquitous mitochondrial oxidative enzymes is not constitutive, but rather is controlled by developmental-stage- and tissue-specific mechanisms. We demonstrate that glucocorticoids serve as one of the signals in the developing kidney. Tissue-specific positive or negative regulatory effects of glucocorticoids, and of other endocrine factors, might thus provide a basis to explain developmental changes in the expression of MCAD and other oxidative enzyme genes in organs with high energy demands. Further analysis of the regulatory sequences of these genes should help to delineate the molecular mechanisms involved in the maturation of mitochondrial energy production.

This work was supported by a grant from the Fondation de France. F.D. was supported by a grant from the Fondation pour la Recherche Médicale. D.P.K. is a Lucille P. Markey Scholar, and this work was supported in part by the Markey Foundation, the March of Dimes Birth Defects Foundation, and NIH grant DK 45416. We thank Dr. Colin C. Thorpe for advice concerning the MCAD enzyme assay.

REFERENCES

1 Tanaka, K., Yokota, I., Coates, P. M., Strauss, A. W., Kelly, D. P., Zhang, Z., Gregersen, N., Andersen, B. S., Matsubara, Y., Curtis, D. and Chen, Y. T. (1992) Hum. Mutat. **1**, 271–279
2 Kelly, D. P., Gordon, J. I., Alpers, R. and Strauss, A. W. (1989) J. Biol. Chem. **264**, 18921–18925
3 Hainline, B. E., Kahlenbeck, D. J., Grant, J. and Strauss, A. W. (1993) Biochim. Biophys. Acta **1216**, 460–468
4 Nagao, M., Parimoo, B. and Tanaka, K. (1993) J. Biol. Chem. **268**, 24114–24124
5 Girard, J., Ferré, P., Pegorier, J. P. and Duee, P. H. (1992) Physiol. Rev. **72**, 507–562
6 Henning, S. J. (1981) Am. J. Physiol. **241**, G199–G214
7 Djouadi, F., Wijkhuisen, A. and Bastin, J. (1992) Am. J. Physiol. **263**, F237–F242
8 Djouadi, F., Bastin, J., Gilbert, T., Rötig, A., Rustin, P. and Merlet-Benichou, C. (1994) Am. J. Physiol. **267**, C245–C254
9 Wirthensohn, G. and Guder, W. G. (1986) Physiol. Rev. **66**, 469–489
10 Bastin, J., Delaval, E., Freund, N., Djouadi, F., Razanoelina, M., Bismuth, J. and Geloso, J. P. (1988) Biochem. J. **252**, 337–341
11 Razanoelina, M., Freund, N., Bismuth, J., Geloso, J. P. and Delaval, E. (1991) J. Dev. Physiol. **16**, 283–286
12 Delaval, E., Andriamanantsara, S., Freund, N., Bastin, J. and Geloso, J. P. (1985) J. Dev. Physiol. **7**, 365–372
13 Bastin, J., Djouadi, F., Geloso, J. P. and Merlet-Benichou, C. (1990) Am. J. Physiol. **259**, F895–F901
14 Nahoul, K., Daffos, F., Forestier, F. and Duhennin, L. (1989) J. Steroid Biochem. **33**, 613–619
15 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
16 Kelly, D. P., Kim, J. J., Billadello, J. J., Hainline, B. E., Chu, T. W. and Strauss, A. W. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 4068–4072
17 Grant, P. M., Tellam, J., May, V. L. and Strauss, A. W. (1988) Nucleic Acids Res. **14**, 6053–6066
18 Lehman, T. C., Hale, D. E., Bhala, A. and Thorpe, C. (1990) Anal. Biochem. **186**, 280–284
19 Sierra, F., Tian, J.-M. and Schibler, U. (1993) in Gene Transcription: A Practical Approach (Hames, B. D. and Higgins, S. J., eds.), pp. 125–152, Oxford University Press, Oxford
20 Greenberg, M. E. and Bender, T. P. (1994) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., eds.), pp. 4.10.1–4.10.11, John Wiley & Sons, New York
21 Leone, T. C., Cresci, S., Carter, M. E., Zhang, Z., Lala, D. S., Strauss, A. W. and Kelly, D. P. (1995) J. Biol. Chem. **270**, 16308–16314
22 Carter, M. E., Gulick, T. G., Moore, D. D. and Kelly, D. P. (1994) Mol. Cell. Biol. **14**, 4360–4372
23 Carter, M. E., Gulick, T., Raisher, B. D., Caira, T., Ladias, J. A. A., Moore, D. D. and Kelly, D. P. (1995) J. Biol. Chem. **268**, 13805–13810
24 Celsi, G., Zheng-Ming, W., Akusjarvi, G. and Aperia, A. (1993) Pediatr. Res. **33**, 5–9
25 Nanthakumar, N. N. and Henning, S. J. (1993) Am. J. Physiol. **264**, G306–G311
26 Valcarce, C., Navarette, R. M., Encabo, P., Loeches, E., Satrustegui, J. and Cuezva, J. M. (1988) J. Biol. Chem. **263**, 7767–7775
27 Aprille, J. R. (1990) in Endocrine and Biochemical Development of the Fetus and Neonate (Cuezva, J. M., Pascual-Leone, A. M. and Patel, M. S., eds.), pp. 101–112, Plenum Press, New York
28 Cuezva, J. M., Valcarce, C., Luis, A. M., Izquierdo, J. M., Alconada, A. and Chamorro, M. (1990) in Endocrine and Biochemical Development of the Fetus and Neonate (Cuezva, J. M., Pascual-Leone, A. M. and Patel, M. S., eds.), pp. 113–135, Plenum Press, New York
29 Luis, A. M., Izquierdo, J. M., Ostronoff, L. K., Salinas, M., Santaren, J. F. and Cuezva, J. M. (1993) J. Biol. Chem. **268**, 1868–1875
30 Izquierdo, J. M., Ricart, J., Ostronoff, L. K., Egea, G. and Cuezva, J. M. (1995) J. Biol. Chem. **270**, 10342–10350
31 Izquierdo, J. M., Luis, A. M. and Cuezva, J. M. (1990) J. Biol. Chem. **265**, 9090–9097
32 Izquierdo, J. M. and Cuezva, J. M. (1993) FEBS Lett. **323**, 109–112

Received 8 September 1995/20 November 1995; accepted 11 December 1995