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Expression of Arginase II and Related Enzymes in the Rat Small Intestine and Kidney[1]

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Arginase, which catalyzes the conversion of arginine to urea and ornithine, and consists of a liver-type (arginase I) and a non-hepatic type (arginase II). Arginine is also used for the synthesis of nitric oxide and creatine phosphate, while ornithine is used for the synthesis of polyamines and proline, and thus collagen. Arginase II mRNA and protein are abundant in the intestine (most abundant in the jejunum and less abundant in the ileum, duodenum, and colon) and kidney of the rat. In the kidney, the levels of arginase II mRNA do not change appreciably from 0 to 8 weeks of age. In contrast, arginase II mRNA and protein in the small intestine are not detectable at birth, appear at 3 weeks of age, the weaning period, and their levels increase up to 8 weeks. On the other hand, mRNAs for ornithine aminotransferase (OAT), ornithine decarboxylase, and ornithine carbamoyltransferase (OCT) are present at birth and their levels do not change much during development. Arginase II is elevated in response to a combination of bacterial lipopolysaccharide, dibutyryl cAMP, and dexamethasone in the kidney, but is not affected by these treatments in the small intestine. Immunohistochemical analysis of arginase II, OAT, and OCT in the jejunum revealed their co-localization in absorptive epithelial cells. These results show that the arginase II gene is regulated differentially in the small intestine and kidney, and suggest different roles of the enzyme in these two tissues. The co-localization of arginase II and the three ornithine-utilizing enzymes in the small intestine suggests that the enzyme is involved in the synthesis of proline, polyamines, and/or citrulline in this tissue.

Key words: arginase II, kidney, ornithine aminotransferase, ornithine decarboxylase, small intestine.

Arginase which catalyzes the hydrolysis of arginine to urea and ornithine, consists of two isoforms. Arginase I (liver-type) is expressed almost exclusively in the cytosol of the liver of ureotelic animals and catalyzes the last step of urea synthesis. In mammals, its gene is induced in the late fetal period in coordination with other urea cycle enzymes, and is regulated by dietary protein and hormones such as glucagon and glucocorticoids (*1, 2* for reviews). Its cDNAs and genes have been isolated and their structures determined (*2* for a review). The induction of the rat gene by glucocorticoids is of a delayed secondary type and is mediated by the induction of C/EBPβ (*3*). Recently, we found that arginase I is coinduced with the inducible form of nitric oxide synthase (iNOS) by bacterial lipopolysaccharide (LPS) in cultured rat peritoneal macrophages and in the lung and spleen *in vivo*. These findings suggested a novel role for the enzyme in down-regulating nitric oxide (NO) synthesis in activated macrophages (*4*). Arginase II (non-hepatic type) differs from arginase I in its catalytic and molecular properties. It is primarily expressed in the kidney, small intestine, and lactating mammary gland, and at low levels in other tissues (*5* for a review). The enzyme is located in the mitochondrial matrix (*6, 7*). The cDNAs for human and rat enzyme have been isolated (*6, 8, 9*) and the human gene has been mapped to chromosome 14q24.1-24.3 (*10*). Arginase II, like iNOS, is induced in murine macrophages and macrophage-like RAW 264.7 cells by LPS, suggesting that arginase II, through competition for arginine substrate, may be involved in down-regulating NO production (*6, 11, 12*). In order to better understand the reason for the high expression of this gene in the intestine and kidney, we analyzed the regulation of the genes for rat arginase II and related enzymes in these tissues.

Here, we report that in the kidney, arginase II mRNA levels remain almost unchanged after birth, whereas in the small intestine, the mRNA and protein levels increase markedly as the animal develops. The enzyme level increases in response to LPS, dibutyryl cAMP, and dexamethasone in the kidney, but is not induced by these

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Abbreviations: AS, argininosuccinate synthetase; iNOS, inducible form of nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; OAT, ornithine aminotransferase; OCT, ornithine carbamoyltransferase; ODC, ornithine decarboxylase.

compounds in the small intestine. The immunohistochemical localization of the enzyme in the small intestine reveals its co-localization with ornithine aminotransferase (OAT) and ornithine carbamoyltransferase (OCT) in epithelial cells.

MATERIALS AND METHODS

Plasmids—A nearly full-length cDNA for rat arginase II was isolated by PCR using mRNA from the small intestine of a male Wistar rat. PCR was carried out using rat arginase II primers (sense and antisense primers, 20 mers) corresponding to nucleotides 86-1211 (GenBank, accession number U90887). The product was inserted into the *Hinc*II site of pGEM-3Zf (+) (Promega, Madison, WI, USA), yielding pGEM-rAII-2. A partial cDNA for OAT was isolated by PCR using mRNA from the kidney of a male Wistar rat. PCR was carried out using rat OAT primers (sense and antisense primers, 22 mers) corresponding to nucleotides 670-1180 (GenBank, accession number M11842). The obtained product was inserted into the *Hinc*II site of pGEM-3Zf (+), yielding pGEM-rOAT-1. A partial cDNA for rat ornithine decarboxylase (ODC) was isolated by PCR using mRNA from the kidney of a male Wistar rat. PCR was carried out using rat ODC primers (sense and antisense primers, 22 mers) corresponding to nucleotides 1440-1905 (GenBank, accession number J04791). The product was inserted into the *Hinc*II site of pGEM-3Zf (+), yielding pGEM-rODC-1. The identities of the obtained cDNA plasmids were confirmed by sequencing. The construction of plasmids containing cDNAs for rat arginase I (*4*), rat argininosuccinate synthetase (AS) (*13*), rat OCT (*14*), rat iNOS (*15*), and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) (*16*) has been previously reported.

Antibodies—Two kinds of antibodies against arginase II were prepared in rabbits. One is an antibody against recombinant human arginase II. The putative mature protein of human arginase II (residues 25-354) (*6*) with an NH_2-terminal histidine tag (the QIA expressionist kit, Qiagen, Hilden, Germany) was expressed in *Escherichia coli* M15 (Qiagen). His-tagged arginase II was purified using a nickel NTA column according to the protocol supplied by the manufacturer. The second antibody was raised against a synthetic peptide corresponding to residues 336-354 of rat arginase II (GenBank, accession number U90887), conjugated to keyhole limpet hemocyanin. The preparation of rabbit antisera against human arginase I (*17*), human OCT (*18*), and rat OAT (*19*) and their specificities has been previously reported.

Animals—Specific pathogen-free male (5 weeks of age) and pregnant Wistar strain rats were fed pelleted rat chow (Rabo MR stock, 18.3% protein, Nihon Nohsan Kogyo, Yokohama). The male rats were injected intraperitoneally with *E. coli* LPS (serotype 0127: B8, Sigma) at 20 mg/kg body weight, dexamethasone at 5.0 mg/kg body weight plus dibutyryl cAMP at 25 mg/kg body weight, or with combinations of these reagents. Animals were sacrificed by exsanguination from the abdominal aorta under ether anesthesia.

RNA Blot Analysis—Total RNAs from rat tissues were prepared by the guanidium thiocyanate-phenol-chloroform extraction procedure (*20*). After electrophoresis in formaldehyde-containing agarose gels, the RNAs were transferred to nylon membranes (Schleicher & Schuell, Germany). Hybridization was performed using digoxigenin-labeled antisense RNAs as probes. Chemiluminescence signals derived from hybridized probes were detected on X-ray films using a DIG luminescence detection kit (Boehringer), and the chemiluminescence signals were quantitated with a Mac BAS bioimage analyzer (Fuji Photo Film, Tokyo).

Immunoblot Analysis—Rat tissues were homogenized in 10 volumes of 50 mM Tris-HCl (pH 7.4) containing 300 mM NaCl, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at $25,000 \times g$ for 30 min at 4°C, and the supernatants were used as tissue extracts. The tissue extracts were subjected to SDS-PAGE, and proteins were electrotransferred to nitrocellulose membranes. Immunodetection was performed using an ECL kit (Amersham, Buckinghamshire, UK) according to the protocol supplied by the manufacturer.

Immunohistochemical Staining—Male Wistar rats, weighing 120-150 g, were anesthetized with diethyl ether and perfused for 15 min at room temperature with a fixative solution through the ascending aorta. The fixative solution consisted of 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4. To prepare paraffin sections, tissues were dehydrated through a graded series of ethanol and xylene, and embedded in paraffin. Paraffin sections (2 μm thick) were cut, deparaffinized, washed with PBS, and immunostained. To unmask antigens, paraffin sections were preincubated with 0.05% trypsin at 37°C for 20 min. Trypsin-treated paraffin sections were pretreated with 5 mM periodate for 10 min at room temperature to inhibit endogenous peroxidase activity.

Frozen sections were also prepared. Rats were perfused with PBS through the ascending aorta, and excised tissues were embedded in the OTC compound (Miles, Elkhart, IN) and frozen in liquid nitrogen. Frozen sections (7 μm thick) were cut and air-dried.

Frozen sections and paraffin sections were incubated for 2 h with antiserum or non-immunized serum (control) diluted with PBS containing 0.5% bovine serum albumin (fraction V). After washing with PBS, the sections were incubated with sheep anti-rabbit Ig[$F(ab')_2$] conjugated with peroxidase (Amersham) (100-fold diluted) for 1 h. Peroxidase activity was visualized cytochemically using 3,3′-diaminobenzidine as a substrate. Sections were slightly counterstained with hematoxylin.

RESULTS

Expression of mRNAs for Arginase II and Related Enzymes in Rat Tissues—The distribution of the mRNAs for arginase II and related enzymes in adult rat tissues is shown in Fig. 1. Arginase I mRNA of about 1.7 kb was present almost exclusively in the liver (Fig. 1A). On the other hand, a major mRNA species of about 1.8 kb and a minor species of 4.0 kb for arginase II were abundant in the gut and kidney. In the gut, the mRNA was most abundant in the jejunum, followed by the ileum, duodenum, and colon, but was barely detectable in the stomach.

OAT catalyzes a key step in the interconversion between ornithine, glutamate and proline. OAT mRNAs of about 2.2 and 4 kb were found to be expressed in the small intestine and kidney where arginase II is expressed (Fig. 1B). OAT

mRNA was also found in the liver. ODC catalyzes a key step in polyamine synthesis from ornithine. ODC mRNAs of about 2.2 and 2.6 kb were found to be expressed in the small intestine and less strongly in the kidney and liver.

Immunoblot analysis of arginase isoforms in rat tissues confirms the distribution based on mRNA expression, as shown in Fig. 2. When the antibody against recombinant human arginase I was used, the 34 kDa arginase I protein was detected specifically in the liver; the antibody did not bind to any protein in any of the other tissues (panel A). On the other hand, when antibody against the rat arginase II peptide was used, the arginase II protein of about 36 kDa was detected in the kidney and jejunum (panel B). Although the antibody cross-reacted slightly with some proteins larger than the enzyme, it did not bind to arginase in the liver. Arginase II was much enriched in the mitochondrial fraction of the kidney, consistent with the mitochondrial localization of the enzyme. When the antibody against recombinant human arginase II was used, both arginase I and arginase II were immunodetected (panel C). Thus, the latter antibody preparation cross-reacts with arginase I.

Expression of Arginase II and Related Enzymes in the Small Intestine during Development—Developmental changes of the mRNAs for arginase II and related enzymes in the small intestine were measured (Fig. 3, A and B). Arginase II mRNA was not detectable at birth, appeared at 3 weeks of age, and increased up to 8 weeks of age. On the other hand, OAT mRNA was abundant in the first two

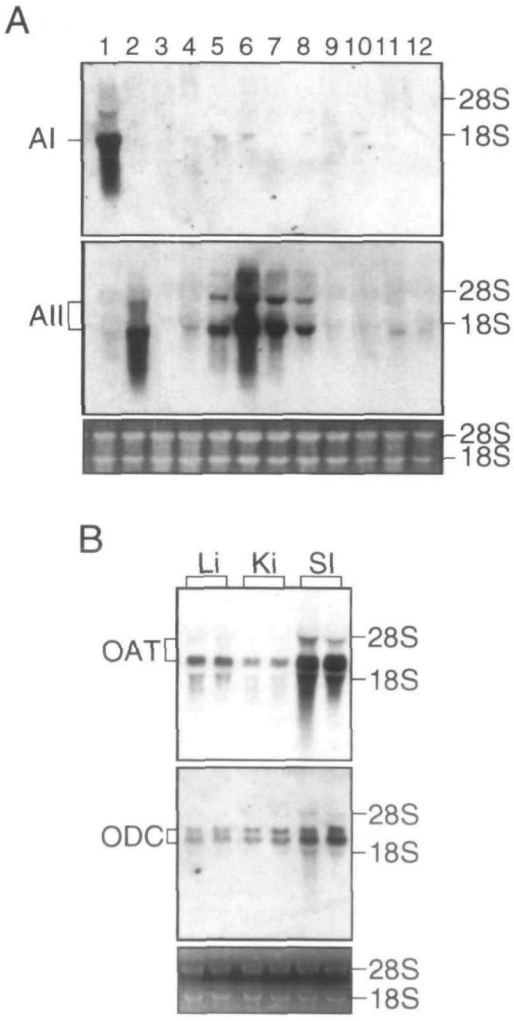


Fig. 1. **Distribution of mRNAs for arginine metabolic enzymes in rat tissues.** (A) Total RNAs (10 μg) from liver (lane 1), kidney (lane 2), spleen (lane 3), stomach (lane 4), duodenum (lane 5), jejunum (lane 6), ileum (lane 7), colon (lane 8), heart (lane 9), lung (lane 10), brain (lane 11), and skeletal muscle (lane 12) were electrophoresed in formaldehyde-containing 1% agarose gels and transferred to nylon membranes. The filters were hybridized using digoxigenin-labeled antisense RNAs for arginase I (AI) or arginase II (AII) as probes. The bottom panel shows ethidium bromide staining of 28S and 18S rRNAs. Similar results were obtained in three independent experiments. (B) Total RNAs (5 μg) from liver (Li), kidney (Ki), and small intestine (SI) were subjected to blot analysis. Hybridization was performed using digoxigenin-labeled antisense RNAs for OAT and ODC as probes. The bottom panel shows ethidium bromide staining of 28S and 18S rRNAs. Two animals were used.

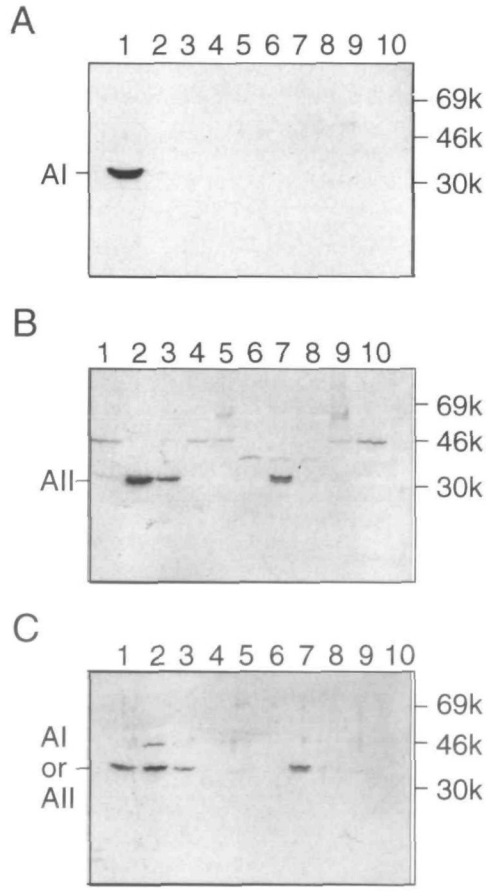
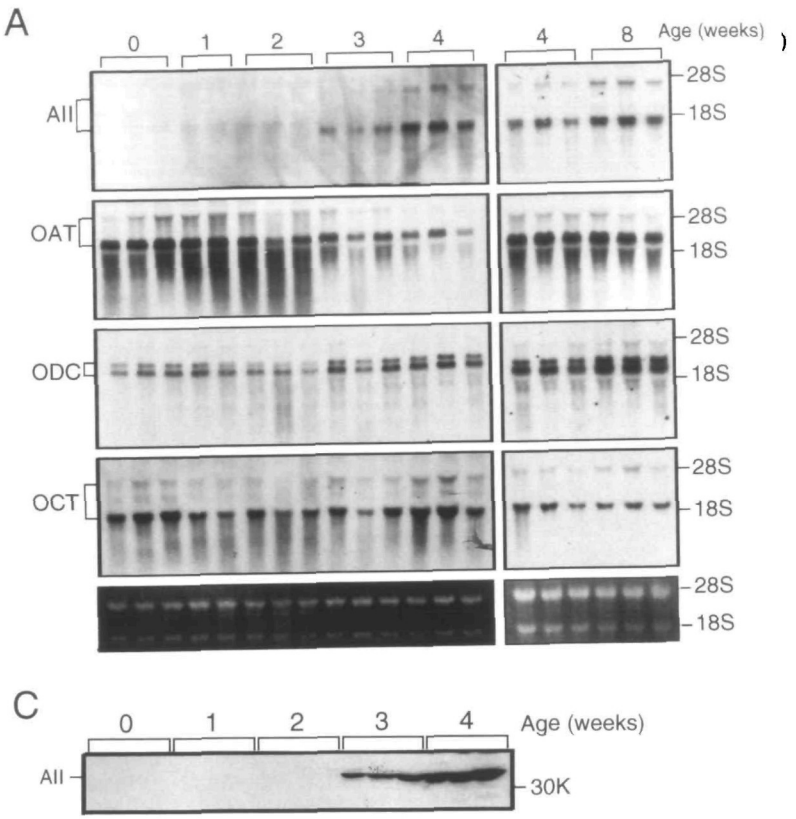


Fig. 2. **Distribution of arginase I and arginase II in rat tissues.** Tissue extracts (50 μg protein) of rat liver (lane 1), kidney (lane 3), spleen (lane 4), stomach (lane 5), duodenum (lane 6), jejunum (lane 7), ileum (lane 8), colon (lane 9), and lung (lane 10) and the mitochondrial fraction of kidney (lane 2; 50 μg protein) were subjected to SDS-10% polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membranes, and the membranes were immunoblotted with rabbit antisera against human arginase I (A), the rat arginase II peptide (B), or recombinant human arginase II (C). Molecular mass markers (Rainbow protein molecular size markers, Amersham) are bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and trypsin inhibitor (21.5 kDa). Similar results were obtained for several blots, except that the faster-migrating band in lane 7 was not seen in other blots and appears to be an artifact.



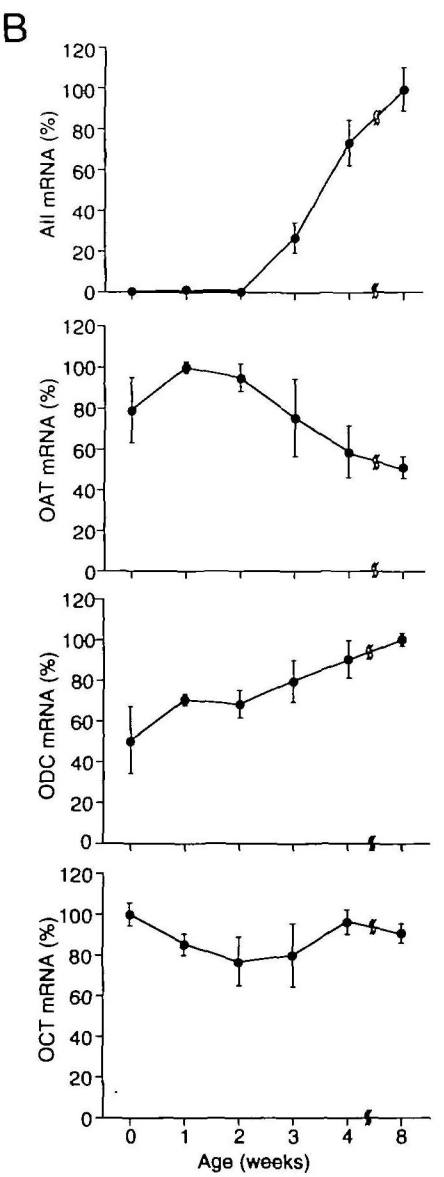


Fig. 3. **Developmental changes of the mRNAs for arginine metabolic enzymes in the rat small intestine.** (A) Total RNAs (5 μg) were extracted from rat small intestine at the indicated ages (weeks). At 0 weeks of age, the tissue was prepared within 5 h of birth. Three animals were used at each time except 1 week of age where two animals were used. Hybridization was performed using digoxigenin-labeled antisense RNAs for arginase II (AII), OAT, ODC, and OCT as probes. The positions of the 18S and 28S rRNAs are shown on the right. The bottom panel shows ethidium bromide staining of the 28S and 18S rRNAs. (B) The results in A were quantitated and represented as means $\pm$ SD ($n=3$), except for 1 week of age where means $\pm$ ranges ($n=2$) are shown. Maximal values are set at 100%. (C) Extracts of rat small intestine (50 μg protein) were subjected to immunoblot analysis using the antiserum against the arginase II peptide, as described for Fig. 2. Three animals were used at each time.

weeks and decreased slowly thereafter. ODC mRNA increased gradually after birth up to 8 weeks of age. The mRNA for OCT, which synthesizes citrulline from ornithine and carbamoyl phosphate, remained almost unchanged during development.

The induction of the arginase II protein during development was examined by immunoblot analysis (Fig. 3C). The enzyme protein was undetectable at birth, appeared at 3 weeks of age, and increased at 4 weeks of age. Therefore, both the arginase II mRNA and protein are induced with similar kinetics during development.

Expression of mRNAs for Arginase II and AS in the Kidney during Development—Developmental changes of the mRNAs for arginase II and AS, a key enzyme in arginine biosynthesis in the kidney, are shown in Fig. 4. In sharp contrast to the small intestine, arginase II mRNA in the kidney was found to be abundant at birth and to remain unchanged up to 4 weeks of age. Similarly, AS mRNA also changed very little during development.

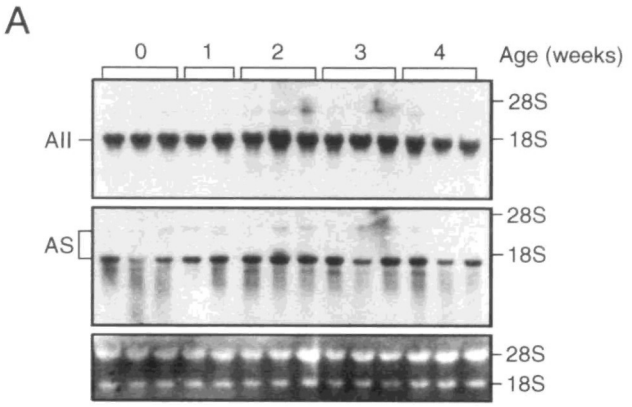
The Immunohistochemical Localization of Arginase II in the Small Intestine—Figure 5 shows the immunostaining of arginase II, OAT, and OCT in the jejunum. Arginase II immunoreactivity appeared strong in the epithelial cells of villi, but was apparently absent from smooth muscle cells, lymphocytes, and plasma cells (panels a and b). Among epithelial cells, enterocytes were strongly positive for arginase II whereas the cytoplasm of goblet cells weakly positive. Mucous granules were negative. In enterocytes, small particulate structures in the cytoplasm were stained, reflecting the mitochondrial localization of the enzyme. OAT and OCT were also stained specifically in enterocytes (panels c and d). Particulate structures similar to those positive for arginase II immunostaining were observed in the cytoplasm. Thus, arginase II, OAT, and OCT co-localize in the mitochondria of epithelial cells of the small intestine.

Induction of Arginase II in the Kidney by LPS, Dibutyryl cAMP, and Dexamethasone—We found previously that the arginase II mRNA, like the iNOS mRNA, is induced in murine macrophage-like RAW 264.7 cells by LPS, and that this induction is enhanced by dexamethasone and dibutyryl

cAMP (*6*). We therefore asked whether the arginase II mRNA in the small intestine and kidney is affected by these reagents. Rats were injected intraperitoneally with LPS or dibutyryl cAMP plus dexamethasone or their combination, and the mRNAs for arginase II and related enzymes were determined by RNA blot analysis.

In the jejunum, when all these reagents were injected, the amount of iNOS mRNA increased 2 h after the treatment, was lower at 6 h, and became undetectable at 12 h (Fig. 6). A higher induction of iNOS mRNA was seen when LPS alone was injected (data not shown). On the other hand, the arginase II mRNA was little affected by these reagents.

In the kidney of rats treated with all these reagents, the iNOS mRNA appeared at 2 h, increased at 6 h, and disappeared at 12 h (Fig. 7, A and B). A higher induction of iNOS mRNA was seen when LPS alone was injected (data not shown). In contrast to the jejunum, arginase II mRNA in the kidney was induced by LPS or by dibutyryl cAMP



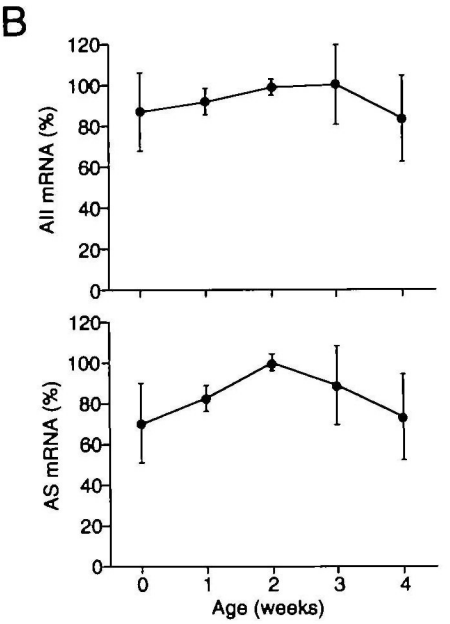


Fig. 4. **Developmental changes of the mRNAs for arginase II and AS in the kidney.** (A) Total RNAs (5 μg) from the kidneys of rats at the indicated ages (weeks) were subjected to blot analysis. Hybridization was performed using antisense RNAs for arginase II (AII) and AS as probes. The positions of the 18S and 28S rRNAs are shown on the right. (B) The results in A were quantitated and represented as means$\pm$SD ($n=3$) except for 1 week of age where means$\pm$ranges ($n=2$) are shown. Maximal values are set at 100%.

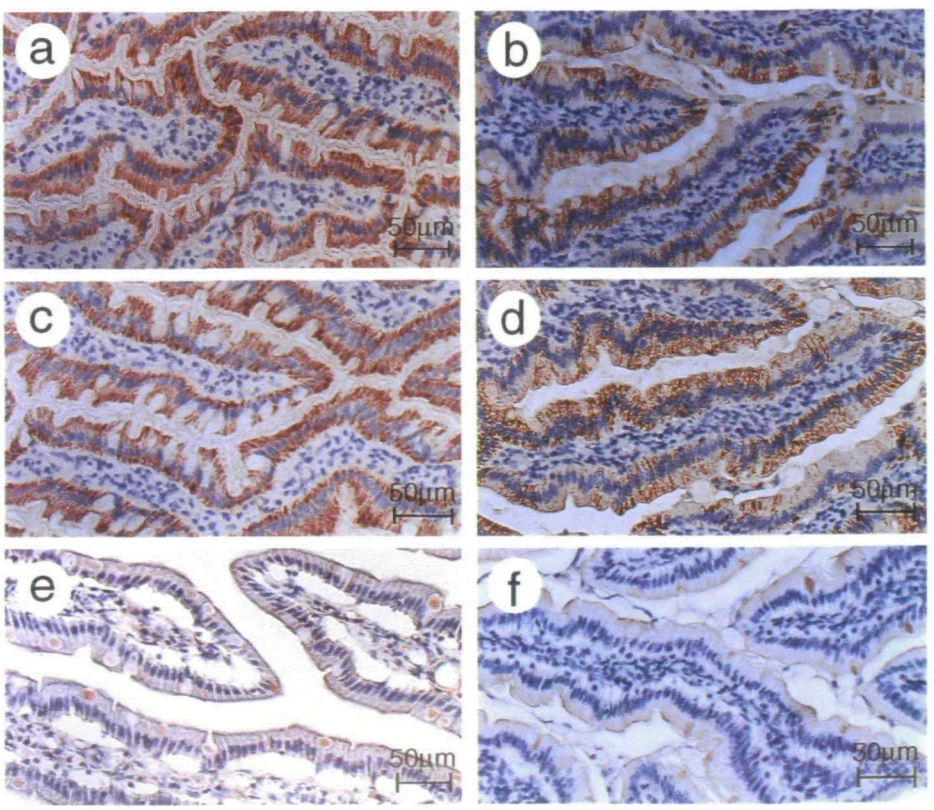


Fig. 5. **Immunostaining of arginase II in the rat jejunum.** Rat jejunum was immunostained with rabbit antisera against the rat arginase II peptide (200-fold diluted) (a), recombinant human arginase II (500-fold diluted) (b), rat OAT (500-fold diluted) (c), or human OCT (500-fold diluted) (d) as described in "MATERIALS AND METHODS." Non-immune rabbit serum was used as control (e and f). a, c, and e are frozen sections, and b, d, and f are paraffin sections. Original magnifications: $\times$200. Bar: 50 μm. Similar immunostainings were observed in several independent experiments.

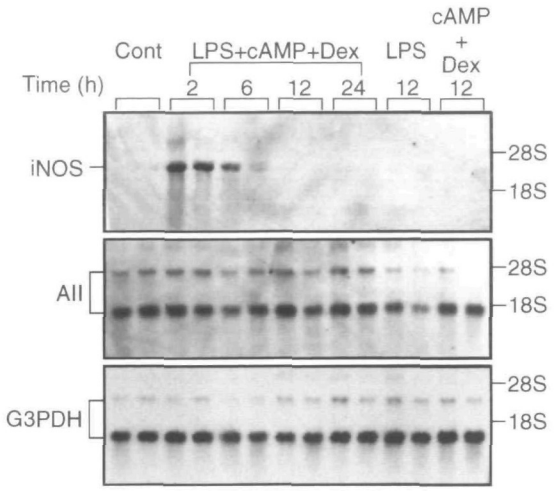


Fig. 6. **Effect of LPS, dibutyryl cAMP, and dexamethasone on arginase II mRNA in the rat jejunum.** Rats were injected with combinations of *E. coli* LPS, dibutyryl cAMP (cAMP), and dexamethasone (Dex) as described in "MATERIALS AND METHODS," and total RNAs were isolated from the jejunum after the indicated periods. The RNAs (2.0 μg) were subjected to blot analysis for iNOS and arginase II mRNA (AII). G3PDH mRNA was used as a control. The positions of the 18S and 28S rRNAs are shown on the right. The mRNAs of each lane were obtained from different rats.

plus dexamethasone, and more strongly by their combination. When all these reagents were injected, the amount of arginase II mRNA increased up to 12 h and decreased slightly at 24 h. On the other hand, OAT mRNA was little affected by these reagents. ODC mRNA was not affected by LPS but was induced by dibutyryl cAMP and dexamethasone, or a combination of the three reagents. When the animals were injected with all three reagents, ODC mRNA was induced rapidly and reached a plateau 6 h after injection. Thus, the induction of ODC mRNA roughly resembles that of arginase II mRNA.

The effect of the reagents on the arginase II protein in the kidney was examined by immunoblot analysis (Fig. 7C). The induction pattern of the enzyme protein was similar to that of the mRNA, except that the induction of the protein by the combination of the three reagents was slower than that of the mRNA (compare to Fig. 7, A and B).

DISCUSSION

Arginase II is expressed most strongly in the small intestine and kidney in adult rats. However, our present study shows that the developmental regulation of the gene differs markedly between these two tissues. The enzyme mRNA and protein are dramatically induced at 3-4 weeks of age in the small intestine, whereas the mRNA levels remains almost unchanged up to 8 weeks of age in the kidney. Konarska and Tomaszewski (*21*) have similarly reported that arginase activity in the rat small intestine is rapidly induced at 3-5 weeks of age. The present results show that the induction of arginase activity is brought about by the induction of the arginase II mRNA and protein, and presumably to transcriptional activation of the enzyme gene. These results suggest that the roles of arginase II in these two tissues are quite different. The rapid induction of the arginase II mRNA at 3-4 weeks of age is associated with the change of diet from milk to laboratory chow. The marked changes in arginase II expression contrast with the changes in the three ornithine-utilizing enzymes in the small intestine, as OAT mRNA levels decrease slightly during development, ODC mRNA levels increase slightly, and OCT mRNA levels remain unchanged. Therefore, there is no close co-relationship between arginase II mRNA and any of the mRNAs for the three ornithine-utilizing enzymes. Quite recently, De Jonge *et al.* (*22*) analyzed the developmental expression of arginine-metabolizing enzymes in the rat small intestine and found that the arginine-biosynthetic enzymes AS and argininosuccinate lyase are abundant at birth and decrease to hardly detectable levels in two weeks after birth. They also reported that the AS protein is located in the enterocytes of new-born rats, and that the enzyme is concentrated in the upper half of the villus. Based on these results, they suggested that arginine biosynthesis from citrulline most likely takes place in the small intestine rather than in the kidney before weaning. After weaning, arginine will be taken up by the small intestine and utilized by the newly-induced arginase II. The present study shows that arginase II is localized in the mucosal epithelial cells of the small intestine where OCT (*23*, this paper), OAT (*19, 24*, this paper), and ODC (*25*) are also present. Therefore, the ornithine that is formed by arginase II may be utilized for the synthesis of citrulline, proline (and thus collagen), and polyamines by OCT, OAT, and ODC, respectively. Among the three ornithine-utilizing enzymes, OCT and OAT are located in the mitochondrial matrix where arginase II is present, whereas ODC is located in the cytosol. Therefore, ornithine formed by the arginase II reaction must be transferred from the mitochondria to the cytosol to be utilized by ODC for polyamine synthesis.

Microdissection studies of rat kidney by Levillain and Hus-Citharel (*26*) showed that urea production from arginine takes place mainly in the proximal straight tubules and in the collecting ducts. Our recent immunohistochemical studies (*27*) demonstrated that arginase II is present in the proximal straight tubules of the outer stripe of the outer medulla and in a subpopulation of the proximal tubules in the cortex of the rat kidney. The distribution of the arginase II protein in the outer stripe of the outer medulla is very similar to that of the OAT protein (*27*) and ODC mRNA (*28, 29*). These results suggest that arginase II in the proximal tubules supplies ornithine for proline and polyamine synthesis in this tissue. However, Levillain and Hus-Citharel (*26*) reported recently that the highest ODC activity is found in the proximal convoluted tubules, a segment devoid of arginase. The reason for this discrepancy in the localization of the ODC mRNA and activity in the kidney is unknown.

Not only the arginase II gene is developmentally regulated differently in the small intestine and kidney, but it is also regulated differently by pleiotropic agents. In the small intestine, the arginase II mRNA shows little change in response to LPS or dexamethasone plus dibutyryl cAMP or their combination. Under these conditions, the iNOS mRNA is induced. In the kidney, on the other hand, the arginase II mRNA and protein are induced by LPS or dexamethasone plus dibutyryl cAMP, or their combination. The iNOS mRNA is also induced. If these two enzymes are induced in the same cells, arginase II may compete with

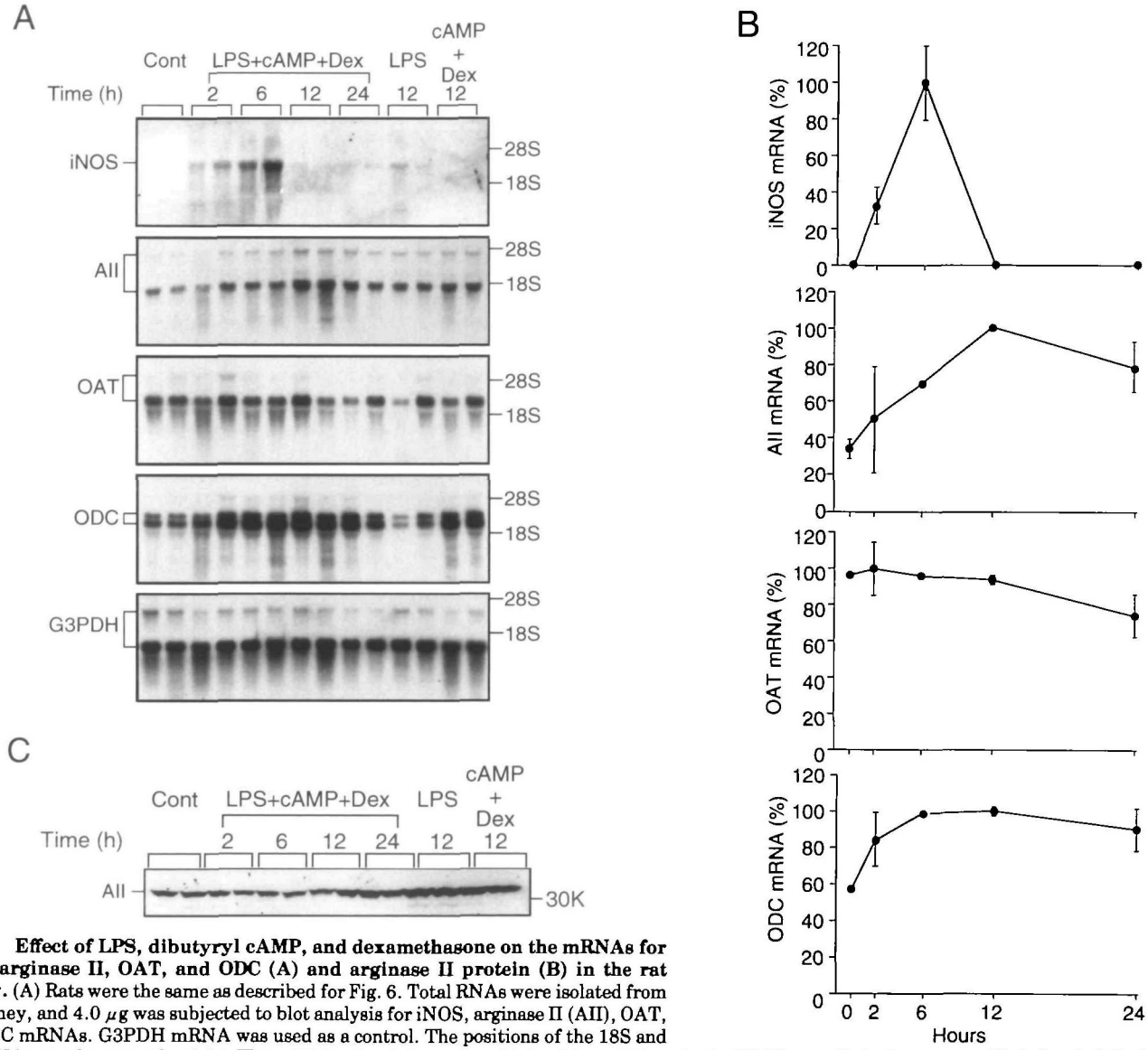


Fig. 7. **Effect of LPS, dibutyryl cAMP, and dexamethasone on the mRNAs for iNOS, arginase II, OAT, and ODC (A) and arginase II protein (B) in the rat kidney.** (A) Rats were the same as described for Fig. 6. Total RNAs were isolated from the kidney, and 4.0 μg was subjected to blot analysis for iNOS, arginase II (AII), OAT, and ODC mRNAs. G3PDH mRNA was used as a control. The positions of the 18S and 28S rRNAs are shown on the right. The mRNAs of each lane were obtained from different rats. (B) The results in A were quantitated and plotted as means $\pm$ ranges ($n=2$). Maximal values are set at 100%. (C) Extracts of rat kidney (50 μg protein) were subjected to immunoblot analysis using antiserum against the arginase II peptide, as described for Fig. 2.

iNOS for arginine and down-regulate NO production. Immunohistochemical analysis of these enzymes in the kidney of rats treated with these reagents remains to be performed. Assuming that arginase II and ODC are induced in the same cells, arginase II appears to be capable of acting in concert with ODC in polyamine synthesis under these conditions. It remains to be studied whether and why polyamine synthesis is stimulated by these reagents in the kidney.

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