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Alternate Promoters in the Rat Aromatic L-Amino Acid Decarboxylase Gene for Neuronal and Nonneuronal Expression: An In Situ Hybridization Study

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Abstract: Aromatic L-amino acid decarboxylase (AADC) is found in both neuronal cells and nonneuronal cells, and a single gene encodes rat AADC in both neuronal and nonneuronal tissues. However, two cDNAs for this enzyme have been identified: one from the liver and the other from pheochromocytoma. Exons 1a and 1b are found in the liver cDNA and the pheochromocytoma cDNA, respectively. In the third exon (exon 2), there are two alternatively utilized splicing acceptors specific to these exons, 1a and 1b. Structural analysis of the rat AADC gene showed that both alternative promoter usage and alternative splicing are operative for the differential expression of this gene. To demonstrate whether alternative promoter usage and splicing are tissue specific and whether the exons 1a and 1b are differentially and specifically transcribed in nonneuronal and neuronal cells, respectively, in situ hybridization histochemistry for the rat brain, adrenal gland, liver, and kidney was carried out using these two exon probes. The exon 1a probe specifically identified AADC mRNA only in nonneuronal cells, including the liver and kidney, and the exon 1b probe localized AADC mRNA to monoaminergic neurons in the CNS and the adrenal medulla. Thus, both alternative promoter usage and differential splicing are in fact operative for the tissue-specific expression of the rat AADC gene. **Key Words:** Aromatic L-amino acid decarboxylase—In situ hybridization—Splicing—Two promoter usage—Neuronal and nonneuronal expression.
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Aromatic L-amino acid decarboxylase (AADC) catalyzes the second step in the biosynthesis of catecholamines as well as serotonin. The enzyme is expressed both in monoamine-producing neuronal cells and in kidney, liver, and thymus cells (Christenson et al., 1972). It was previously reported that rat AADC is encoded by a single gene in both neuronal and nonneuronal tissues (Albert et al., 1987). However, two rat AADC cDNAs, one from the liver (Tanaka et al., 1989) and the other from pheochromocytomas (Krieger et al., 1991), have been isolated, and they differ only in 5′ untranslated sequences. Recent publications (Hahn et al., 1991, 1993; Albert et al., 1992) showed that the rat AADC gene is composed of two promoters and 16 exons spanning >80 kb in the genome. The first exon (exon 1a) carries the majority of the 5′ untranslated sequence of the liver cDNA, and the second exon (exon 1b) carries that of the pheochromocytoma cDNA (Hahn et al., 1993). In the third exon, there are two alternatively utilized splicing acceptors specific to the first and second exons. Therefore, both alternative promoter usage and alternative splicing may be operative for the differential expression of this gene (Hahn et al., 1993). In spite of these findings, cellular and tissue-specific localization and the alternative usage of exons 1a and 1b for the differential expression of this gene in neuronal and nonneuronal cell types have not been experimentally demonstrated.

In the present report, we show with in situ hybridization histochemistry of rat tissues that the exon 1a probe exclusively hybridizes to nonneuronal cells and that exon 1b only hybridizes to neuronal cells. The rat AADC cDNA hybridized to both types of cells, and polyclonal antibodies to rat AADC uniformly identified these cells as well.

MATERIALS AND METHODS

Animals

Sprague–Dawley male rats weighing 200–225 *g* obtained from Charles River (Boston, MA, U.S.A.) were used.

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Abbreviations used: AADC, aromatic L-amino acid decarboxylase; ADM, adrenal medulla; LC, locus ceruleus; PBS, phosphate-buffered saline; RN, raphe nucleus; SN, substantia nigra; SSC, sodium chloride–sodium citrate.

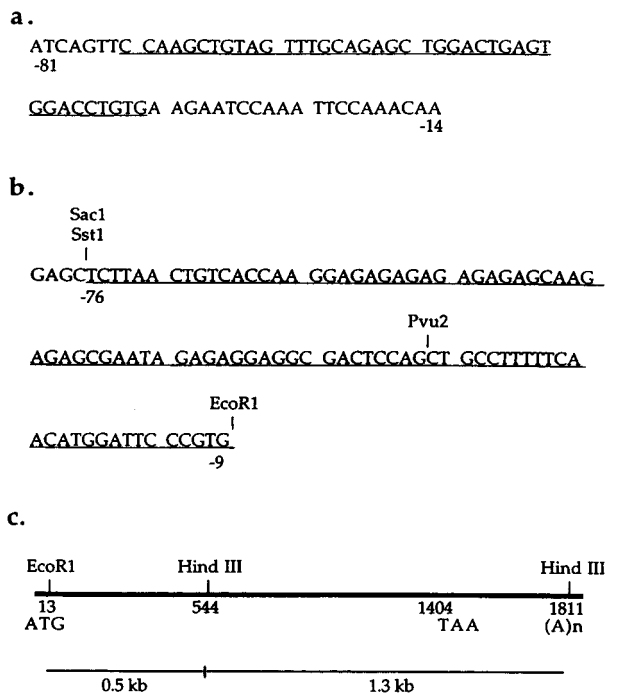

FIG. 1. Rat AADC probes used for in situ hybridization. **a:** Forty-mer oligonucleotides (underlined) of exon 1a. **b:** *Sac*I–*Eco*RI fragment of exon 1b. **c:** Combination of 0.5 and 1.3 kb of AADC cDNA, which covers total AADC cDNA.

Probes for in situ hybridization

A 40-mer oligonucleotide sequence of exon 1a as shown in Fig. 1a was used as the exon 1a probe, and the exon 1b probe was the total exon 1b sequence that was obtained by digestion with *Sst*I and *Eco*RI (Fig. 1b). The cDNA probe consisted of a mixture of 0.5- and 1.3-kb AADC cDNA fragments as shown in Fig. 1c.

In situ hybridization

To demonstrate whether exon 1a is exclusively localized in nonneuronal cells of the liver and the kidney and exon 1b only in neuronal cells, exon 1a and 1b probes were used for in situ hybridization of the following monoaminergic neurons and cells: the substantia nigra (SN) (dopaminergic neurons), the locus ceruleus (LC) (noradrenergic neurons), the raphe nucleus (RN) (serotonergic neurons), and adrenal medulla (ADM) (catecholaminergic cells). For in situ hybridization of nonneuronal cells, the liver and the kidney were used. Furthermore, an AADC cDNA probe was also used to confirm the identical coding sequence of the remaining AADC exons.

A detailed procedure for in situ hybridization was described in our previous publication (Weiser et al., 1993). In brief, animals were deeply anesthetized with sodium pentobarbital (120 mg/kg) and perfused transcardially with saline containing 0.5% sodium nitrate and 10 U/ml of heparin sulfate followed by cold formaldehyde in 0.1 *M* sodium phosphate buffer, pH 7.2. The brains were postfixed in the fixative for 1 h and stored in 30% sucrose overnight. Free-floating sections (40 μm), obtained on a freezing microtome, were placed in vials containing 2 × sodium chloride–sodium citrate (SSC; 1 × SSC is 0.15 *M* NaCl and 0.015 *M* sodium

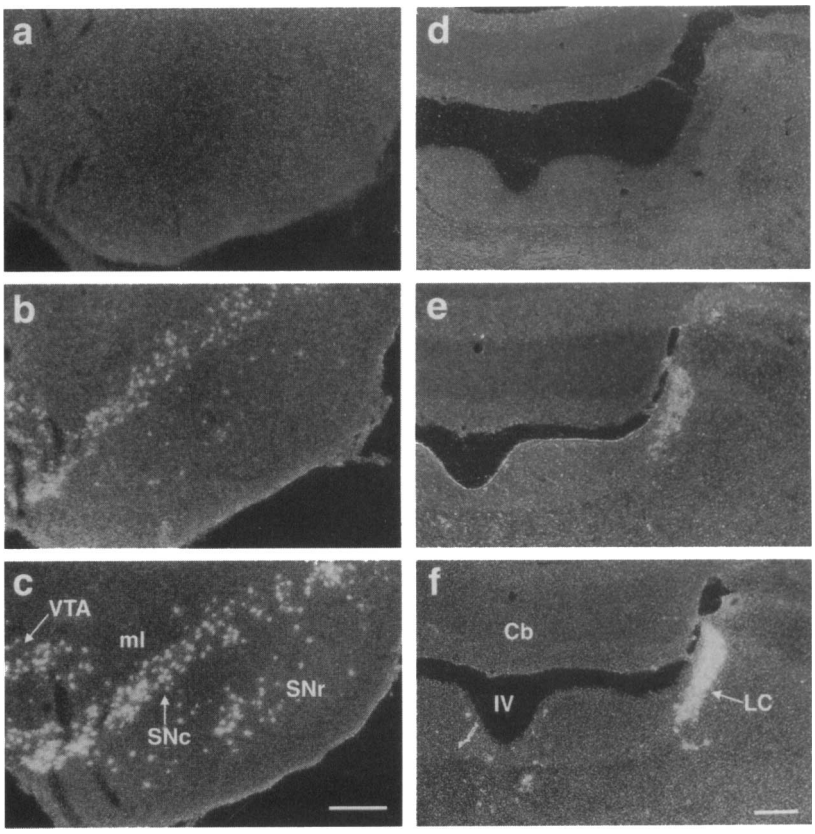

FIG. 2. Dark-field photoemulsion autoradiograms of coronal sections at the level of the SN and the LC by in situ hybridization with nonneuronal (exon 1a) and neuronal (exon 1b) probes and the cDNA probe of rat AADC. **a:** SN with exon 1a probe. **b:** SN with exon 1b probe. **c:** SN with cDNA probe. **d:** LC with exon 1a probe. **e:** LC with exon 1b probe. **f:** LC with cDNA probe. The nonneuronal probe of exon 1a did not hybridize to neuronal cells (a and d), but the neuronal probe of exon 1b and the cDNA probe specifically hybridized to neuronal cells (b, c, e, and f). SNc, SN pars compacta; SNr, SN pars reticulata; ml, medial lemniscus; VTA, ventral tegmental area; IV, fourth ventricle; Cb, cerebellum. Bar = 0.1 mm.

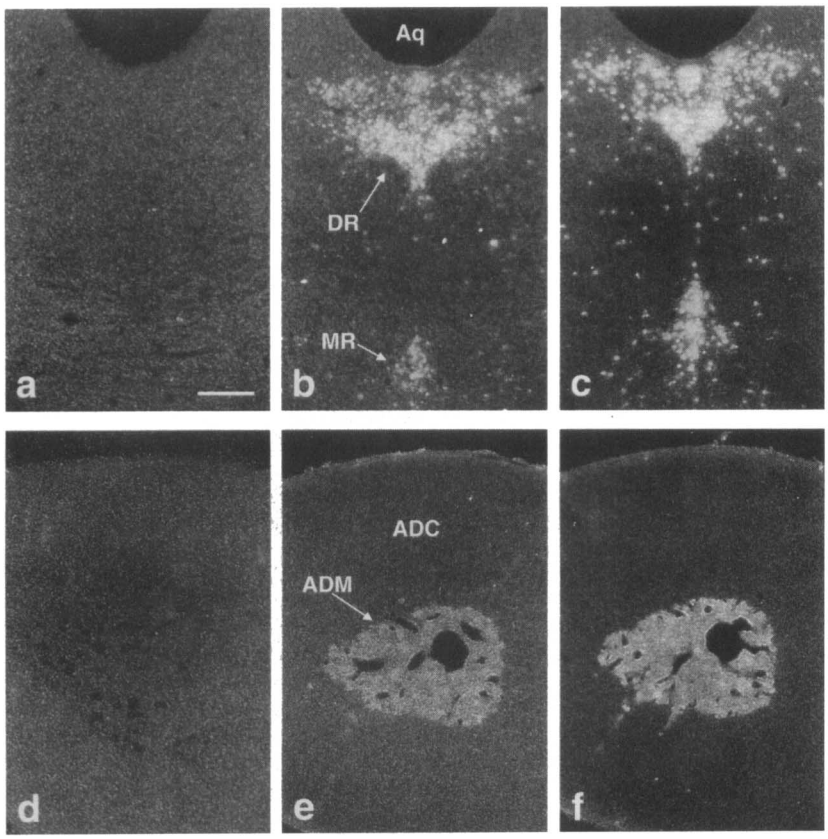

FIG. 3. Dark-field photoemulsion autoradiograms of coronal sections at the level of the RN and cross-sections of the adrenal gland by in situ hybridization with nonneuronal (exon 1a) and neuronal (exon 1b) probes and the cDNA probe of rat AADC. **a:** RN with exon 1a probe. **b:** RN with exon 1b probe. **c:** RN with cDNA probe. **d:** Adrenal gland with exon 1a probe. **e:** Adrenal gland with exon 1b probe. **f:** Adrenal gland with cDNA probe. DR, dorsal raphe; MR, median raphe; Aq, aqueduct; ADC, adrenal cortex. Bar = 0.1 mm.

citrate) and 50 m*M* dithiothreitol. Tissues were prehybridized in 50% formamide, 10% dextran, 2 × SSC, 1 × Denhardt's solution, 10 m*M* dithiothreitol, and 0.5 mg/ml of sonicated and denatured salmon sperm DNA. Denatured ^{35}S-ATP-labeled cDNA probe or oligo probes were added to the vial (10,000,000 cpm per vial), and hybridization was carried out overnight at 48°C for cDNA and 37°C for oligo probes. The sections were washed in serial dilutions of SSC at 48°C for cDNA and 37°C for oligo probes, starting with 2 × SSC and ending with 0.1 × SSC. After a 15-min wash in 0.05 *M* phosphate buffer, sections were mounted and dehydrated. For determination of optimal developing time, slides were opposed to Kodak XAR-5 film for 48–72 h at 4°C. Slides were subsequently dipped in Kodak NTB-2 emulsion and exposed at 4°C for 7 days. After developing in Kodak D-19 developer at 16°C, sections were fixed in Kodak fixer, counterstained with cresyl violet, dehydrated, and coverslipped.

Immunohistochemistry

Polyclonal bovine AADC antibodies (1:30,000 dilution) raised in rabbits (Albert et al., 1987) were used for immunohistochemistry of AADC. The procedure for immunohistochemistry has been described in detail elsewhere (Weiser et al., 1993). In brief, animals were perfused and brain sections were obtained as above. Free-floating sections (40 μm), obtained on a freezing microtome, were washed for 30 min in 0.1 *M* sodium phosphate-buffered saline (PBS) and incubated with 1% bovine serum albumin and 0.2% Triton X in 0.1 *M* PBS. Sections were washed in PBS containing 0.5% bovine serum albumin and incubated overnight with AADC antibody or preimmune serum (1:30,000). Sections were washed in PBS containing bovine serum albumin and incubated for 1 h with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, U.S.A.). The tissue was washed and incubated for 1 h with avidin–biotin–horseradish peroxidase complex according to the Vector kit instructions (Vector Laboratories). The antibodies were visualized by reaction with 3,3′-diaminobenzidine tetrahydrochloride as a chromogen and 0.003% hydrogen peroxide for 5 min. Sections were mounted on gelatin-coated slides, dehydrated through graded ethanols, and coverslipped with Permount.

RESULTS

In situ hybridization for neuronal cells

The AADC gene is expressed in monoaminergic neurons of the CNS and in chromaffin cells of the ADM. As depicted in Figs. 2 and 3, the exon 1a probe (nonneuronal) did not hybridize to monoaminergic cells in the CNS (Figs. 2a and d and 3a) or the ADM (Fig. 3d). In contrast, the exon 1b probe (neuronal) strongly hybridized to all monoamine-producing cells, which include dopaminergic neurons in the SN (Fig. 2b), noradrenergic neurons in the LC (Fig. 2e), serotonergic cells in the RN (Fig. 3b), and chromaffin cells in the ADM (Fig. 3e).

In situ hybridization using the cDNA probe of AADC hybridized with identical patterns to the exon 1b probe for neuronal cells. All monoamine-producing

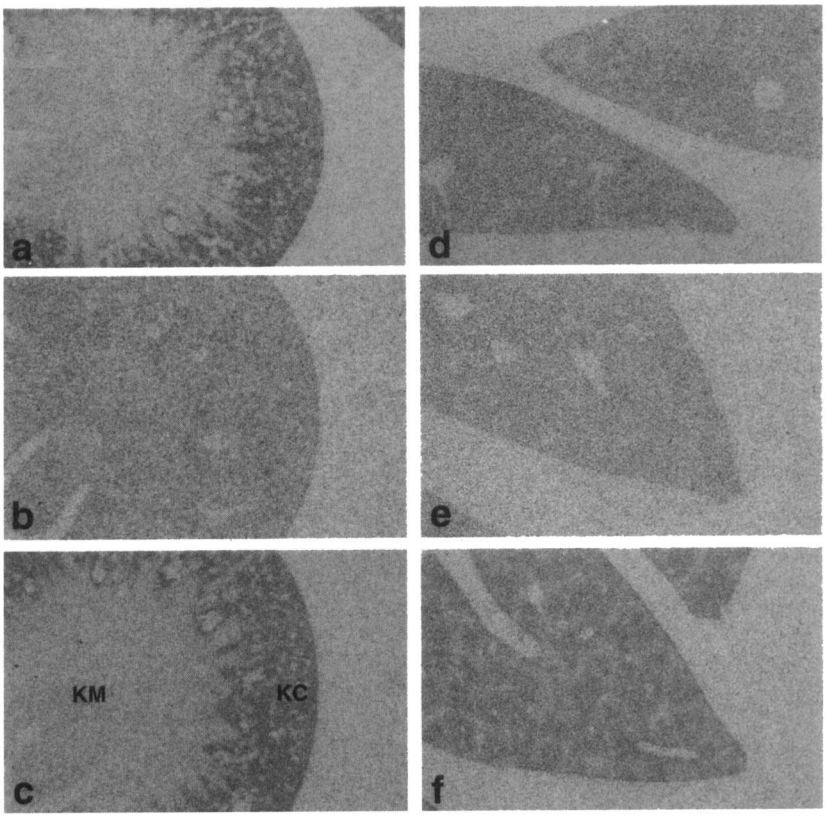

FIG. 4. Bright-field x-ray film autoradiograms of kidney and liver cross-sections by in situ hybridization with nonneuronal (exon 1a) and neuronal (exon 1b) probes and the cDNA probe of rat AADC. **a:** Kidney with exon 1a probe. **b:** Kidney with exon 1b probe. **c:** Kidney with cDNA probe. **d:** Liver with exon 1a probe. **e:** Liver with exon 1b probe. **f:** Liver with cDNA probe. The nonneuronal probe of exon 1a and the cDNA probe specifically hybridized to kidney cortex and liver (a, c, d, and f), but the neuronal probe exon 1b did not hybridize to nonneuronal cells (b and e). KC, kidney cortex; KM, kidney medulla.

cells were strongly positive with the cDNA probe (as depicted in Figs. 2c and f and 3c and f).

In situ hybridization of nonneuronal cells

The probe for exon 1a (nonneuronal) was used to demonstrate specific hybridization to only nonneuronal AADC mRNA. This probe hybridized to cells in the kidney cortex (Fig. 4a) and diffusely in the liver (Fig. 4d). In contrast, the neuron-specific exon 1b probe did not detect AADC mRNA in these tissues (Fig. 4b and e). The cDNA probe hybridized strongly with AADC mRNA in these nonneuronal cells (Fig. 4c and f).

Immunohistochemistry

To confirm that these positively hybridized cells produce AADC protein, immunohistochemical localization of AADC with the specific AADC antibody was carried out. Figure 5 depicts the pattern of immunostaining in all of these monoamine-producing neuronal cells. For nonneuronal cells in the kidney and liver, the AADC antibody detected AADC; however, the staining was not as clear as in neuronal cells. With the antiserum, AADC-positive cells were detected in the kidney cortex (Fig. 6b), whereas control serum did not stain (Fig. 6a). As AADC was detected in the vast majority of liver cells and antibody staining was very weak, it was difficult to distinguish and to compare the specific staining of AADC in liver cells with the antibody or control serum (data not shown).

DISCUSSION

The gene structure and *cis*-acting elements of the promoter region of *Drosophila* AADC (termed dopa-decarboxylase) have been extensively investigated (Bray and Hirsh, 1986; Scholnick et al., 1986; Johnson et al., 1989). The *Drosophila* AADC gene is 4 kb long and contains four exons. Two forms of *Drosophila* AADC, the brain and hypodermal forms, are produced from two different mRNAs that result from alternative splicing (Eveleth et al., 1986; Morgan et al., 1986). However, our previous report revealed a wide divergence of the rat AADC gene from the *Drosophila* AADC gene (Hahn et al., 1991). In the mammalian AADC gene, only one form of coding sequence equivalent to the hypodermal form of the *Drosophila* AADC gene has been identified (Albert et al., 1987; Ichinose et al., 1989; Tanaka et al., 1989; Kang and Joh, 1990; Taketoshi et al., 1990; Krieger et al., 1991; Sumi-Ichinose et al., 1992; Le Van Thai et al., 1993). The rat (Albert et al., 1992; Hahn et al., 1993) and human (Ichinose et al., 1992; Sumi-Ichinose et al., 1992; Le Van Thai et al., 1993) AADC genes undergo similar splicing within their 5′ untranslated regions, and two different AADC transcripts have identical coding regions. Based on the following observations, Krieger et al. (1991) suggested alternative splicing and alternative promoter usage of the rat AADC gene: (a) The rat AADC is identical in neuronal and nonneuronal

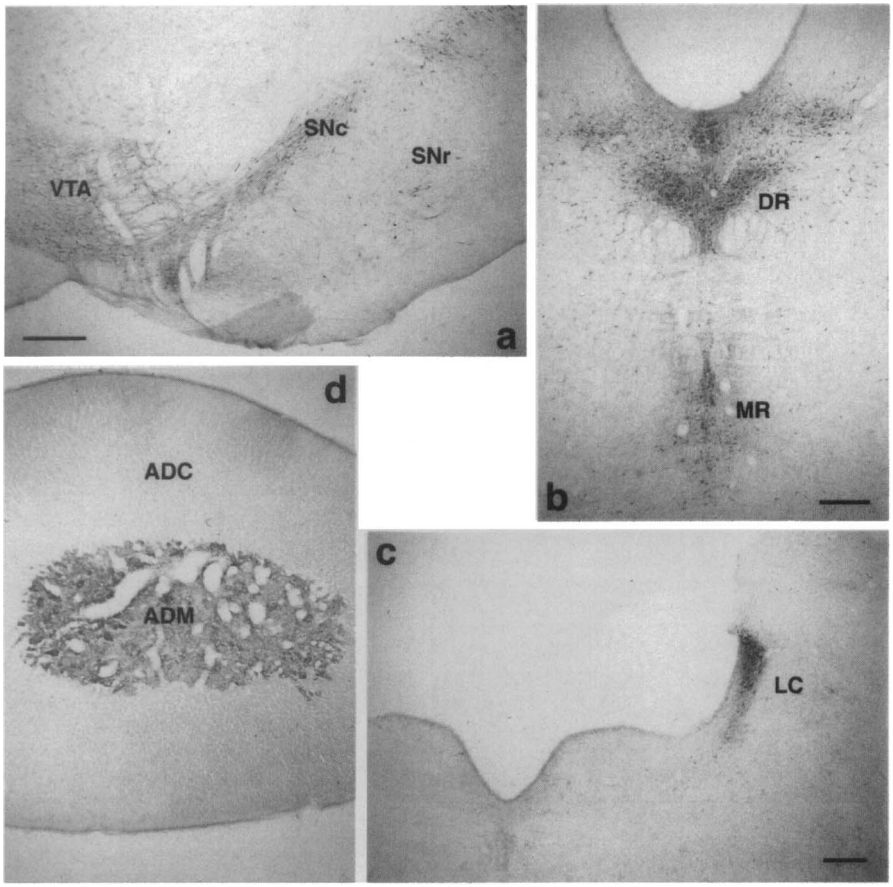

FIG. 5. Bright-field immunohistochemical localization of AADC in neuronal cells. **a:** Dopamine neurons in the SN and the ventral tegmental area. **b:** Serotonin neurons in the RN. **c:** Noradrenergic neurons in the LC. **d:** The ADM. Abbreviations are as in Figs. 2 and 3. Bar = 0.1 mm.

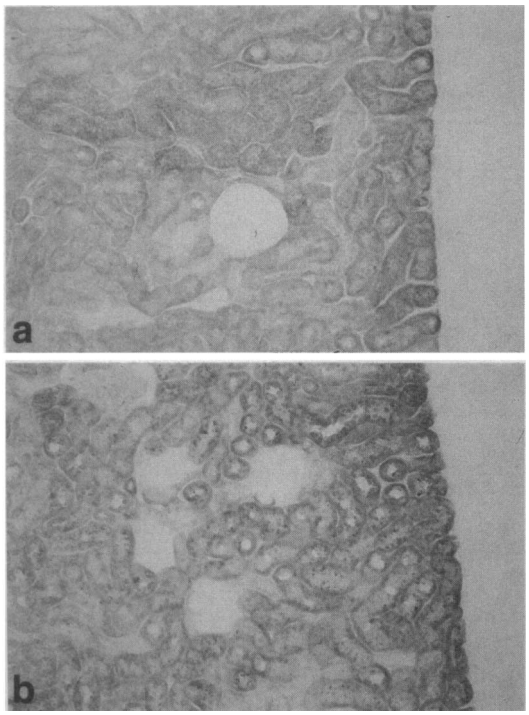

FIG. 6. Immunohistochemical localization of AADC in kidney cortex cells (**a**) with control serum and (**b**) with polyclonal anti-AADC serum.

cells (Christenson et al., 1972); (b) a single gene may code for rat AADC (Albert et al., 1987); and (c) two reported rat AADC cDNA sequences, the liver and pheochromocytoma forms (Tanaka et al., 1989; Krieger et al., 1991), differ from each other only at the 5′ untranslated region. The structural analysis of the rat AADC gene confirmed the alternative promoter usage of the first two exons [exon 1a and 1b in Hahn et al. (1993) and exon 1 and 2 in Albert et al. (1992)].

In the present study, we show for the first time the cellular and tissue-specific localization of the exon 1a and 1b transcription products by in situ hybridization and confirm the differential expression of this gene in neuronal and nonneuronal cell types. This characteristic difference between neuronal and nonneuronal cell types will allow the identification of these two types of cells during development (Teitelman et al., 1983) and other undefined AADC-expressing cell types, such as pancreatic islet cells (Teitelman et al., 1981).

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