Different localization of spermidine/spermine N^1-acetyltransferase and ornithine decarboxylase transcripts in the rat kidney

S. Bettuzzi[a], M. Marinelli[a], P. Strocchi[b], P. Davalli[a], D. Cevolani[b], A. Corti[a,*]

[a]*Dipartimento di Scienze Biomediche, Università di Modena, via G. Campi 287, 41100 Modena, Italy*
[b]*Dipartimento di Farmacologia, Università di Bologna, via Irnerio 48, 40126 Bologna, Italy*

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Abstract In situ hybridization histochemistry of transverse sections from male rat kidney showed that the mRNA of the regulatory enzyme of polyamine degradation, spermidine/spermine N^1-acetyltransferase, has a spotty distribution in the cortex, is low and diffused in the outer stripe and high and diffused in the inner stripe of the outer medulla. At the cellular level, this mRNA is solely expressed by the epithelium of the distal straight and convoluted nephron tubules. Since biosynthetic ornithine decarboxylase mRNA is solely found in the proximal straight tubules, it is proposed that polyamine biosynthesis and degradation occur at separate sites along the nephron.

Key words: Ornithine decarboxylase; Spermidine/spermine N^1-Acetyltransferase; Polyamine; Kidney (rat)

1. Introduction

Alipathic polyamines are polycationic compounds normally present in all cells where they appear to affect a vast array of processes. Many functional roles have been proposed for polyamines but only their participation in normal or pathological cell growth and differentiation is supported by unequivocal evidence [1].

The first step in polyamine biosynthesis is the conversion of ornithine to putrescine (1,4-diaminobutane) which is catalyzed by ornithine decarboxylase (EC 4.1.1.17, ODC), a rate controlling enzyme. Transfer of one or two aminopropyl groups from decarboxylated *S*-adenosylmethionine to one or both of the nitrogens of putrescine leads to formation of the two higher polyamines, spermidine and spermine, respectively. Constitutive spermidine and spermine synthases catalyze the two transfer reactions. Decarboxylated *S*-adenosylmethionine is produced by *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), the second rate controlling enzyme of this biosynthetic pathway [1].

Polyamine catabolism in eukaryotic cells occurs by an interconversion pathway whereby two enzymes, spermidine/spermine N^1-acetyltransferase (SSAT) and FAD-requiring polyamine oxidase, by acting sequentially upon spermine or spermidine, remove the aminopropyl groups, thus converting spermine back to spermidine, and spermidine back to putrescine. Inducible SSAT regulates this catabolic pathway. The product of spermidine acetylation, acetylspermidine, in addition to being oxidized to putrescine by polyamine oxidase, can be excreted from the cell. Putrescine can be excreted as well, or degraded by diamine oxidase [2,3].

The intracellular concentrations of polyamines are highly controlled by intricate, partly unique [4], mechanisms involving the inducible enzymes of both the biosynthetic and degradative pathways. In fact, both SSAT and ODC activity are finely regulated by changes in enzyme protein concentration, resulting, in part, from changes in the abundance of their transcripts [1,2].

It has been previously reported that in the rat kidney ODC activity is not diffused all over the organ but localized in a restricted area [5]. More recently, the ODC mRNA has been found to be specifically associated to the epithelial cells of the proximal straight tubules belonging to the outer stripe of the outer medulla and the medullary rays [6,7]. In the present paper we show that the transcript encoding catabolic SSAT is expressed in a portion of the rat nephron which is separate from that expressing the transcript encoding biosynthetic ODC. This is a novel finding which may imply that polyamine levels undergo dramatic changes along the nephron, suggesting a previously unreported role(s) for these compounds in the renal physiology.

2. Materials and methods

2.1. Animals

Male Wistar rats (3 months old) from a commercial source were used.

2.2. Enzyme assays

ODC activity was determined as detailed elsewhere [8]. The high speed supernatant of cortical or medullary kidney tissue was incubated in a mixture containing (final concentrations) 50.0 mM Tris-HCl, pH 7.2; 6.0 μM EDTA; 40.0 μM pyridoxal-5′-phosphate; 0.50 μCi DL-[1-^{14}C]ornithine (59 mCi/mmol, Amersham, UK). After 60 min of incubation at 37°C, the $^{14}CO_2$ released was quantitated by scintillation counting. The same supernatants were used for SSAT detection. This was based on measurement of label incorporation from [^{14}C]acetyl-CoA into monoacetyl-spermidine. The assay system [9], containing 50 mM Tris-HCl, pH 7.8, 10.0 μM [1-^{14}C]acetyl-CoA and 3.0 mM spermidine, was incubated 10 min at 30°C.

2.3. In situ hybridization procedure

The in situ hybridization procedure was essentially as detailed elsewhere for rat prostate [10]. Kidneys were rapidly dissected out and frozen in liquid nitrogen. Tissue sections (10 μm) were fixed in 4% paraformaldehyde, 0.1 PBS (0.14 M NaCl, 10 mM sodium phosphate, pH 7.2), acetylated with 0.25% (v/v) acetic anhydride in 0.1 M triethanolamine (pH 8.0). Consecutive sections of the kidney were hybridized with either a ^{35}S-labeled oligodeoxyribonucleotide probe for SSAT mRNA (5′-CTT GAT CTT CCA TGT ATT CAT ATT TAG CCA GTT CCT TGA TCA GTC GCA GGA TG-3′ [11,12] or ODC mRNA (5′-GGG AAG TAC TTG TCC AGA GCT GGG TTG ATT ACA CTG GTG ATC TCC-3′, [13]). Being the sequence of rat SSAT mRNA still unknown, the SSAT oligonucleotide was taken from a region in which hamster [11] and mouse [12] cDNAs show complete homology.

*Corresponding author. Fax: (39) (59) 372653.

Abbreviations: ODC, L-ornithine decarboxylase; SSAT, spermidine/spermine N^1-acetyltransferase.

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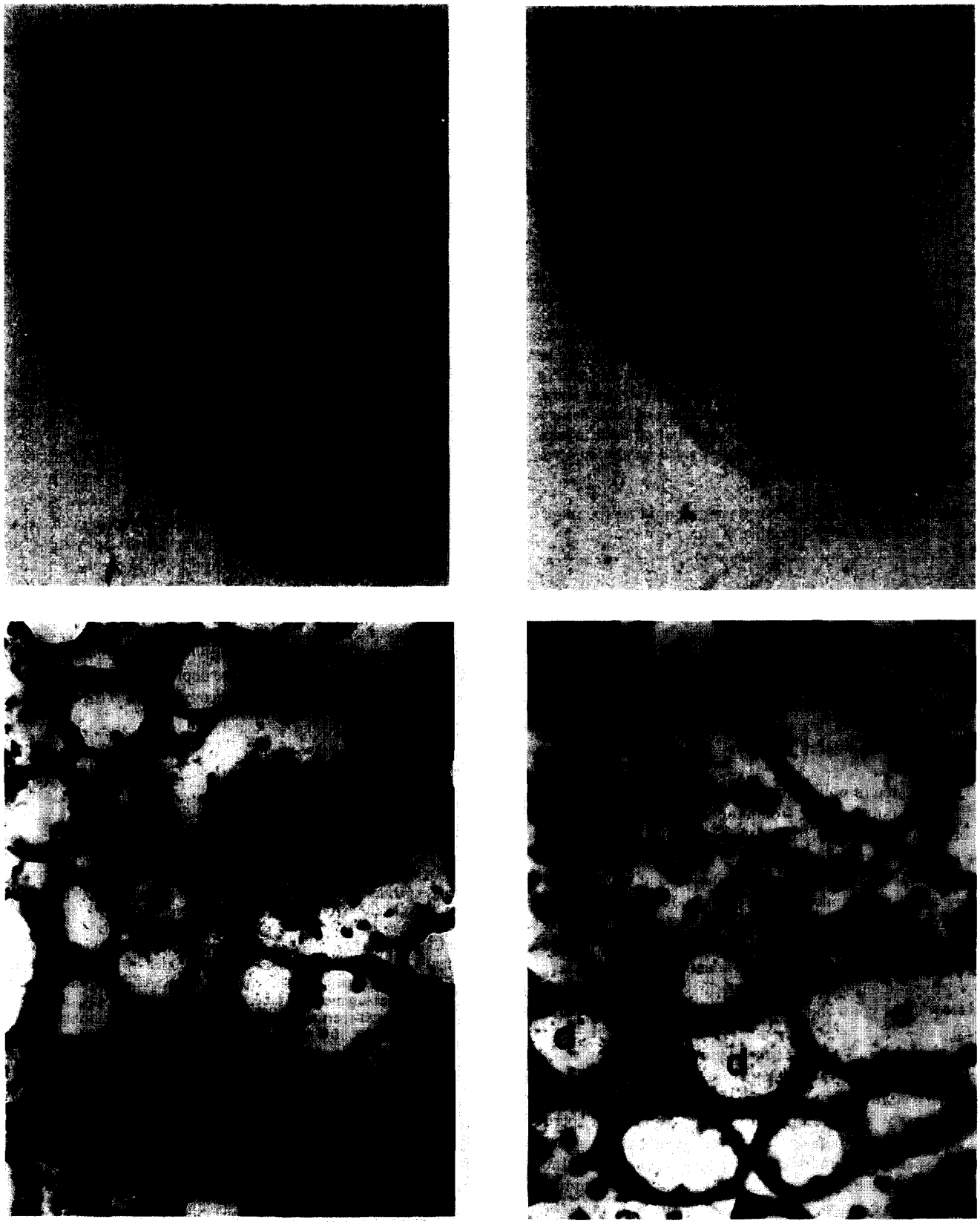

Fig. 1. (A) and (B), in situ hybridization autoradiograms of transverse sections from whole rat kidney. (A) was hybridized with the SSAT mRNA probe, (B), adjacent to (A), with the ODC mRNA probe. (C) and (D), high power microphotographs of two fields from the cortex of sections hybridized with the SSAT probe, treated with photographic emulsion and counterstained. Tubules exhibiting the hybridization signal (d) are in close proximity of the glomeruli (g) and have the appearance of convoluted distal segments (see text). Magnification: × 7.5 (A) and (B); × 520 (C); × 750 (D).

The probes were radiolabeled at the 3′-end with [α-^{35}S]dATP (Amersham, Bucks, UK) using the enzyme TDT (Boehringer, Mannheim, Germany). Each section was covered with the hybridization buffer [10] containing about 20 fmol ^{35}S-labeled probe, washed at 42°C for 1 h with 2 × standard saline citrate solution (SSC) and for 1 h at room temperature in 1 × SSC [10]. After dipping into ice-cold water, slides were dried and exposed to film (Amersham, Hyperfilm-^{3}H) which was developed 3 days later.

Slides for photographic emulsion were coated with Ilford K5 emulsion, exposed for 40–50 days, developed and stained with haematoxylin-eosin.

The specificity of hybridization was tested by searching first for possible homology with other known sequences in the Genbank/EMBL databases; then, by hybridizing kidney sections with (a) oligodeoxyribonucleotides of unrelated sequence but with a length and GC content equal to that of SSAT or ODC probe, which gave no specific signal; (b) a second oligodeoxynucleotide against another portion of SSAT or ODC mRNA, which gave the same pattern of specific signal.

3. Results

In order to understand whether or not the enzyme of polyamine degradation, SSAT, localizes in the same region of the rat kidney where the biosynthetic ODC has been found [5–7], portions of the cortical and medullary regions of this organ were carefully separated and assayed both for ODC and SSAT activities. Table 1 shows that the medullary ODC activity was almost 30-fold higher than the cortical one, which is a much greater difference than reported previously by others [5], probably due to the fact that in these experiments, the whole medullary tissue was assayed, while in ours, the outer portion of the medulla, where ODC transcript accumulates (see below), was assayed.

Contrary to ODC, the SSAT activity was higher in the cortex than in the medulla. The difference in SSAT activity between the two portions of the kidney is probably much higher than shown here. In fact, due to non-enzymatic acetylation of amines and other enzyme activities forming acetylated polyamines, the amplitude of changes in SSAT activity, assayed by the standard systems (see section 2), are underestimated [2].

The latter results prompted us to investigate the relative distribution of the transcripts of the two enzymes within the rat kidney. Fig. 1A shows the autoradiographic image of a transverse section from an adult male rat kidney, hybridized with ^{35}S-labeled antisense SSAT oligonucleotide. It appears clearly that the SSAT transcript is differently distributed in the various areas of the section (see scheme of Fig. 2). In the region apparently corresponding to the renal cortex, the hybridization signal is focally localized, at high levels, in many small spots. In the adjoining region, spots are no more present and the signal is weak and diffuse. In the inner portion of the kidney it is still diffuse but very intense.

Sections adjacent to those tested for SSAT mRNA were hybridized with the ODC antisense [^{35}S]oligonucleotide. Fig. 1B shows that the ODC mRNA is exclusively localized in a region adjoining the cortex that includes structures recalling the medullary rays. This characteristic distribution of the ODC transcript is identical to that previously reported by others [6,7]. By photographic emulsion and counterstaining procedure these authors showed that the ODC probe hybridizes to the epithelial cells of proximal straight tubules (Fig. 2). In similar experiments, not shown here, we obtained evidence for the same cellular localization. Since these nephron segments are solely comprised within the outer stripe of the outer medulla and the

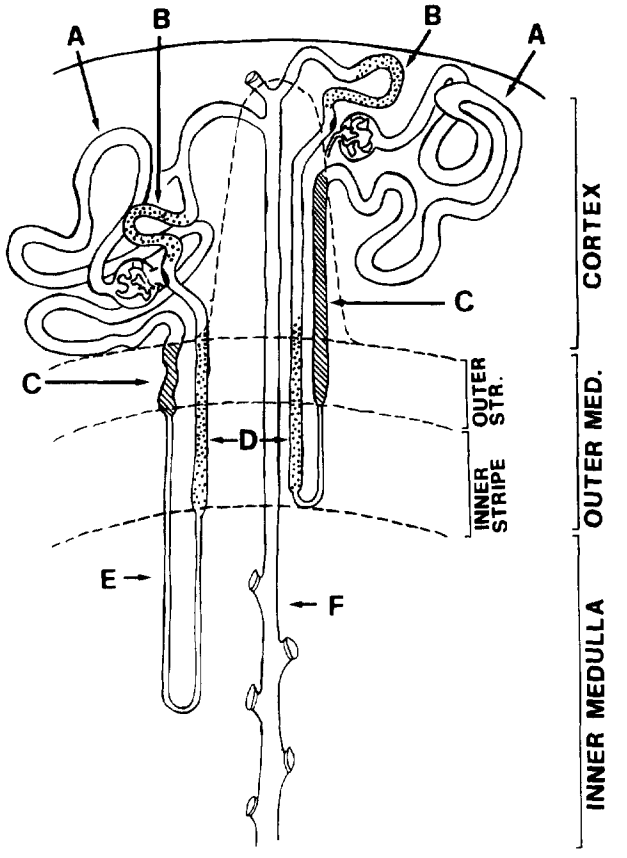

Fig. 2. Schematic representation of the regions in which the kidney can be morphologically and functionally divided [12]. Proximal (A) and distal (B) convoluted tubule; proximal (C) and distal (D) straight tubule; Henle's loop descending limb (E); collecting duct (F). Segments (B) and (D) express SSAT mRNA, segments (C), ODC mRNA.

medullary rays (Fig. 2), these are the regions outlined by the ODC signal in Fig. 1B. Under our conditions, this area is perfectly superimposable to that expressing low levels of the SSAT transcript (Fig. 1A). Thus the area of the section where the SSAT mRNA is low and diffuse essentially corresponds to the outer stripe of the outer medulla and, possibly, the medullary rays (although the SSAT signal does not allow to identify clearly this structure); the area, adjoining the latter, where the transcript is high and diffuse, is the inner stripe of the outer medulla; the area where it is focally expressed is the cortex; the innermost region, where the signal is undetectable, is the inner medulla (Fig. 1A and Fig. 2).

The cellular localization of the SSAT hybridization signal in the cortex was studied by the photographic emulsion and counterstaining procedure. The microphotographs of Fig. 1C,D show that the SSAT probe hybridizes to the epithelial cells of tubules grouped at the vascular pole of the glomeruli. Their location, relatively low number (the distal convoluted tubule is shorter than the proximal counterpart), irregular shape, apical position of the nuclei in most of their columnar epithelial cells, are good evidence that they represent the convoluted parts of distal tubules.

Although, by the same method, it appeared that also in the outer medulla (inner and outer stripe, Fig. 2) the SSAT transcript localized within the tubular epithelium (not shown), it

Table 1
ODC and SSAT activities in the cortical and medullary portions of the rat kidney

ODC (pmol $^{14}CO_2$/h/mg protein*)		SSAT (pmol N^1-AcSPD**/h/mg protein*)	
Cortex	Medulla	Cortex	Medulla
24 ± 3.8	620 ± 89	532 ± 123	344 ± 66

*Mean ± standard deviation from duplicate determinations of 3–5 experiments.
**N^1-Acetylspermidine.

could not be established unequivocally which segment(s) of the nephron was involved. However, by examining the distribution pattern of the SSAT mRNA in the different zones of the section (Fig. 1A) and the portions of the tubules specifically comprised in each zone (Fig. 2), it can be concluded that the SSAT mRNA signal detected in the outer medulla is most likely associated to the distal straight tubule rather than to the other segments. In fact, the distal straight tubule is present in all regions exhibiting the SSAT hybridization signal and absent where the signal is undetectable (inner medulla, Fig. 1A and Fig. 2), whereas the proximal straight tubule ends at the transition from the outer to the inner stripe (where the SSAT signal abruptly increases) and both the descending limb of the Henle's loop and the collecting duct extend into the inner medulla (Fig. 2), which does not express detectable SSAT mRNA (Fig. 1A).

The intensification of the signal occurring on transition from the outer to the inner stripe of the outer medulla might result from higher levels of the transcript being expressed in the portion of the distal tubule traversing the inner stripe. As a matter of fact, this segment exhibits morpho-functional features distinguishing it from the outer stripe segment.

4. Discussion

Detection of ODC and SSAT activities in separate portions of the rat kidney, grossly corresponding to the cortex and the outer stripe of the outer medulla, suggested that the first regulatory enzyme of polyamine biosynthesis and that of their degradation have a distinct localization within this organ. In situ hybridization experiments showed that the ODC and SSAT transcripts are specifically associated to the straight portion of the proximal tubules and straight and convoluted portions of the distal tubules, respectively. Polyamines seem, therefore, to undergo separate metabolic fates in the various segments in which the nephron of the rat kidney can be morphologically and functionally divided [14].

The following picture can thus be tentatively postulated. In the cells of the renal corpuscule and proximal convoluted tubule, where the hybridization signal for both ODC and SSAT mRNAs is undetectable, the metabolism of polyamines would be scarcely active; in the straight part of the proximal tubule, polyamine biosynthesis would be markedly active; in the cells of the distal straight and convoluted tubules polyamines, possibly released from the cells of the former segment into the tubular fluid, would be actively degraded.

Being polycationic compounds that can be rapidly synthesised and disposed off by the cell, free polyamines may participate in establishing the correct balance of charges between the compartments of the nephron that are involved in the transport of charged chemical species. The different metabolic fate of polyamines along the nephron may be related to the different roles played by the successive segments in this secretory/reabsorption activity.

Experiments are in progress to find evidence for this possible relationship.

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References

[1] Pegg, A.E. (1988) Cancer Res. 48, 759–774.
[2] Casero, R.A., Jr. and Pegg, A.E. (1993) FASEB J. 7, 653–661.
[3] Seiler, N. (1987) Can. J. Physiol. Pharmacol. 65, 2024–2035.
[4] Canellakis, E.S., Viceps-Madore, D., Kyriakidis, D.A. and Heller, J.S. (1979) Curr. Top. Cell. Regul. 15, 155–202.
[5] Levine, J.H., Leaming, A.B. and Gross, A.J. (1980) Life Sci. 27, 1653–1657.
[6] Blackshear, P.J., Manzella, J.M., Stumpo, D.J., Wen, L., Huang, J.K., Oyen, O. and Young, W.S.III (1989) Mol. Endocrinol. 3, 68–78.
[7] Crozat, A., Palvimo, J.J., Julkunen, M. and Janne, A. (1992) Endocrinology 130, 1131–1144.
[8] Davalli, P., Carpenè, E., Astancolle, S., Viviani, R. and Corti, A. (1992) Biochem. Pharmacol. 44, 721–726.
[9] Erwin, B.G., Persson, L. and Pegg, A.E. (1984) Biochemistry 23, 4250–4255.
[10] Marinelli, M., Quaglino, D., Bettuzzi, S., Strocchi, P., Davalli, P. and Corti, A. (1994) Biochem. Cell Biol. 72, 515–521.
[11] Pegg, A.E., Stanley, B.A., Wiest, L. and Casero, R.A., Jr. (1992) Biochim. Biophys. Acta 1171, 106–108.
[12] Fogel-Petrovic, M., Kramer, D.L., Ganis, B., Casero, R.A., Jr. and Porter, C.W. (1993) Biochim. Biophys. Acta 1216, 255–264.
[13] Van Kranen, H.J., Van de Zande, L., Van Kreijl, Bischop, H. and Wieringh, B. (1987) Gene 60, 145–155.
[14] Kriz, W. and Bankir, L. (1988) Eur. J. Physiol. (Pflüger's Arch.) 411, 113–120.