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ORIGINAL ARTICLE

Expression and distribution of genes encoding for polyamine-metabolizing enzymes in the different zones of male and female mouse kidneys

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Abstract The role of polyamines in renal physiology is only partially understood. Moreover, most of the data on the enzymes of polyamine metabolism come from studies using whole kidneys. The aim of the present study was to analyze the mRNA abundance of the genes implicated in both the polyamine biosynthetic and catabolic pathways in different renal zones of male and female mice, by means of the quantitative reverse transcription-polymerase chain reaction. Our results indicate that there is an uneven distribution of the different mRNAs studied in the five renal zones: superficial cortex, deep cortex, outer stripe of the outer medulla (OS), inner stripe of the outer medulla (IS), and the inner medulla + papilla (IM). The biosynthetic genes, ornithine decarboxylase (ODC) and spermine synthase, were more expressed in the cortex, whereas the mRNAs of the catabolic genes spermine oxidase (SMO) and diamine oxidase were more abundant in IS and IM. The genes involved in the regulation of polyamine synthesis (AZ1, AZ2 and AZIN1) were expressed in all the renal zones, predominantly in the cortex, while AZIN2 gene was more abundant in the OS. ODC, SMO, spermidine synthase and spermidine/spermine acetyl transferase expression was higher in males than in females. In conclusion, the genes encoding for the polyamine metabolism were specifically and quantitatively distributed along the corticopapillary axis of male and female mouse kidneys, suggesting that their physiological role is essential in defined renal zones and/or nephron segments.

Keywords Polyamines · Gene expression · Mouse kidney · qRT-PCR · Renal zones · Sexes

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Introduction

Polyamines are organic polycations implicated in cell growth, proliferation and differentiation (Thomas and Thomas 2001; Gerner and Meyskens 2004; Pegg 2009). They are present in all mammalian cells, and their levels are tightly regulated by different pathways, including biosynthesis, catabolism, uptake, and efflux (Pegg 2009). As shown in Fig. 1, ornithine is converted into the diamine putrescine by ornithine decarboxylase (ODC), the key enzyme of the polyamine biosynthetic pathway (Pegg 2006). ODC is negatively regulated by the antizymes (AZs), small proteins that are induced by high levels of intracellular polyamines (Hayashi et al. 1996; Coffino 2001). There are three members in the AZ family named AZ1, AZ2, and AZ3 (Mangold 2005); whereas, AZ1 and AZ2 are ubiquitously expressed in mammal tissues (Ivanov et al. 1998) AZ3 expression is testis specific (Ivanov et al. 2000; Tosaka et al. 2000). Antizyme

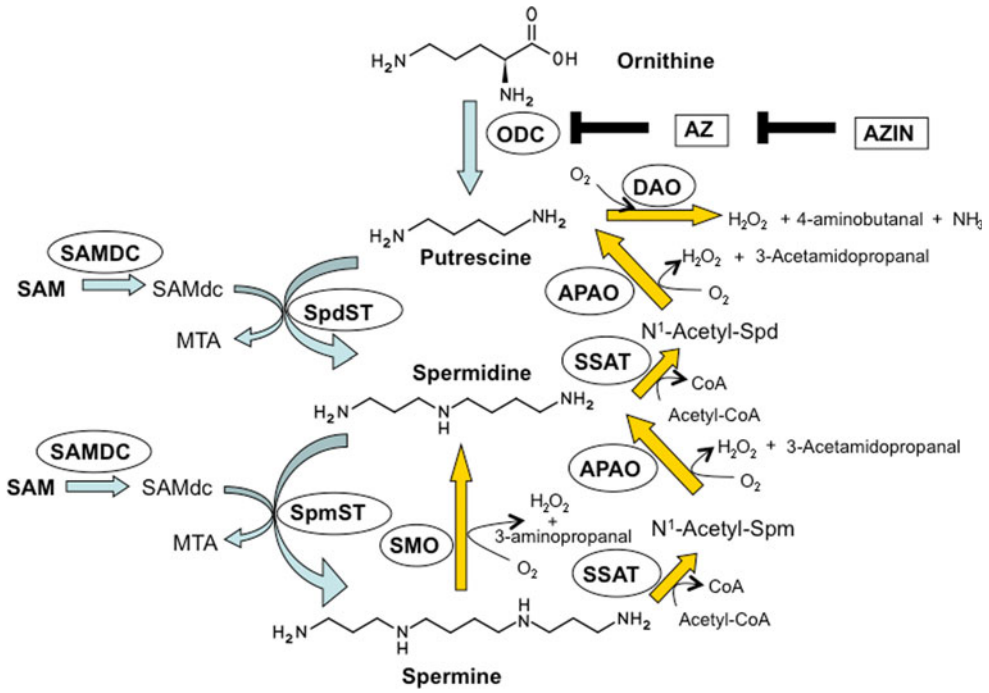

Fig. 1 Scheme of the polyamine metabolism in mammalian cells. The diamine putrescine is synthesized from ornithine by ornithine decarboxylase (*ODC*). This enzyme is negatively regulated by antizymes (*AZs*) and positively by antizyme inhibitors (*AZINs*). Spermidine is synthesized by spermidine synthase (*SpdST*) that catalyzed the transfer of the aminopropyl group from decarboxylated *S*-adenosylmethionine (*SAMdc*) to putrescine. SAMdc is formed by the decarboxylation of *S*-adenosylmethionine (*SAM*) by SAM decarboxylase (*SAMDC*). The addition of another aminopropyl group to spermidine by spermine synthase (*SpmST*) generates spermine. Two enzymes, spermidine/spermine *N*1-acetyltransferase (*SSAT*) and *N*1-acetyl polyamine oxidase (*APAO*), participate in the retroconversion pathway that generates spermidine and putrescine from spermine and spermidine, respectively. Spermine can be converted into spermidine by spermine oxidase (*SMO*) and putrescine can be catabolized by diamine oxidase (*DAO*). All these oxidases utilize oxygen as substrate and generate hydrogen peroxide

inhibitors (AZINs) are the proteins highly homologous to ODC but devoid of ODC activity, which bind to AZs and negate the effect of these on ODC (Fujita et al. 1982; Mangold 2006; Kahana 2009; López-Contreras et al. 2010). AZIN1, the first characterized AZIN (Fujita et al. 1982), is a ubiquitous protein that participates in polyamine metabolism and cell growth (Mangold 2006; Kahana 2009). AZIN2 mRNA was detected early in human and mouse brain and testes (Pitkanen et al. 2001; López-Contreras et al. 2006), although lower but detectable AZIN2 mRNA levels were also found in other mouse tissues such as adrenal glands, pancreas, lung, heart and kidney (Ramos-Molina et al. 2012). The enzymes *S*-adenosylmethionine decarboxylase (SAMDC), spermidine synthase (SpdST), and spermine synthase (SpmST) participate in the synthesis of spermidine and spermine from putrescine and decarboxylated SAM (Pegg 1984, 2009; Pegg and Michael 2010). The polyamine retroconversion route is controlled by two enzymes: spermidine/spermine-*N*1-acetyltransferase (SSAT) (Casero and Pegg 1993; Pegg 2008) and a FAD-dependent acetyl-polyamine oxidase (APAO) (Casero and Pegg 2009). The enzymes diamine oxidase (DAO) and spermine oxidase (SMO) are also implicated in diamine and polyamine catabolism (Agostinelli et al. 2004; Vujcic et al. 2002; Wang et al. 2003).

Polyamines are present in the mammalian kidney at levels similar to those found in other tissues, but the influence of these compounds in renal physiology is far from being understood. Although plasma polyamine levels are altered in patients with chronic renal failure (Igarashi et al. 2006; Igarashi and Kashiwagi 2010), the relevance of this finding is presently unknown. In rodents, the increase in renal ODC and putrescine contents has been proposed to be at least partially responsible for kidney hypertrophy, but this has not been a consistent observation (Berger and Porter 1986; Tovar et al. 1995). In the mouse kidney, there is a clear sexual dimorphism in some enzymes of the polyamine metabolism, especially in ODC. Both renal ODC activity and putrescine excretion are considerably higher in male than in female mice (Grahn et al. 1973; Henningsson and Rosengren 1975). Testosterone administration markedly increases ODC activity in the female kidney (Henningsson et al. 1978; Levillain et al. 2003), this enhancement being dependent on androgen receptors (Pajunen et al. 1982). Although it is clear that the enzymes required for polyamine biosynthesis, interconversion and

catabolism are present in rodent kidneys, very few studies, with the exception of those of ODC, have been carried out on these enzymes (Murakami et al. 1988; Manteuffel-Cymborowska et al. 1992; Jotova et al. 1999; Bettuzzi et al. 2001; Levillain et al. 2003). In addition, most of the data concerning renal polyamine metabolism have been obtained from studies in whole organ. Since the kidney is composed of a variety of cells (Kaissling and Kriz 1979; Pfaller and Rittinger 1980; Pfaller 1982), whole organ data are of limited value for the interpretation of the functions of the polyamines. As an alternative, isolated nephron segments and kidney zones have been used to study the regional distribution of polyamines and their related enzymes in the rabbit, rat, and mouse kidneys. These studies demonstrated that polyamines are unevenly distributed within the rat and rabbit kidney (Levillain et al. 2000b) and that ODC is mainly localized in the proximal tubules of mouse and rat nephrons (Levillain and Hus-Citharel 1998; Levillain et al. 2000a; Bettuzzi et al. 2001). In order to gain more information about the renal polyamine metabolism, we thought essential to analyze mRNA abundance of different genes implicated in the polyamine metabolism in mouse kidney zones by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Materials and methods

Animals

Eight- to nine-week-old adult female (30–32 g body weight) and male (35–40 g body weight) Swiss mice from Janvier (Le Genest-saint-Isle, France) were given free access to tap water and standard laboratory food (2018 Teklad Global, 18 % Protein Rodent Diet, Harlan, Gannat, France). The animals were housed in a controlled environment maintained at 21 ± 1 °C with a 12-h light, 12-h dark cycle, lights on at 0700 hours. Mice were anesthetized (i.p.) using 0.1 ml/30 g body wt sodium pentobarbital diluted 1:2 in 0.9 % NaCl solution (Nembutal 6 %, Clin Midy, Paris, France).

Animal care complied with French regulations for the protection of animals used for experimental and other scientific purposes and with European Community regulations (Council of Europe, no. 123, Strasbourg, 1985). The author (O. Levillain) is authorized by the "Direction Départementale des Services Vétérinaires" (authorization no. 69266391) and the local Animal Care Committee to use animals for these experiments (protocol no. BH 2009-14).

Dissection of renal zones

After laparotomy, the right and left kidneys were rapidly excised and decapsulated. The blood contained in each kidney was removed with blotting paper (blood-free). A slice of 1–2 mm thickness was cut along the corticopapillary axis from each blood-free kidney of male and female mice. Along the corticopapillary axis, five renal zones were clearly distinguished and dissected at 4 °C under a stereomicroscope: superficial cortex (Cs), deep cortex (Cd), outer stripe of the outer medulla (OS), inner stripe of the outer medulla (IS), and inner medulla including papilla (IM). Dissected pieces of Cs brought sufficient amount of tissue in each mouse to harvest large amount of total RNA. In contrast, for the other renal zones, the tissue of two mice was pooled. The dissected tissues were frozen, weighed, and conserved at −80 °C.

RNA isolation

Total RNAs were extracted from each renal zone using Trizol reagent according to the manufacturer's procedure. Briefly, each renal zone was mixed in the proportion of 150 mg frozen tissue per 1 ml Trizol at 4 °C with a Turrax homogenizer. Total RNAs were extracted with chloroform, purified by isopropanol precipitation, and washed with 70 % ethanol. RNA pellets were resuspended in sterilized water (Eurobio, Courtaboeuf, France) and stored at −80 °C. The concentration and purity of RNAs were determined by measuring the absorbance at 260 and 280 nm using a BioPhotometer (Eppendorf France S.A.R.L., Le Pecq, France). The integrity of RNAs was checked by 1 % agarose gel electrophoresis (Eurobio).

Reverse transcription

Reverse transcription (RT) of 1 µg of total RNAs was performed using 200 U of MMLV-RT, 4 µl of MMLV-RT 5× buffer, 15 nmoles of deoxynucleotide triphosphates, 1 µl RNAseOUT, and 1 µg oligo dT in a final volume of 20 µl to initiate cDNA synthesis. The reaction consisted of 5 min at 65 °C (RNA and oligo dT), then 60 min at 37 °C (all mix) followed by 15 min at 70 °C. After chilling, the cDNAs obtained were frozen and maintained at −80 °C until PCR.

Quantitative real-time RT-PCR

PCR amplification was carried out using a SYBR Green® PCR Master Mix (Applied Biosystems) and a 7500 Real Time instrument (Applied Biosystems). Different set of primers and cDNA were used and the fluorescence data were collected and analyzed by means of 7500 SDS software (Applied Biosystems). In most cases, the primers were selected to achieve similar amplification efficiency, by testing them with plasmids containing the ORFs of the different genes of polyamine metabolism analyzed. The

expression level of each gene was normalized against β-actin and L19 ribosomal protein. The following primers were used:

Gene	Forward (5′–3′)	Reverse (5′–3′)
β-Actin	GATTACTGCTCTGGCTCCTAGCA	GCTCAGGAGGAGCAATGATCTT
L19	GGCTTGCCTCTAGTGTCCTC	CTGATCTGCTGACGGGAGTT
ODC	ATGGGTTCCAGAGGCCAAA	CTGCTTCATGAGTTGCCACATT
AZ1	GAGTTCGCAGAGGAGCAACT	CCAAGAAAGCTGAAGGTTCG
AZ2	AGTAAGTGTCCCCAGCTCCA	ATCTTCGACAGTGGGTGAGG
AZIN1	CTTTCCACGAACCATCTGCT	TTCCAGCATCTTGCATCTCA
AZIN2	GCTTAGAGGGAGCCAAAGTG	CTCAGCAAGGATGTCCACAC
SAMDC	AAGACTGACAAGCAGGAAGC	TGCTTTCAGTAAGAGGGTG
SpdST	TGCCCGAGTTCACCCGGAAG	CTGGTCCAGGACTGGTGGTC
SpmST	TGACTTGGCATATACCCGTG	ACAATATGCCTCCATCGCCG
SSAT	GAGGATGGCTTTGGAGAACA	CATGGCGAACCCAACAATGC
APAO	CTCTCACTCCAAGACACCTG	CAGCAATAAACCCACAGAGC
SMO	CTGCCCTACACAGAGAGCTC	GGGTGGTGGAGTAGTACTTG
DAO	CCAGAATGACCCCTGGGATC	TGTGCAGGAAGCCTACTGTC
AKR	AGCGGCAGGATCTCTTCATTG	GGTAGAGATCCAGGTAGTCC
ASS	CGCACCACATCCCTGGAACTC	CTCGTAGATACCTCGGGAC
THP	TACCTGAATGACAGACAATG	TGTAGGTGGCATGGGTTTCG
NKCC2	GTTCGACTGAACGAGCTCTT	AGGAAGGTTCTTGGTCAGG

Materials

Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and Trizol were purchased from Invitrogen (France). SYBR Green® PCR Master Mix was from Applied Biosystems (Warrington, UK). Primers were purchased from Sigma-Aldrich (Cambridge, UK).

Statistical analysis

Data are expressed as mean ± SE. The significance of the differences observed was assessed by Student's t test or one-way ANOVA, followed the Newman–Keuls post hoc multiple range test. $P < 0.05$ was considered statistically significant.

Results

The mRNA levels of the different genes studied were determined in microdissected renal zones of male and female mouse kidneys. These included two cortical samples (Cs and Cd) and three medullary samples (OS, IS, and IM) (See Fig. 2). To validate the correct dissection of the renal zones from the mouse kidney, we analyzed the expression pattern of different kidney markers. Figure 3 shows that aldose keto-reductase (AKR) mRNA was mainly found in IM, which is in agreement with the fact that AKR protein is essentially expressed in the medullary and papillary collecting ducts (Sands et al. 1989). Argininosuccinate synthetase (ASS) mRNA was mainly found in the cortical zone in accordance with the reported almost exclusive expression in the renal cortex (Morris et al. 1991; Levillain 2012). The uromodulin (THP) and Na-K-2Cl cotransporter (NKCC2) mRNA content was high in the outer medulla. This agrees with reports that showed a strong expression of THP mRNA in the outer medullary segment of rat kidney (Bachmann et al. 1990) and that NKCC2 is expressed mainly at the thick ascending limb cells in rats and in the maculla densa cells in rabbits (Obermüller et al. 1996). In some renal zones, the values of ASS, THP, and NKCC2 mRNAs were slightly higher in males.

Figure 4 shows mRNA levels of the various genes implicated in the polyamine biosynthetic pathway in the different kidney zones. It can be seen that there was an uneven distribution of ODC mRNA among the different zones, showing a very high expression in the cortical zones of the male kidneys. In fact, in Cd, the level of ODC mRNA was about 25-fold higher than in IM. In addition, the levels of ODC mRNA in the cortical zones of male kidneys were about 10- to 20-fold higher than those found in females. With regard to SAMDC mRNA levels, no differences between sexes or renal zones were observed. SpdST mRNA was detected in all renal zones but no significant differences among them were found. However, the

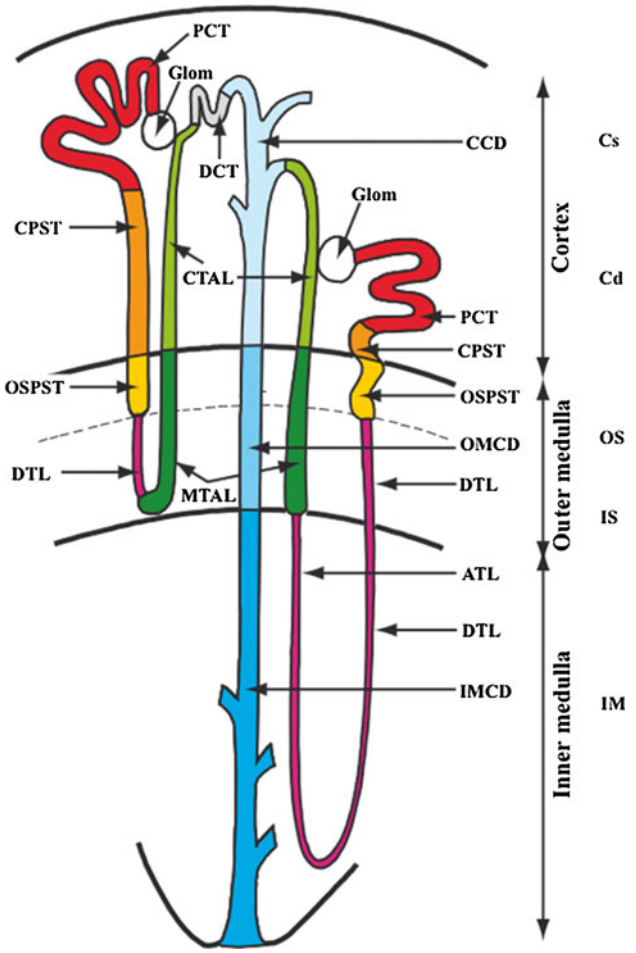

Fig. 2 Scheme localizing the different renal zones and their relation with the nephron segments. Scheme localizing the tubules in superficial and deep nephrons. The cortex is divided into the superficial (*Cs*) and the deep layer (*Cd*), while the medulla is divided into the outer medulla that is subdivided into the outer stripe (*OS*) and the inner stripe (*IS*), and the inner medulla + papilla (*IM*). The smallest structural unit in the kidney is the nephron which begins in the glomerulus (*Glom*) and is followed by the renal tubule composed of several morphologically and functionally different segments. The first segment is the proximal tubule, which is subdivided into three unequal parts: the proximal convoluted tubule (*PCT*), the cortical proximal straight tubule (*CPST*), and the outer medullary proximal straight tubule (*OSPST*). The proximal tubule is followed by the Henle's loop composed of the descending thin limb (*DTL*), the ascending thin limb (*ATL*), the medullary thick ascending limb (*MTAL*), and the cortical thick ascending limb (*CTAL*). The connection between the thick limb and the collecting duct (*CD*) is ensured by the distal convoluted tubule (*DCT*). The collecting duct runs through the cortex (*CCD*), the outer medulla (*OMCD*), and the inner medulla (*IMCD*). Superficial nephrons differ from deep nephrons in the length of their Henle's loop

levels in Cs, OS and IM in males were about twofold higher than in females. SpmST mRNA was also present in all renal segments without significant differences between sexes. For this gene, the expression levels in the cortical zones Cs and Cd were considerably higher than those found in IM. Taking into consideration the relative values of the different transcripts corresponding to the polyamine biosynthetic genes, it is clear that the polyamine biosynthesis appears to be more active in the cortical than in the medullary zones of the kidney. The transcript levels of the different genes related to the regulation of ODC are shown in Fig. 5. The two ODC paralogs, the antizyme inhibitors AZIN1 and AZIN2, were expressed in all renal zones with no significant differences between sexes. In all zones, except in IS, AZIN1 mRNA levels were about 10-fold higher than those of AZIN2. This ratio was about threefold in IS. Among the different antizymes of ODC, AZ1 was also more expressed than AZ2 (about 10- to 20-fold) in all renal zones, and no significant differences between sexes were evident. AZ3 mRNA could not be detected (data not shown). Overall, the regulatory genes of ODC are evenly expressed among the different renal zones, in spite that ODC is mostly expressed in the renal cortex.

The expression of the genes of the polyamine retroconversion pathway in the different renal zones is shown in Fig. 6. SSAT and APAO mRNA levels were slightly higher in the cortical zones and a moderate sexual dimorphism in OS and IS was also evident for SSAT, and in Cs, Cd, OS, and IS for APAO. On the other hand, the transcripts of the catabolic genes SMO and DAO were more abundant in the medullary zones, especially in the case of DAO, the levels in IS and IM were about eightfold higher than in the other zones (Fig. 6).

Discussion

The present study has been conducted to establish the renal distribution of expression of the genes involved in polyamine anabolism and catabolism and to determine whether sexual dimorphism in the expression of these genes occur in the mouse kidney. Importantly, we found that the anabolic and catabolic pathways of the polyamines are almost spatially separated, with the former being in the renal cortex and the latter mainly located in the medulla. In addition, a sexual dimorphism in the renal expression of ODC and SpdST was observed, whereas moderate differences in the expression of SSAT and APAO occurred in specific and restricted renal zones.

The enzymes required for the biosynthesis and catabolism of polyamines are present in rodent kidneys, but most detailed studies have been focused on ODC, the key regulatory enzyme of polyamine biosynthesis (Pajunen et al. 1982; Seely and Pegg 1983; Kontula et al. 1984; Jänne et al. 1991; Crozat et al. 1992). In addition, most of the data concerning the expression of the genes involved in renal polyamine metabolism have been obtained from studies carried out on the whole organ. Since the kidney contains a great variety of cell types (Kaissling and Kriz 1979; Pfaller

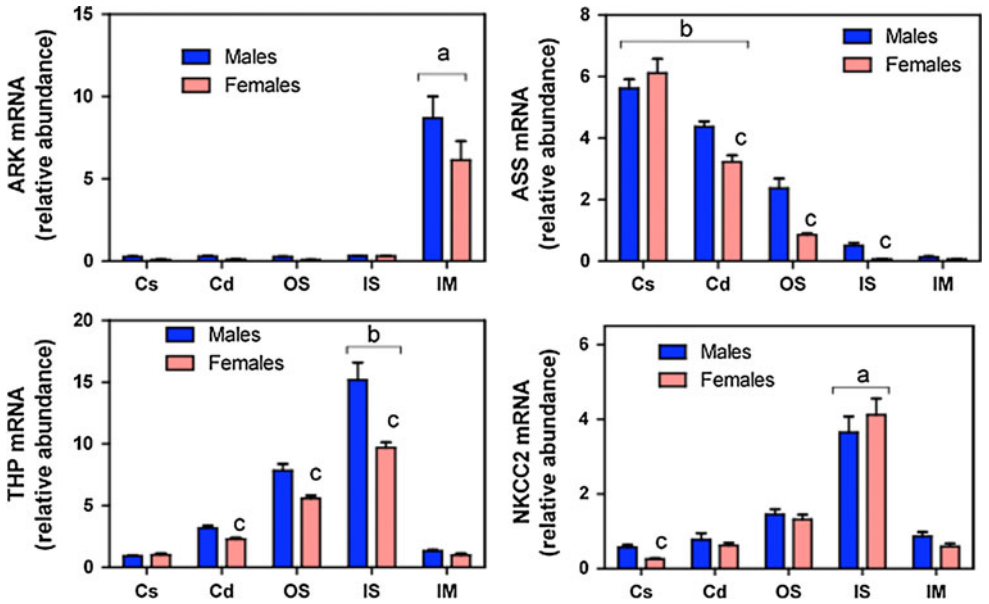

Fig. 3 Relative abundance of mRNAs of several markers of the five zones in male and female mouse kidney. Transcript levels were analyzed by quantitative real time RT-PCR and normalized to β-actin. Results are the mean ± SE of triplicate determinations of RNA samples from the renal zones of four adult male or female mice for Cs and eight mice for the other renal zones ($n = 4$ experiments). *Cs* superficial cortex, *Cd* deep cortex, *OS* outer stripe of the outer medulla, *IS* inner stripe of the outer medulla, *IM* inner medulla and papilla, *AKR* aldose keto-reductase, *ASS* argininosuccinate synthetase, *THP* Tamm-Horsfall protein (uromodulin), *NKCC2* Na^+-K^+-$2Cl^-$ co-transporter. *a* $P < 0.001$ versus the other zones, *b* $P < 0.01$ versus the other zones, *c* $P < 0.01$ versus males

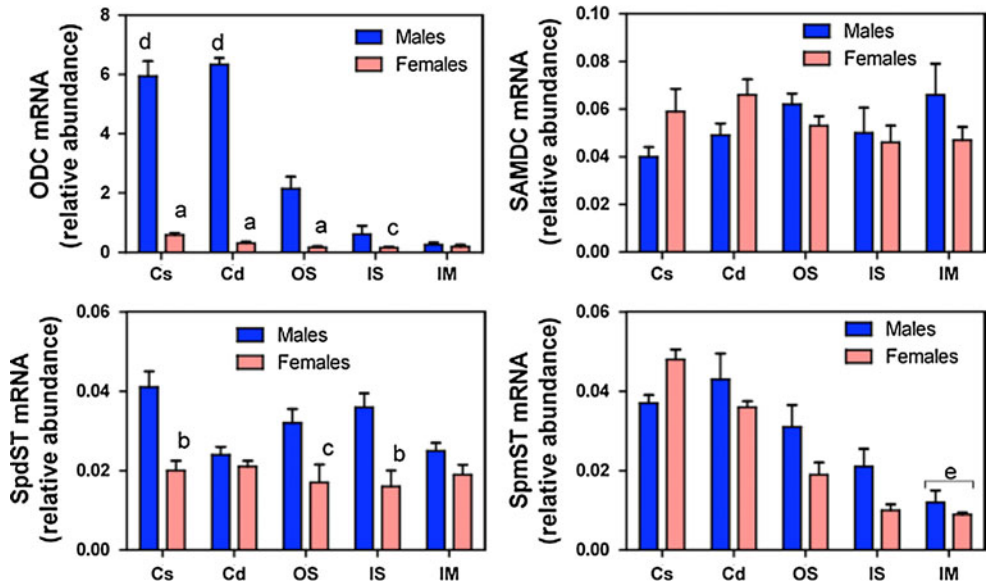

Fig. 4 Relative abundance of mRNAs of polyamine biosynthetic enzymes in the five zones of male and female mouse kidneys. Transcript levels were analyzed by quantitative real time RT-PCR and normalized to β-actin. Results are mean ± SE as per Fig. 3. *ODC* ornithine decarboxylase, *SAMDC* *S*-adenosylmethionine decarboxylase, *SpdST* spermidine synthase, *SpmST* spermine synthase. Other abbreviations as per Fig. 3. *a* $P < 0.001$ versus males; *b* $P < 0.01$ versus males; *c* $P < 0.05$ versus males; *d* $P < 0.001$ versus OS, IS, and IM; *e* $P < 0.001$ versus Cs and Cd

and Rittinger 1980; Pfaller 1982), whole organ data are of limited value to identify precisely the renal sites that express the genes involved in polyamine metabolism and, in a latter extent, to correlate the gene/enzyme distribution with the renal content of polyamines. Previous studies using isolated nephron segments or kidney sections support this statement, since it has been demonstrated that different metabolites and enzymes related to the polyamine

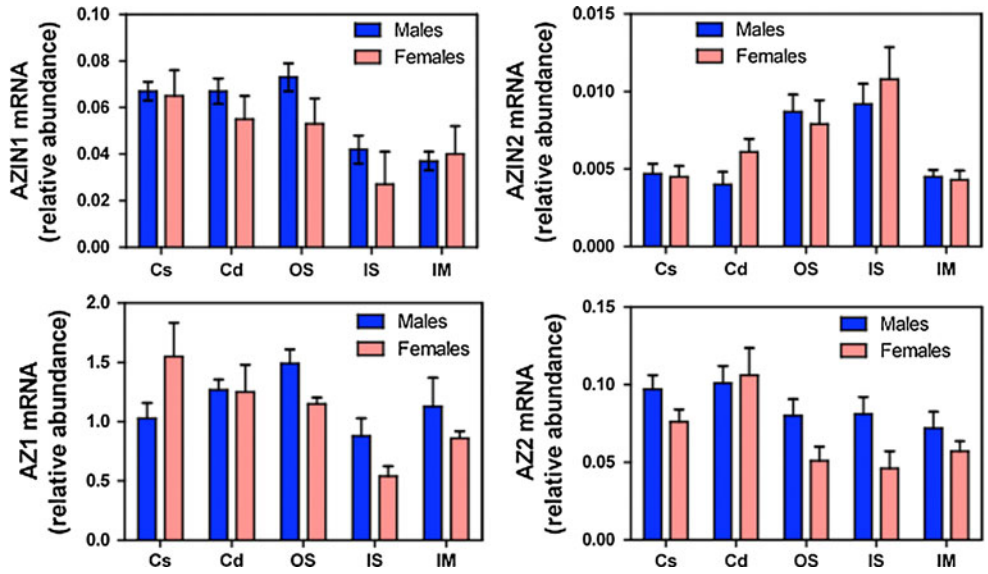

Fig. 5 Relative abundance of antizyme (*AZ*) and antizyme inhibitor (*AZIN*) mRNAs in the five zones of male and female mouse kidneys. Transcript levels were analyzed by quantitative real time RT-PCR and normalized to β-actin. Abbreviations as per Fig. 3. Results are mean ± SE as per Fig. 3

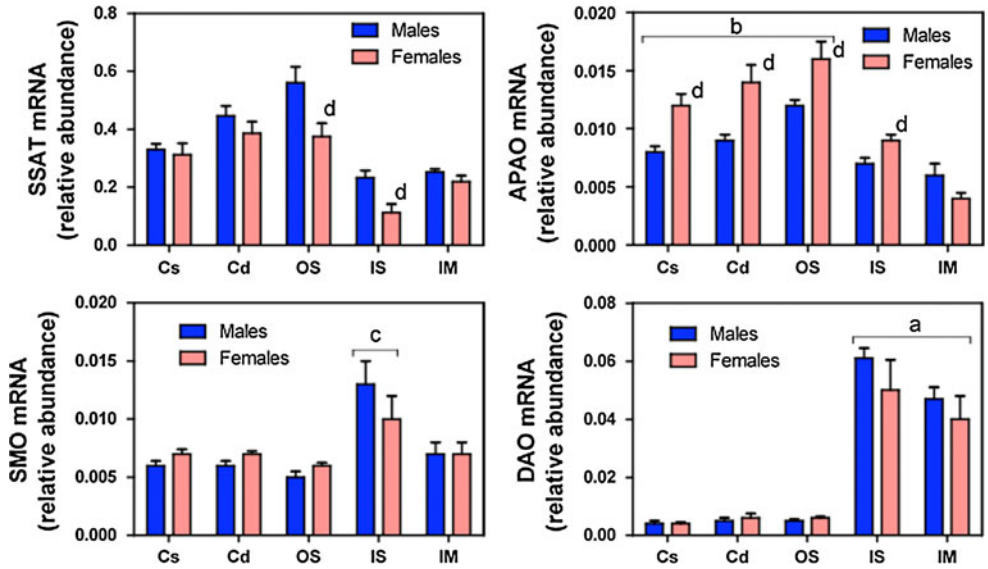

Fig. 6 Relative abundance of mRNAs of polyamine interconversion and catabolic enzymes in the five zones of male and female mouse kidneys. Transcript levels were analyzed by quantitative real time RT-PCR and normalized to β-actin. Results are mean ± SE as per Fig. 3. *SSAT* spermidine/spermine *N*1-acetyl transferase, *APAO* acetylpolyamine oxidase, *SMO* spermine oxidase, *DAO* diamine oxidase. *a* $P < 0.001$ versus Cs, Cd, and OS; *b* $P < 0.05$ versus IS and IM; *c* $P < 0.05$ versus the other groups; *d* $P < 0.05$ versus males

metabolism are unevenly distributed along the cortico-papillary axis of the kidney in different species (Levillain and Hus-Citharel 1998; Levillain et al. 2000a; Levillain 2012). In particular, each polyamine exhibited its own distinct distribution pattern (Levillain et al. 2000b). Therefore, in order to extend the knowledge on the expression of the genes related with the polyamine metabolism (and given that the mouse kidneys are of small size), we decided to measure their mRNA levels using qRT-PCR in five distinct kidney regions of male and female mice. This technique has the advantage of allowing the quantification of transcripts of many different genes in small renal zones, although it should be mentioned that in some cases the levels of mRNAs may not directly correspond with protein levels due to translational or post-translational regulatory mechanisms (Matsufuji et al. 1995;

Shantz and Pegg 1999). Indeed, the analysis of the protein levels and the enzyme activities of the genes encoding for polyamine metabolism would be more informative on the functionality of these pathways. Nevertheless, as the mouse kidneys are of small size, the latter technique requires too much tissue, while for the former good antibodies are not available in many cases. Taken together, qRT-PCR appeared to be the best approach to identify the distribution pattern of each gene encoding for a polyamine metabolizing enzyme in the different renal zones dissected from the mouse kidneys.

Our results indicate that ODC mRNA is more abundant in the cortex, which is enriched in proximal tubules, mainly proximal convoluted tubules, and that there is a marked sex difference, with males having much higher values than females. These data are in agreement with previous findings (Pegg et al. 1982; Crozat et al. 1992; Koibuchi et al. 1993a, b; Levillain and Hus-Citharel 1998; Levillain et al. 2000b, 2003). Of the other polyamine biosynthetic genes, SAMDC is probably expressed in all renal structures since the levels of SAMDC mRNA are equally distributed in all renal zones. The homogeneous distribution of SpdST mRNA, especially in the female kidney, also suggests that several nephron segments express this gene. The profile of SpmST distribution along the corticopapillary axis reveals a constant and progressive decline in the level of SpmST mRNAs. This profile suggests that the expression of this gene is more important in the proximal tubules and most probably in the proximal convoluted tubules. One can also expect that a decreasing gradient of SpmST gene expression takes place in the collecting ducts. This profile is quite similar to that of ASS and ODC mRNA distribution. Collectively, these results suggest that polyamine biosynthetic activity may be potentially more active in the renal cortex than in the medulla. However, other factors such as substrate availability in the different renal sections may influence the rate of polyamine biosynthesis. In fact, both arginase II mRNA and protein, related with ornithine production, are more highly expressed in the deep cortex and in the outer stripe of the outer medulla than in the other renal sections (Levillain et al. 2005; Levillain 2012). Although the blood constitutes a source of amino acids for polyamine synthesis in several nephron segments, the proximal convoluted tubules are known to reabsorb amino acids (e.g., ornithine, methionine) from the ultrafiltrate to supply the needs of ODC and SAMDC (Silbernagl 1992). The disproportionate expression of ODC in the renal cortex of male mice in relation to that of the other biosynthetic enzymes indicates that in this region, SAMDC may be the limiting enzyme in the polyamine biosynthetic process. This can explain why putrescine is the only polyamine that is more abundant in male than in female kidneys (Sánchez-Capelo et al. 1994; Levillain et al. 2003). The facts that the urinary excretion of putrescine in male mice is considerably higher than in females (Melanitou et al. 1987; Cremades et al. 1992) and that the administration of testosterone to female mice increases the urinary excretion of putrescine (Grahn et al. 1973) support the contention that a high proportion of the putrescine synthesized in the cortex is excreted. However, it cannot be excluded that part of the putrescine formed in the renal cortex by ODC could be removed via efferent arterioles and leave the kidney in the venous blood as reported for amino acids, glucose, and other molecules.

Although it is well known that ODC can be regulated by AZs and AZINs (Kahana 2009), little is known on the expression of the different members of these families of proteins in the mouse kidney. Our results indicate that AZ1 mRNA is the most expressed form of the AZs in the different renal zones, with no significant differences between sexes. This is in agreement with previous findings that showed that the level of AZ1 mRNAs was similar in male and female mouse kidneys, although it was stimulated by treatment with pharmacological doses of testosterone (Levillain et al. 2003). However, it is possible that the levels of AZ1 protein in the renal zones could be different due to the strong translational control of AZ1 mediated by polyamines through ribosomal frameshifting (Matsufuji et al. 1995). In addition, the presence of AZ1 and AZ2 in renal zones having relatively low levels of ODC suggests that AZs may participate in the regulation of polyamine transport in these cells, since it has been reported that AZs inhibit polyamine uptake (Mitchell et al. 1994; Suzuki et al. 1994). Given that AZ1 has been reported to be localized in the mitochondria (Gandre et al. 2003) and AZ2 in the nucleus (Murai et al. 2009), we wonder whether these proteins in these organelles may play other physiological roles that remain to be identified. Concerning AZINs, it is interesting to mention that AZIN2 is expressed in mouse kidney confirming a previous finding (Ramos-Molina et al. 2012) and that the level of expression is similar in both male and female mice. The relatively high level of AZIN2 mRNAs in IS suggests that the medullary thick ascending limb notably expresses this gene. This zone is mainly composed of medullary thick ascending limbs followed by outer medulla collecting ducts, thin limbs, and vascular bundles. This profile is similar to that of THP mRNA distribution. In relation with all these biosynthetic aspects, previous work on renal ODC and polyamines in six different models of kidney hypertrophy in mice (including compensatory renal hypertrophy produced by unilateral nephrectomy, experimental diabetes, potassium depletion, and treatment with hormones such as testosterone, thyroxine and fluorocortisone), excluded that polyamine are required for kidney hypertrophy (Tovar et al. 1995).

Regarding the genes implicated in the catabolic pathway, the renal distribution of SSAT mRNA suggests that the proximal tubules express this gene with an increasing intensity from proximal convoluted tubules towards outer medullary proximal straight tubules. These tubules are not the only ones that express SSAT gene. The distribution pattern of SSAT differs from that found in the rat kidney, where SSAT was localized in the distal convoluted tubule and in the whole thick ascending limbs (cortical and medullary thick ascending limbs) (Bettuzzi et al. 1995). In mice, in situ RNA hybridization experiments showed that the SSAT transcript is expressed only by the epithelial cells of the straight and convoluted distal tubules of the nephron (Bettuzzi et al. 2001). Increased expression of SSAT has been correlated with tubular cell damage in kidney ischemia–reperfusion injury (Zahedi et al. 2003). The renal distribution of APAO mRNA is quite similar to that of SSAT. The possible co-localization of SSAT and APAO mRNAs is in agreement with the functional roles of these enzymes in the retroconversion of higher polyamines. The expression profiles of SMO and DAO suggest that the catabolism of spermine and putrescine may be important in the descending and ascending thin limbs and/or in the collecting ducts of the inner medulla. The distribution of DAO in the mouse kidney strongly differs from that of the pig kidney. In the latter, DAO expression is confined to the epithelial cells of the proximal tubules (Schwelberger et al. 1998). Since both enzymes produce H_2O_2, one may speculate that they can exert oxidant actions, but their exact physiological functions remain unknown. On the other hand, although it was postulated that DAO is the amiloride binding protein component of the epithelial transport of Na^+ (Novotny et al. 1994), the real role of DAO in this process has not been elucidated. Amine oxidases (DAO, SMO, and APAO) generate from the natural polyamines toxic metabolites such as ammonia, aldehyde(s), and hydrogen peroxide (H_2O_2) (Agostinelli et al. 2004, 2007). They can induce cytotoxicity, provoke deleterious actions, increase the production of reactive oxygen species (ROS), which alter protein functions, enhance lipid peroxidation, provoke changes in mitochondrial respiration, and induce opening of the mitochondrial permeability transition (MTP). In fact, lipid peroxidation is involved in basic deteriorative mechanisms, e.g., membrane damage, enzyme damage, cell damage, and nucleic acid mutagenicity. In addition, H_2O_2 has multiple physiological functions in signal transduction cascades and plays a role in the pathology of several disorders, including cancer and neurodegenerative diseases. The production of H_2O_2 in mitochondria during the oxidation of amines might be extremely important in inducing mitochondria-mediated apoptosis (Agostinelli et al. 2007). The contribution of the polyamine pathway in renal physiology and diseases remains to be determined. Interestingly, recent findings have indicated that increased polyamine catabolism through the generation of by-products of polyamine oxidation contributes to kidney damage (Zahedi et al. 2010). Although it can be argued that excess in H_2O_2 production can be prevented by catalase, the uneven distribution of this enzyme in the kidney, expressed in the proximal tubules but undetectable the other cortical tubules and in the medulla (Oberley et al. 1990; Zhou and Kang 2000), where the polyamine catabolic genes are more expressed, make unlikely that possibility for the renal medulla.

It is noteworthy that the anabolic and catabolic pathways of the polyamines are almost spatially separated, with the former being in the renal cortex and the latter mainly located in the medulla. The axial heterogeneity of polyamine synthesis along the mouse nephron resembles that previously reported for arginine synthesis and hydrolysis (Levillain 2012) as well as for other metabolic pathways such as gluconeogenesis (Burch et al. 1978b; Vandewalle et al. 1981; Ross and Guder 1982; Yamada et al. 1986), glycolysis (Ross and Guder 1982), glutamine synthesis (Burch et al. 1978a; Ross and Guder 1982), ammoniagenesis (Curthoys and Lowry 1973; Ross and Guder 1982; Guder and Ross 1984), and amino acid metabolism (Guder and Morel 1992).

Collectively, our results reveal that although all genes of the polyamine metabolism are expressed in the mouse kidney, the heterogeneous profile of expression suggests that the requirements of polyamines in each renal region are specific and probably related to the characteristics of the metabolism and function of the different type of cells existing in these regions.

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Conflict of interest The authors declare that they have no conflict of interest.

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