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Polyamines are unevenly distributed within the rat and rabbit kidney

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Summary. Aliphatic polyamines have generally been measured on the whole kidney. Since the kidney is composed of a variety of cells, whole organ data are of limited value for the interpretation of the functions of the polyamines. The aim of this study was to establish the distribution pattern of putrescine, spermidine and spermine within the kidneys of male and female rats and rabbits. It is shown that the polyamines are unevenly distributed along the cortico-papillary axis. Each amine exhibited its own distinct distribution pattern. The polyamines are predominantly located in the cortex. Putrescine levels increased gradually from the cortex to the papillary tip in rabbits, whereas, in rats, fluctuations in putrescine level were marked. In the six zones of the rabbit kidney studied, spermidine and spermine concentrations were markedly higher in females than in males. This difference was less marked in rats.

Keywords: Amino acids – Polyamines – Putrescine – Spermidine – Spermine – Sex differences – Mammalian species

Introduction

The aliphatic polyamines putrescine, spermidine and spermine, occur ubiquitously at high concentrations in most animal and plant tissues. They are involved in many cellular and physiological processes including growth and differentiation (Cohen, 1998) by interacting with a great variety of negatively charged compounds and structures of cells, such as nucleic acids (Raina and Jänne, 1975), membranes (Schuber, 1989), ribosomes (Raina and Jänne, 1975), lipids (Shayman and Radin, 1991) and other small molecules, such as inositol phosphates (Mernissi-Arifi et al., 1996).

Putrescine, spermidine and spermine concentrations have been determined only in homogenized kidney from different species (Rosenthal and Tabor, 1956; Tovar et al., 1995). In contrast, efforts were undertaken to locate

polyamines within the animal and human brain (Shaskan et al., 1973; Shaskan and Snyder, 1973; Shaw and Pateman, 1973; Seiler and Schmidt-Glenewinkel, 1975; Morrison et al., 1995), ram spermatozoa (Rubinstein and Breitbart, 1994) and tiger salamander retina (Valentino et al., 1996).

The mammalian kidney is an example of a heterogeneous organ that contains a great variety of cell types. The special architecture and topographical relationships between tubules and tubules/vascular structures lead us to distinguish several renal zones. Each of these zones is characterized by typical nephron segments (Kaissling and Kriz, 1979; Bankir et al., 1987). At present, it is not known how polyamines are distributed within the kidney, and whether they are concentrated specifically in renal zones, as was reported earlier for urea and organic osmolytes (Yancey and Burg, 1989; Guder et al., 1990), amino acids (Silbernagl et al., 1996) and guanidino compounds (Levillain et al., 1997). Furthermore, no data are available on the renal content of polyamines and on polyamine metabolism in the rabbit kidney. The aim of this study was to establish the distribution pattern of putrescine, spermidine and spermine within the kidneys of rats and rabbits. These biogenic amines were determined in six areas of rat (omnivorous) and rabbit (herbivorous) kidney of both sexes.

The results indicate that the polyamines are heterogeneously distributed within the kidney. Each amine exhibited its own specific distribution pattern along the cortico-papillary axis. The highest concentrations were found in the cortex. Sex and species differences are a characteristic of renal polyamines.

Materials and methods

Animals

Male and female Sprague Dawley rats (8 weeks old weighing 280 g and 250 g, respectively) from Iffa Credo (L'Arbresle sur Orge, France) and male and female New Zealand rabbits (7–8 weeks old weighing 1.5–1.7 kg and 1.8–2.0 kg, respectively) from Elevage Scientifique des Dombes (Chatillon sur Chalaronne, France) were used. They had free access to tap water and standard laboratory food (Souriffarat 20% proteins for rats and Lapin labo: 16% proteins for rabbits, Genthon S. A., France). The animals were maintained in a 12 h-dark, 12 h-light cycle as standardized laboratory conditions.

The rats were anaesthetized by intraperitoneal injection of pentobarbital (Nembutal 6%, 0.1 ml per 100 g body weight). The rabbits were anaesthetized with a pentobarbital-heparin solution (Leo, 5,000 U/ml: 1/7, v/v) injected into the marginal vein of the ear.

Dissection of the kidney

After laparotomy, the abdominal aorta was clamped above the kidneys to prevent access of most of the blood to the kidneys. Kidneys were rapidly excised, decapsulated and tissue slices were prepared from the cortex to the papilla. Six renal zones were distinguished along the cortico-papillary axis: cortex (C), outer stripe of the outer medulla (OS), inner stripe of the outer medulla (IS), the upper and the lower zones of the inner medulla (IM_1 and IM_2, respectively) and the papilla (Pap). Only small pieces of C, OS and IS were sampled from each kidney whereas the whole of the inner medulla and papilla was used. Five male and eight female rats were killed per experiment to provide enough

Table 1. Renal content of polyamines in rabbit and rat

Renal zones	Male rabbit	Female rabbit	Male rat	Female rat
Cortex	282 ± 30	592 ± 23*	611 ± 87	826 ± 6*
Outer stripe	192 ± 10#	482 ± 21*#	562 ± 53	804 ± 14*
Inner stripe	165 ± 15#	483 ± 36*#	499 ± 47	591 ± 13#£
Inner medulla 1	159 ± 22#	550 ± 38*	498 ± 67	590 ± 14#£
Inner medulla 2	136 ± 6#	499 ± 47*	503 ± 43	594 ± 11#£
Papilla	193 ± 10#	496 ± 53*	375 ± 33#	509 ± 6*β

Results are given as mean ± SEM and expressed in nmol polyamines (putrescine + spermidine + spermine) per gram wet weight. Results were statistically analyzed by ANOVA with Fisher's PLSD. When considering sex, species or regional differences, the calculated Test-F values of the ANOVA correspond to $P < 0.001$ or more. *sex difference; #, £, β statistically different from C, OS and 5 zones respectively. N = 4 male and 6 female rabbits and 7 and 5 groups of 5 male and 8 female rats, respectively. For abbreviations see the Method section.

inner medullary and papillary tissue. In contrast, one rabbit yielded sufficient tissue. The dissected tissue was immediately frozen on dry-ice to reduce the enzyme activities involved in polyamine metabolism. Samples were weighed before polyamine extraction.

Extraction and assay of polyamines

The renal tissue was homogenized in 10 volumes of $HClO_4$ 0.2 N at 4°C. After centrifugation (4,000 × g for 30 min at 4°C), the clear supernatant was used. 200 μl aliquots of the perchloric extract were reacted with dansyl chloride, and the fluorescent dansyl derivatives were separated by HPLC using a C_{18} reversed phase column, as previously described in detail elsewhere (Seiler et al., 1996).

Calculations and statistical analyses

Results are expressed in nmol per g wet weight (mean ± SEM). Statistically significant differences were calculated by analysis of variance (ANOVA, Statview II SE) and the Fisher PLSD test.

Results

General aspects of renal polyamine distribution

Polyamines were distributed along the cortico-papillary axis and their distribution patterns showed regional, sex and species differences (Table 1 and Fig. 1). Irrespective of the renal zone, the polyamine content in male rats was 2- to 3.7-fold higher than in the corresponding zone of male rabbits. In female rats, the polyamine content in cortex, outer medulla (OS + IS) was only slightly higher than in female rabbits (1.2- to 1.7-fold; $p < 0.02$). Both in rats and rabbits, the females exhibited higher polyamine levels compared with males. This difference was more marked in rabbits than in rats.

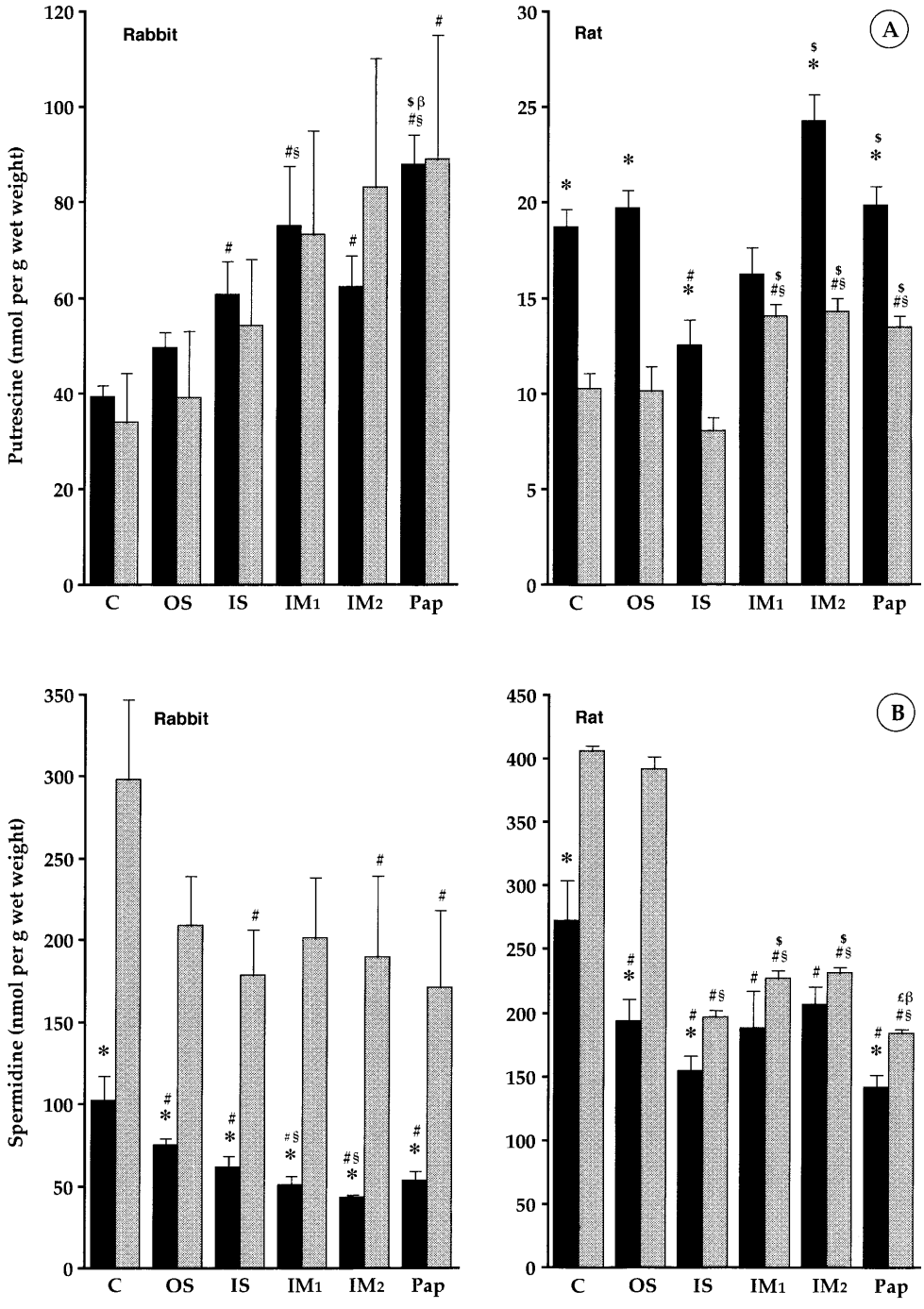

Fig. 1. Renal distribution of putrescine (**A**), spermidine (**B**) and spermine (**C**) within the rabbit and rat kidney. Results are given as mean ± SEM. Closed bars (male) and dotted bars (female). Results were statistically analyzed by ANOVA with Fisher's PLSD. When considering sex, species or regional differences, the calculated Test-F values of the ANOVA correspond to $P < 0.001$ or more. *sex difference; #, §, $, £, β statistically different from C, OS, IS, IM_1 and IM_2, respectively. N = 4 male and 6 female rabbits, and 7 and 5 groups of 5 male and 8 female rats, respectively

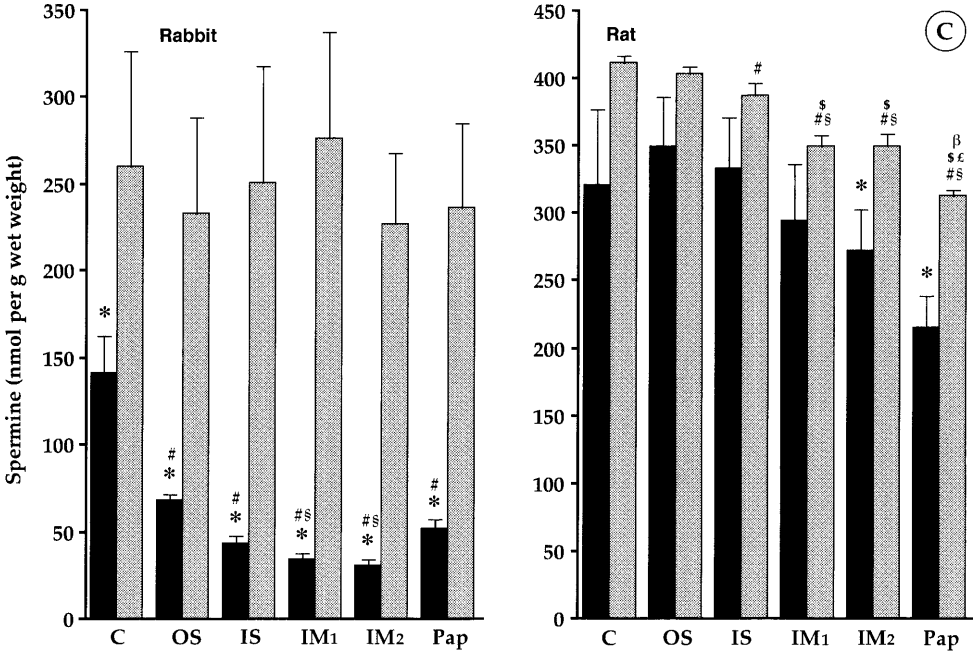

Fig. 1C

Renal putrescine content

Putrescine levels displayed considerable regional variations within the rabbit kidney (Fig. 1A. $p < 0.027$). In both males and females, putrescine concentration increased gradually from the cortex to the papilla (Fig. 1A). Individual variations were more pronounced in female than in male rabbits.

Putrescine distribution throughout the rat kidney exhibited sex and regional differences (Fig. 1A). Male rats had higher putrescine levels than females in all renal zones, except in the inner medulla (IM_1). In their outer medulla, the inner stripe contained the lowest putrescine concentration compared to the other zones ($p < 0.05$). In the cortex and the outer medulla (OS + IS) of female rats, putrescine levels were not significantly different but, were 39% lower ($p < 0.05$) than in the inner medulla and the papilla.

Rabbits exhibited higher putrescine levels than rats: they were 2- to 4-fold higher in male rabbits, compared to male rats, and 4- to 8-fold higher in female rabbits, compared to female rats (Fig. 1A).

Renal spermidine content

In rabbits, spermidine levels indicated marked sex and moderate regional differences along the cortico-papillary axis (Fig. 1B). In six renal zones, female rabbits exhibited 3- to 4-fold higher spermidine contents than males. High levels of spermidine were observed in cortex of females compared to IS, IM_2 and papilla ($p < 0.05$). In males, the cortex had a higher spermidine level

than in the other zones ($p < 0.001$) and spermidine gradually diminished from the cortex to the papilla.

In rats, spermidine distribution showed marked regional and sex differences. The cortical zone of both sexes and the outer stripe of the outer medulla of female rats contained the highest levels of spermidine compared to the other medullary zones (Fig. 1B). Females had a higher renal content of spermidine than males; this difference was more pronounced in the cortex and OS than in IS and papilla ($p < 0.02$).

In the different renal zones, male rats exhibited a 2- to 3-fold higher concentration of spermidine than male rabbits (Fig. 1B). In contrast, females presented smaller differences, except in the cortex and the outer stripe of the outer medulla.

Renal spermine content

Female rabbits exhibited 2- to 5-fold higher spermine levels than male rabbits indicating marked sex differences (Fig. 1C). Spermine concentration was constant in the six zones of the female rabbit kidney. In contrast, in males, spermine was predominantly found in the cortex ($p < 0.0001$) and decreased from the cortex to the inner medulla (IM_2).

In rats, the distribution pattern of spermine along the cortico-papillary axis was quite similar in males and females (Fig. 1C). Females showed a higher renal content of spermine and exhibited more regional differences ($p = 0.05$) compared to males. The highest level of spermine was found in the cortex and the lowest in the papilla ($p < 0.02$). In males, spermine tended to decrease from the inner stripe of the outer medulla to the papilla, but the results were not statistically significant because of considerable intragroup variations.

Regardless of the area under consideration, rats had higher renal concentrations of spermine than rabbits (Fig. 1C). This difference was more pronounced when males of both species are compared. Of the three polyamines, spermine was the most abundant in the kidney.

Discussion

Measurements of aliphatic polyamines in the kidney have been performed up to now on the whole organ. Since the kidney is composed of a variety of cells, whole organ data are of limited value for the interpretation of functions of the polyamines. We decided, therefore, to establish the distribution pattern of putrescine, spermidine and spermine in kidneys of male and female rabbits and rats. It was found that the polyamines are unevenly distributed along the cortico-papillary axis of rat and rabbit kidneys. Species and sex differences were marked. Species differences may be attributed to dietary, metabolic and behavioral differences, since rats are omnivorous and nocturnal while rabbits are herbivorous and diurnal. Sex differences are presumably related to hormonal control of the ornithine decarboxylase (ODC, EC 4.1.1.17). It is well

known that administration of testosterone to female mice causes a dramatic induction of ODC (Berger et al., 1984).

Regional differences in the polyamine content might be related to the sites of their synthesis, uptake and their physiological roles. In a defined renal zone, polyamines originate presumably from cells of nephron segments, tubular lumens, blood vessels, vasa recta and interstitial spaces, but since the nephron represents the largest fraction of renal tissue (Kaissling and Kriz, 1979; Pfaller, 1982), it is likely that most of the polyamines are stored within the tubules. In addition to the important cellular heterogeneity, a quantitative morphology of the subcellular organelles indicates that the cortical and outer medullary (OS) tissues contain a variety of membrane, structures (mitochondria, vacuoles) and more mitochondrial DNA than the inner medullary and papillary tissues (Kaissling and Kriz, 1979; Pfaller, 1982). Thus, it is likely that the high density of negatively charged macromolecules in cortex and outer stripe of the outer medulla, compared to inner medulla and papilla, may explain the elevated spermidine and spermine concentrations.

Concerning polyamine synthesis, ODC was predominantly found in rat and mouse proximal convoluted tubule (Pegg et al., 1982; Levillain and Hus-Citharel, 1998) while Spd/Spm N^1-acetyltransferase mRNA was localized in the medullary thick ascending limb and the distal convoluted tubule (Bettuzzi et al., 1995). There is no tight correlation between the renal distribution of ODC and the putrescine content in rats. One may, therefore, assume that the polyamines move from their sites of synthesis to their sites of storage. A more likely explaination is the predominant formation of putrescine from spermidine via the interconversion pathway. It is known that in highly differentiated, non-growing organs (such as the brain), putrescine formation from spermidine is quantitatively more important than its formation from de novo ornithine (Seiler, 1988). Finally, the renal uptake of polyamines (Seiler and Dezeure, 1990) from the bloodstream and the storage of spermidine and spermine could compensate for the synthetic capacities (i.e. a low ODC activity). Uptake may, thefore, be an important source of polyamines for the kidneys.

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