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Gene relaxation and aging: changes in the abundance of rat ventral prostate SGP-2 (clusterin) and ornithine decarboxylase mRNAs

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Abstract

Sulfated glycoprotein 2 (SGP-2) mRNA progressively increased in the ventral prostate of the aging rat, reaching, at 24 months, 4-fold higher than at 3 months. Ornithine decarboxylase (ODC) mRNA peaked at 6 months (4-fold increase), and at 12 and 24 months was maintained at higher levels than at 3 months. ODC enzymatic activity was enhanced at 6 months to a much smaller extent than its own mRNA, the values at 12 and 24 months dropping to below those at 3 months. Putrescine (Put), spermidine (Spd) and spermine (Sp) concentrations also peaked at 6 months (100% increase for Put, 50% for Sp and Spd). At 24 months, Put and Spd were diminished, and Sp was unchanged with respect to the 3-month values. Under the same conditions, glyceraldehyde-3-phosphate dehydrogenase mRNA did not undergo significant alterations.

Key words: Clusterin; SGP-2; Ornithine decarboxylase; Polyamine; Rat ventral prostate

1. Introduction

The progressive decline in physiological functions and resistance to diseases that characterizes the aging process appears to be related to a gradual relaxation in the control of gene expression, resulting in a slow loss of cell differentiation. Previous studies have reported direct and indirect evidence of changes in the expression of specific genes during aging (reviewed in [1]).

We have been investigating the possible cellular functions of sulfated glycoprotein 2 (SGP-2, clusterin) [2–7], a heterodimeric glycoprotein of approximately 80 kDa that has attracted the interest of many researchers because of its broad distribution in animal tissues and body fluids and its involvement in a variety of biological processes as diverse as programmed cell death, extracellular lipid transport, and complement cascade regulation (reviewed in [8–12]).

Changes in the levels of SGP-2 transcript have been studied by us [3,5,6] in several systems in parallel with changes in the levels of the transcript for ornithine decarboxylase (EC 4.1.1.17; ODC). The latter is a regulatory enzyme that catalyzes the production of the diamine putrescine, a biosynthetic precursor of aliphatic polyamines, spermidine and spermine [13,14]. These are ubiquitously distributed endogenous polycations required for normal or pathological cell growth. In fact, ODC responds typically, although not exclusively [15], to trophic stimuli, with increases in the amount of enzyme molecules, often, but not always, in consequence to corresponding enhancements in the amount of its mRNA [13,14].

We have reported previously that the dramatic augmentation of SGP-2 mRNA occurring in the ventral prostate of the rat following androgen depletion, is accompanied by a marked diminution in ODC mRNA [6]. Similarly, in an another model of programmed cell death, rat thymocytes under the influence of glucocorticoid, SGP-2 mRNA increases [3] while ODC mRNA decreases [16]. In contrast, in phytohaemagglutinin-stimulated human peripheral blood lymphocytes we detected a decrease in the SGP-2 transcript that was accompanied by an increase in ODC mRNA [5].

In view of the fact that the levels of androgens and their receptors may undergo changes during the aging process, we addressed the question of whether SGP-2, ODC gene expression, and other parameters related to polyamine metabolism, are affected by aging in the rat ventral prostate.

2. Materials and methods

2.1. Animals

Male rats of the Wistar strain aged 3–24 months, from a commercial source, were used. They were kept under controlled temperature, humidity and lighting conditions (lights on at 08.00 h and lights off at 20.00 h) and with free access to water and food.

2.2. Molecular probes and hybridization conditions

The cDNA coding for SGP-2 was cloned in pUC13 [2]. The recombinant plasmid, called pSB28, was digested with *Eco*RI and *Stu*I to release the 1513 bp cDNA insert containing the entire coding region for SGP-2 and 67 bp of the 5′ region and 112 bp of the 3′ region. After isolation by agarose gel electrophoresis and purification by Geneclean (Bio 101), the 1513 bp fragment was labelled by the oligolabelling kit from Promega in the presence of [α-^{32}P]dCTP to a specific radioactivity of about 2×10^9 dpm/μg DNA. The cDNA coding for ODC was obtained by pODCE10, kindly donated by Dr. van Kranen [17]. After

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Abbreviations: SGP-2, sulfated glycoprotein 2; ODC, ornithine decarboxylase; Put, putrescine; Spd, spermidine; Sp, spermine.

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digestion of the plasmid with *Eco*RI and *Hin*dIII, a 950 bp cDNA coding for ODC was excised, purified and labelled as in the case of SGP-2. The 1.2 kb fragment coding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained by digestion of the plasmid with *Pst*I. It was then purified and labelled as before. Northern blot analysis was performed according to Maniatis et al. [18]. Total RNA (10 μg) was blotted on Hybond membranes from Amersham and crosslinked with UV light. The prehybridization and hybridization conditions were as described elsewhere [2]. Quantitation of the autoradiograms was obtained by densitometric scanning (LKB Ultrascan XL densitometer).

2.3. RNA preparation

RNA extraction was performed using the guanidinium thiocyanate method of Chirgwin et al. [19].

2.4. Enzyme assay

ODC activity was determined by quantitating the release of $^{14}CO_2$ from [1-^{14}C]ornithine by the method of Jänne and Williams-Ashman [20] with minor modifications. Aliquots (about 0.2 mg protein) of the high speed supernatants of tissue homogenates were incubated at 37°C for 60 min with 50 mM Tris-HCl, pH 7.5, 40 μM pyridoxal-5'-phosphate, 5.0 mM dithiothreitol, 160 μM L-ornithine, 0.5 μCi DL-[1-^{14}C]ornithine (57 mCi/mmol) in a final volume of 0.125 ml.

2.5. Determination of polyamines

Polyamines were extracted from tissues with perchloric acid and assayed by HPLC with fluorescence detection after pre-column derivatization with dansyl chloride [21].

2.6. Determination of protein

Protein concentrations were detected according to Lowry et al. [22].

3. Results

We report here the changes in the abundance of SGP-2, ODC and GAPDH mRNAs and in ODC activity and polyamine concentrations, occurring in the ventral prostate of the rat at 3, 6, 12 and 24 months of age. Fig. 1 shows that in this organ the aging process is accompanied by a progressive marked increase in the levels of the mRNA for SGP-2 as detected by Northern blot analysis of total RNA. Indeed, accumulation of this transcript increased almost linearly, by 4-fold, in animals aging from 3 to 24 months. Under the same circumstances the mRNA for ODC increased by about 4-fold, from 3 to 6 months, and decreased thereafter, although remaining higher than at 3 months (Fig. 1). Similarly to other rat tissues, Northern analysis of rat ventral prostate total RNA with rat ODC cDNA gave rise to three hybridization bands corresponding to three ODC mRNA species; since each single band appeared to be similarly affected by aging (Fig. 1) the three bands were quantitated together.

The transcript for GAPDH did not change significantly in the ventral prostate of the aging rat (Fig. 1).

The activity of ODC determined in the same tissues (Fig. 2) exhibited a pattern similar to that of ODC mRNA, except that the increase at 6 months was much smaller and the activity at 12 and 24 months descended well below that detected at 3 months. Also the patterns of concentrations of putrescine and polyamines paralleled those of ODC mRNA and activity (Fig. 2). At

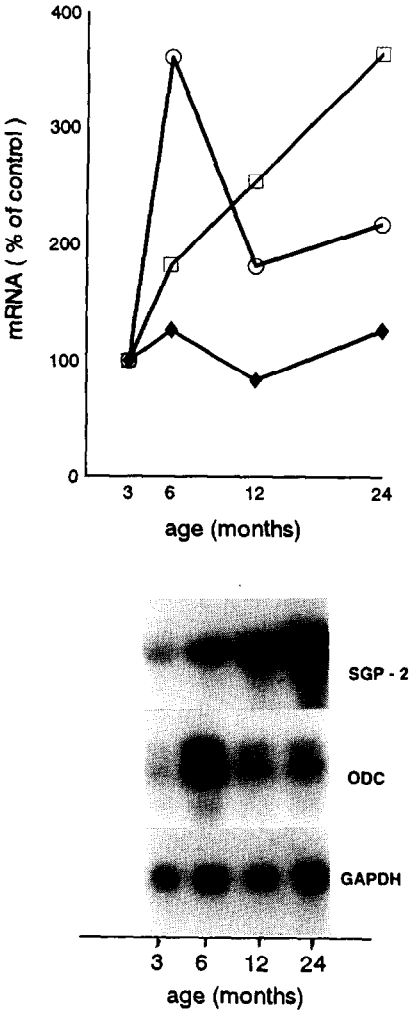

Fig. 1. Northern blot analysis of total RNA (10 μg) from rat ventral prostate extracted at the ages indicated and hybridized to SGP-2 (□), ODC (○) or GAPDH (◆) cDNAs. Autoradiograms were quantitated by densitometric scanning. Patterns of repercentage values (values at 3 months = 100) from one single representative experiment out of three, are shown. The densitometric values of the bands of the three ODC mRNA species were pooled.

6 months, putrescine increased by more than 100% and spermidine and spermine by more than 50%. At 12 months all three amines had returned to the 3 month levels. At 24 months the concentration of spermine remained constant, while that of spermidine decreased slightly, and that of putrescine was about 40% less than at 12 months.

4. Discussion

Age-dependent alterations in the expression of specific genes, either due to stochastic events and/or to a genetically determined program, appear to be part of the mechanisms underlaying the aging process. We show here that

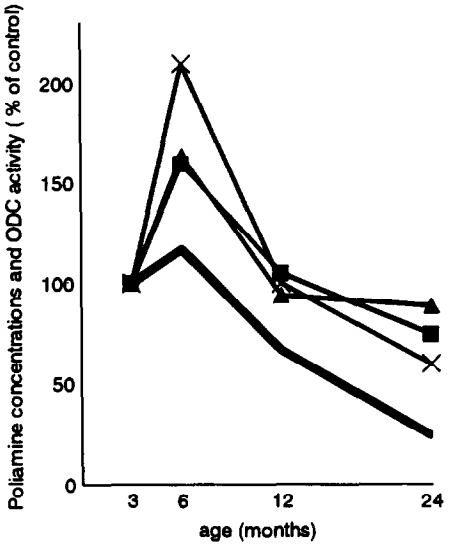

Fig. 2. ODC activity (–), putrescine (×), spermidine (■) and spermine (▲) concentrations in the ventral prostate of aging rats. Patterns of percentage values (values at 3 months = 100) from one single representative experiment out of three, are shown. At 3 months, ODC activity was 1.070 nmol $^{14}CO_2$ released/mg protein/h; amine concentrations were: putrescine 237, spermidine 1,742, spermine 1,002 pmol/mg fresh tissue.

in the rat ventral prostate (and not in the liver or testis; Marinelli et al. manuscript in preparation), the transcript for SGP-2 increases almost linearly with increasing age; under the same conditions, the pattern of ODC mRNA peaks at 6 months, while GAPDH mRNA remains essentially constant. In other experiments not shown here, accumulation of SGP-2 mRNA in the ventral prostate of 21-month-old rats was 11-fold higher than in 6-month-old animals.

The extent, specificity and regular progression of SGP-2 mRNA accumulation strongly suggest that the SGP-2 gene participates in the genotypic alterations accompanying aging of the rat ventral prostate. Due to its distribution in biological systems that play totally different roles in mammalian organisms, many different functions have been proposed for SGP-2. The enhancements in the rate of its expression that often precede the phenomenon of programmed cell death (apoptosis), have provoked suggestions that SGP-2 plays a role in the latter process (reviewed in [8,9,11,12]).

The age-related increase in the abundance of SGP-2 transcript that we show here (i) is not associated with an opposite response of the ODC transcript comparable to that reported by us in several systems [3,5,6]; (ii) develops progressively and not transiently as in the case of androgen depletion [2]; and (iii) is not accompanied by the marked activation of apoptotic processes and organ involution that characterize the latter condition. These features suggest that SGP-2 gene relaxation in the ventral prostate of the aging rat is part of a process which is different from that induced by androgen ablation. Thus, the diminution of the circulating levels of the latter hormones known to occur in the aging rat [23], can hardly be considered the cause of SGP-2 mRNA accumulation. The possible role played by the progressive augmentation in the levels of estradiol occurring under the same conditions [23] is currently being investigated in our laboratory.

Between 6 and 24 months of age, diminutions in ODC mRNA (Fig. 1) and activity (Fig. 2) occur that are similar to those previously reported with AXC/SSh rats [24]. At variance with results obtained in the same laboratory [25], we found decreases and not increases in spermidine and spermine concentrations. Considering the role of polyamines in normal or pathological cell growth, we wonder whether these different responses to aging bear any connection to the proneness of AXC rat prostate to malignant transformation.

The peak exhibited by ODC mRNA, ODC activity, putrescine and polyamine concentrations at 6 months indicates the occurrence, at around that age, of presently unknown physiologic event(s) requiring a transient enhancement of the levels of these polycations.

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