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Rat age-related benign prostate hyperplasia is concomitant with an increase in the secretion of low ramified α-glycosydic polysaccharides

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ABSTRACT

This work analysed the expression of prostate polysaccharides in rats with age-related benign prostatic hyperplasia (BPH) for a better understanding of the possible relationship between prostate polysaccharides secretion and BPH onset. For this, prostatic glands from 1 month-old, 3 months-old, 6 months-old and 12 months-old Sprague-Dawley rats were processed in order to identify their overall polysaccharide content. Additionally, serum testosterone was also determined. One-month old rats showed significantly ($P < 0.05$) lower testosterone levels (0.77 ng/mL±0.12 ng/mL) compared with the other groups, which showed no significant difference among them. PAS staining showed positive polysaccharides markings in both the prostatic lumen and inside of luminal prostatic cells in all groups. Semiquantitative analysis of intraluminal PAS showed that one month-old rats had significantly ($P < 0.005$) lower PAS intensity when compared with all other groups (100.0 ± 0.5, arbitrary units vs. 107.3 ± 0.6, arbitrary units in 3 months-old ones), whereas 12 months-old ones showed significantly ($P < 0.005$) higher values when compared with all other groups (133.6 ± 3.5, arbitrary units in 12 months-old rats vs. 108.6 ± 1.4, arbitrary units in 6 months-old ones). The PAS + content practically disappeared when tissues were pre-incubated with either α-amylase or amyloglucosidase, regardless of a previous incubation with proteinase K. Incubation of prostate extracts from 12 months-old rats for 2 h with α-amylase yielded a significantly higher amount of free glucose (1.47 nmol/mg protein±0.23 nmol/mg protein vs. 0.32 nmol/mg protein±0.01 nmol/mg protein in untreated extracts). Similar results were obtained when extracts were pre-incubated with amyloglucosidase. Contrarily, pre-incubation with *N*-glycosidase induced a significantly ($P < 0.05$), much lower increase of free glucose. Pre-treatment with proteinase K did not significantly modify these results, which indicate that BPH is related to an increase in the secretion of low ramified ductal α-glycosydic polysaccharides that were not protected against lysis by any type of protein protective core. These changes seem to not be related with concomitant variations in serum testosterone levels.

1. Introduction

Age-related benign prostate hyperplasia (BPH) is a very common condition affecting a very high percentage of men above 50 years old and also older male individuals from other species such as rodents [1–3]. Although BPH is a benign process, its importance lies in the fact that it often develops towards malignant situations such as prostate carcinoma [3]. Thus, a close vigilance of the evolution of BPH is needed to avoid the appearance of derived, malignant processes.

Control of BPH should be based on a good understanding of its pathogenesis. However, currently there are several important aspects regarding BPH pathogenesis that are not well understood, impairing thus an optimal surveillance of the process. Among these, one of the most prominent would be centred in BPH-related alterations of prostatic secretions. In this regard, it is well known

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that one of the important components of prostatic secretions are amyloid bodies (ABs; see Refs. [4,5]). The onset of BPH has been related to a noticeable increase in the ABs content of prostatic secretions [4,5], suggesting thus a relationship between ABs metabolisms and the onset of BPH. Moreover, the malignant evolution of BPH towards carcinoma has also been related to a significant change in the composition of ABs. Thus, whereas ABs of both healthy and BPH-affected prostatic glands seem to be mainly composed of insoluble amylaceous compounds, those from malignant carcinoma tissues seem to contain mucin and unidentified crystalloids [4–6]. This difference would indicate a significant change in the sugar metabolism linked to the appearance of malignant prostate cells, which would be in accordance with the already described change in metabolism fluxes related to the appearance of malignity in all types of tumours [7]. However, despite all of our knowledge, the exact composition of ABs is currently not well known. Thus, a complete description of the amylaceous compounds forming BPH-related ABs is lacking, with the exception that they contain glucosamine, galactose and sulphur [8], which could point to the presence of keratan sulphate [6]. Related to this, it has been also observed that BPH is concomitant with an increase in the expression of chondroitin sulphate located at the basal membrane of epithelial ductal cells and periductal myocytes [6]. However, it is not known if chondroitin sulphate is a relevant component of BPH-related ABs.

Taking into account the above-described points, the main aim of this work was to go in depth in the exact composition of prostate polysaccharide secretions linked with the BPH process. The work was centred on the identification of the overall structure present in most prostate polysaccharides through their analysis by both PAS staining and selective polysaccharide degradation by using specific polysaccharolythic enzymes, namely α-amylase, amyloglucosidase and *N*-glycosidase, and further testing the possibility of a close structural linking of these polysaccharides with a protein core by selective digestion with proteinase K.

2. Material and methods

2.1. Animals

The animals used were healthy male Sprague Dawley rats aged 1 month-old (prepuberal, n = 20), 3 months-old (pubertal, n = 20), 6 months-old (mature, n = 9) and 12 months-old (elder, n = 9). The rats were not raised in the university facilities, but they were directly purchased from a commercial supplier (Harlan Interfauna Ibérica S.L.; Sant Feliu de Codines, Spain) at the indicated ages. Once at the laboratory, animals were housed in the university facilities in controlled environmental conditions of 12 h/12 h light/darkness cycle, at 20 °C and 40% humidity, fed with a commercial diet and "ad libitum" access to water. Rats were kept in these conditions for 48 h before euthanasia. Animals were euthanized introducing them in a CO_2 chamber for 5 min. Following the monitorized death, blood was immediately sampled through intracardiac puncture and animals were subsequently dissected to perform prostates collection. Regarding blood collection, samples were allowed to clot at 20 °C for 20 min and, afterwards, serums were separated by centrifugation at 1600×*g* at 4 °C for 10 min. Subsequently, serums were immediately frozen in liquid N_2 and further stored at −80 °C until their analyses were performed. Regarding dissected prostates, only the ventral prostate was used in the study. For this purpose, ventral prostates were carefully dissected and then excised into two portions. One portion was fixed by immersion in a 4% (v:v) formaldehyde solution in phosphate buffered saline (PBS, pH 7.4) at 20 °C by using a volume proportion of 10:1. The minimum time elapsed between the start of fixation and the use of samples was 24 h at 20 °C. Fixed portions were used for histological analyses. The second portions were immediately frozen in liquid N_2 and then they were stored at −80 °C. These portions were used for studies centred in the analysis of the prostate polysaccharide content. The design of our experiment did not require any specific code from the Ethical Commission (EC) of the Autonomous University of Barcelona (UAB), since there was no experimentation procedure linked with the work. Despite this, it was specifically approved by the EC of the UAB after checking that it followed all of the Animal Welfare Guidelines approved by the Spanish and Catalan Governments.

2.2. Determination of serum levels of testosterone

Levels of serum testosterone were determined by means of a commercial ELISA kit (Testosterone rat/mouse ELISA kit; ref. DEV9911; Demeditec Diagnostics GmbH, Kiel, Germany).

2.3. Processing samples for histological procedures

As stated, formaldehyde-fixed samples were first thoroughly washed with PBS and then dehydrated through sequential immersion in ethanol dilutions from 70% (v:v) to 100% (v:v), following standard practices. Dehydrated samples were then treated with xylol for 1 h at 20 °C and embedded in paraffin overnight at 58 °C. Paraffined samples were then cooled at 4 °C for 1 h until the formation of a solid block. These blocks were subsequently sliced by means of a microtome (HistoCore Biocat®; Leica Biosystems; Wetzlar, Germany) to obtain slices with a thickness of both 5-μm and 7-μm. These slices were then placed onto 25 mm × 75 mm xylene-coated slides for their analysis.

2.4. Histological staining procedures

Two separated histological stainings were used in this work. The first was a standard haematoxylin-eosin (H-E) staining in order to determine the histological architecture of samples. The second staining was carried out through the periodic acid-Schiff (PAS) technique, which analysed the polysaccharide content of the histological samples. Both H-E and PAS procedures were carried out following previously well-established procedures as shown in Refs. [9,10], which were applied to both 5 μm and 7 μm samples. Focusing on PAS technique, in some cases, slides were previously incubated with a proteinase K (Sigma Aldrich; St. Louis, USA) solution in PBS at a final dilution of 30 IU/mL to determine putative changes in PAS stain related with the presence/absence of proteins.

Semiquantitative analysis of PAS staining intensity was conducted by using the Image J Fiji image processing package [11]. For this purpose, images of 9 individuals from each age group were analysed. For each animal, 10 circular sections taken at the centre of each ductus pertaining to 10 separate intraductal areas were processed to avoid significant bias of the intensity linked to the background. These circular sections had in all cases a diameter of 5 μm. All images were processed with the same setup values of brightness and contrast to avoid artefactual bias of results and mean intensity of the red channel of each section was taken. Afterwards, the mean values for the 10 obtained measures for each animal were determined. Following this, the obtained mean value from the first animal of the 1 month-old group was transformed to an arbitrary value of 100. Subsequently, all mean values for all other animals, regardless of the age group, were transformed by using the same factor applied to the first individual. Finally, the appropriate statistical procedures explained in the appropriate section of this manuscript were applied to these transformed values.

2.5. Analysis of polysaccharide content of prostatic tissue

Only samples from 12 months-old rats were used for this analysis, since these animals showed the highest levels of polysaccharides. The analysis was based on the incubation of tissue extracts with separated enzymes that had a specific ability to hydrolyze polysaccharides. The used enzymes were α-amylase, amyloglucosidase and *N*-glycosidase. Incubations were performed either in tissues without any previous treatment or with a first incubation with proteinase K to determine the possible presence of protein-trapped polysaccharides that were not hydrolysed in raw conditions. Procedures were the following:

To determine polysaccharide content in raw samples, frozen samples stored at −80 °C were homogenised in an ice-cold 50 mM Tris-HCl homogenization buffer (pH 7.4) containing 1 mM ethylene diamino tetraacetic acid (EDTA), 10 mM ethyelenglycol-bis(2-aminoether)-N,N,N′,N′ tetraacetic acid (EGTA), 25 mM dithyotreitol (DTT), 1.5% (v:v) Triton X-100®, 1 mM phenylmethyl sulfonil fluoride (PMSF), 10 μg/mL leupeptin, 1 mM Na_2VO_4 and 1 mM benzamidine. Homogenization was performed by using an Ultra-Turrax® T25 basic device (IKA®-Werke; Staufen, Germany) at 13.500 rpm in ice-cold conditions and with a proportion of 5 mL buffer:1 mg sample. A 10 μL aliquot of each sample was then taken in order to evaluate the total protein content of samples through the Bradford technique [12] by using a commercial kit (Protein Assay Dye Reagent Concentrate; BioRad; London, UK). Afterwards, homogenised samples were centrifuged at 1200×*g* for 10 min at 4 °C and resultant supernatants were fractionated in 4 separated fractions of equal volume. Three portions were added with a 0.07 M acid acetic-sodium acetate buffer (AA buffer, pH 4.7), whereas the remaining fraction was added with PBS. Dilution of all four fractions was performed until obtaining a final protein concentration of 2.5 mg/mL. Afterwards, two of the fractions diluted in the AA buffer were added with either α-amylase or amyloglucosidase to a final concentration of both enzymes of 0.2 IU/μL, whereas the other fraction diluted in the same buffer was left without any other addition as a control point. Otherwise, PBS diluted samples were also added with *N*-glycosidase until a final activity of 0.2 IU/μL. All samples were incubated for 2 h at 37 °C. Then, enzyme reactions were stopped by immersion of samples in liquid N_2. Samples were subsequently thawed and glucose concentration was determined espectrophotometrically by using a commercial kit (Glucose HK CP ABX, Horiba; Montpellier, France).

The determination of polysaccharide content in samples previously treated with proteinase K was performed after homogenization of frozen samples stored at −80 °C in an ice-cold 50 mM Hepes buffer (pH 5.0) containing 25 mM DTT, 1.5% (v:v) Triton X-100® and 6 mM $CaCl_2$. Homogenization was performed following the same protocol described above. At this moment, a 10 μL aliquot was taken in order to determine the total protein content by using the Bradford technique [12] as described above. Afterwards, proteinase K (Sigma Aldrich; St. Louis, USA) was added to the homogenised samples until a final enzyme activity of 30IU/mL. Samples were then incubated for 10 h at 37 °C. Proteinase K reaction was stopped by adding PMSF to samples to a final concentration of 5 mM. At this moment samples were centrifuged at 1200×*g* for 10 min at 4 °C and resultant supernatants were split in 4 separate fractions of equal volume. These fractions were diluted and incubated without or with α-amylase, amyloglucosidase or *N*-glycosidase following the same protocol described above. Finally, enzyme reactions were stopped by immersion of samples in liquid N_2. Samples were subsequently thawed and glucose concentration was determined espectrophotometrically by using the Glucose HK CP ABX commercial kit (Horiba; Montpellier, France).

2.6. Statistical analysis

Putative statistical differences among results were carried out by using a statistical package (SPSS® Ver 25.0 for Windows; IBM Corp., Armonk, USA). Analysis of both serum testosterone levels and semiquantitative PAS intensity were performed with the application of the Student-Newman Keuls (S–N–K) test. Otherwise, data regarding the effects of incubation with glycosidases in the absence or presence of proteinase K were first tested for normal distribution with the Shapiro Wilks test. In this case, all data showed normal distributions. Afterwards, data was compared by using a two-way analysis of variance (ANOVA), in which the Factor 1 was the incubation with or without any glycosidase and the Factor 2 was the incubation with or without proteinase K. Subsequently, the ANOVA analysis was followed by a *post hoc* Sidak test for pair-wise comparisons.

3. Results

3.1. Histopathological detection of benign prostatic hyperplasia

The histological study of prostate gland in prepubertal rats by using the H/E staining procedure showed the presence of a one-layer ductal tubular epithelium formed by cuboidal cells. This epithelium was surrounded by mesenchyme which contained connective cells and blood vessels (Fig. 1A and B). Furthermore, tubuli were filled by an amorphous, acellular, strongly H/E reactive content (Fig. 1A and B). This image was only slightly modified in pubertal (3 months-old) and mature (6 months-old) rats, although the diameter of tubuli was noticeably greater when compared with prepuberal ones (data not shown; Fig. 1C and D). On the other hand, several 6 months-old animals showed early signs of tubular hyperplasia. These signs were very focalized and punctual, showing the presence of more than one layer of ductal cells (Fig. 1C,D). Cclear signs of hyperplasia were detected in all of the analysed 12 months-old rats. In these animals, hyperplasia was detected by the presence of focal areas of variable size in which ductal epithelium was composed by more than one layer of cells. Moreover, prostatic mesenchyme of these animals was characterised by a noticeable increase in the number of blood vessels, especially around and inside the hyperplasia areas (Fig. 1E and F).

3.2. Serum levels of testosterone

As shown in Figs. 2 and 1 month-old rats showed the lowest levels of serum testosterone (0.54 ng/mL±0.07 ng/mL; mean ± S.E.M.). These levels were significantly ($P < 0.05$) higher in 3 months-old rats, which showed mean values of 2.71 ng/mL±0.36 ng/mL (mean ± S.E.M.). Finally, serum testosterone levels from both 6 months-old and 12 months-old rats were not significantly different to those obtained in 3 months-old animals (Fig. 2).

3.3. Analysis of polysaccharide intraductal content by applying the PAS staining

The PAS staining showed a positive intraductal content in all the studied rats, from prepubertal animals to 12 months-old ones (FigA. 3). Likewise, epithelial ductal cells also showed a PAS + content in all cases (Fig. 3A). This content would be consistent with the presence of ABs. Previous incubation of samples with α-amylase induced a strong decrease and even a complete disappearance of the intraductal PAS staining (Fig. 3C). A similar result was obtained with samples pre-incubated with amyloglucosidase

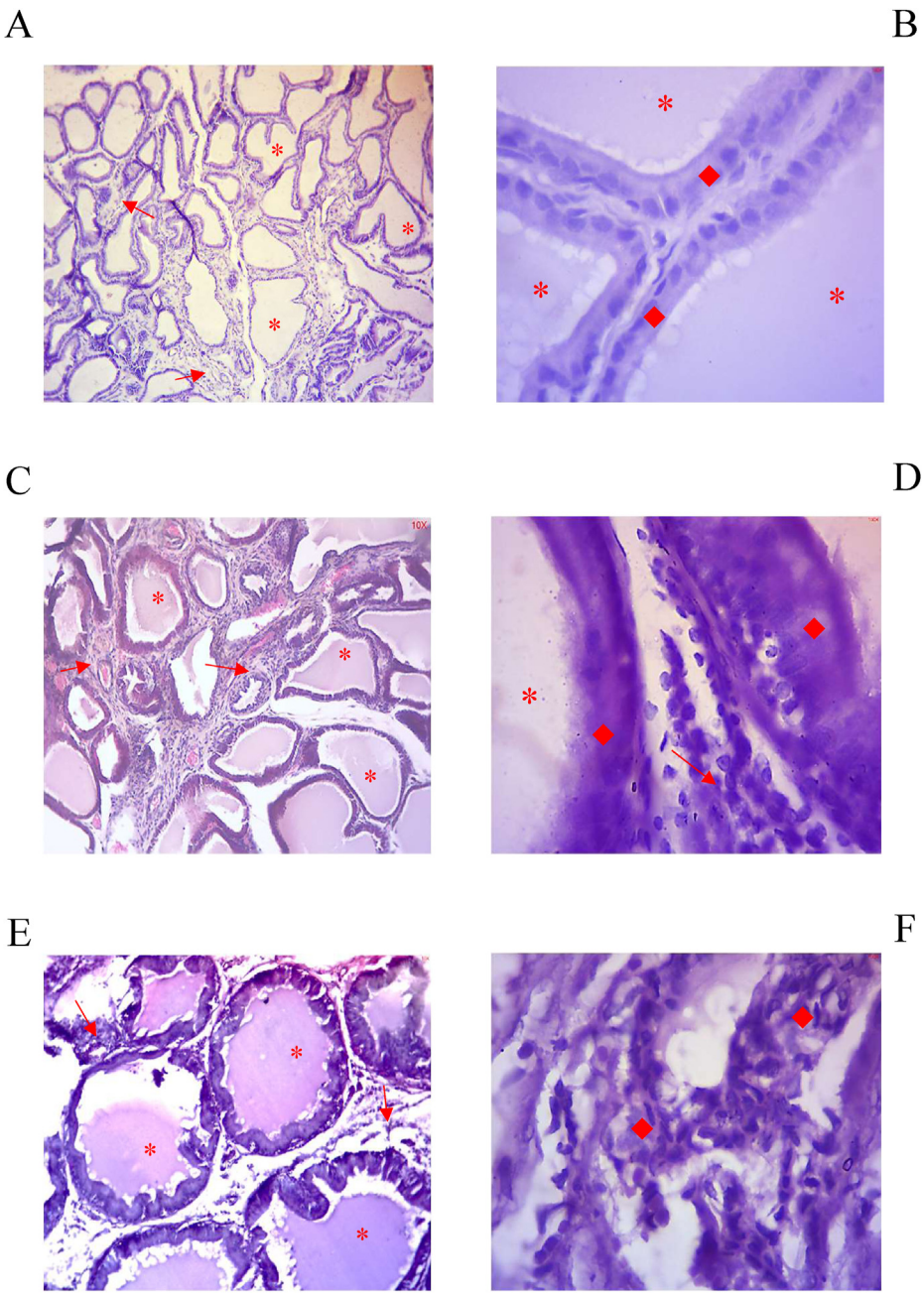

Fig. 1. Prostatic tissue from 1 month-old, 6 months-old and 12 months-old rats stained with the haematoxylin-eosin technique. Prostatic samples from 1 month-old, 6 months-old and 12 months-old rats were fixed and stained as described in the Material and Methods section. A,B: 1 month old rats. C,D: 6 months-old rats. E,F: 12 months-old rats. A,C,E: images observed at 100X augmentations. B,D,F: images observed at 1000X augmentations. *: intraductal areas. →: interstitial tissue. ◆: ductal epithelium. Bars in images A, C and E indicate 100 μm length, whereas bars in images B, D and F indicate 20 μm length. Figure shows representative images obtained from twenty 1 month-old rats, nine 6 months-old animals and nine 12 months-old ones.

previously to the PAS staining (Fig. 3E). Noticeably, an appreciable loss of PAS staining was observed when samples were incubated for 2 h with the dilution medium of both α-amylase and amyloglucosidase without enzymes, although this decrease was in all cases much less intense than the observed when enzymes were applied (Fig. 3B). The previous incubation with proteinase K before further incubation with either α-amylase or amyloglucosidase did not modify the above-described results (Fig. 3D and F).

The semiquantitative analysis of the intraductal PAS staining intensity yielded mean low values in 1 month-old rats (100.0 ± 0.5, arbitrary units; see Fig. 4). These values were significantly ($P < 0.05$) higher in animals from both the 3 months-old group and the 6 months-old one (107.3 ± 0.6, arbitrary units in 3 months-old rats and 108.6 ± 1.4, arbitrary units in 6 months-old ones; Fig. 4), while no significant differences between the 3 months-old rats and 6 months-old ones were detected. Finally, 12 months-old ones showed significantly ($P < 0.005$) higher values when compared with all other age groups (133.6 ± 3.5, arbitrary units; Fig. 4).

3.4. Free glucose release from prostate gland extracts in the presence of α-amylase, amyloglucosidase or N-glycosidase

The incubation of raw prostate gland extracts for 2 h without any enzyme yielded a concentration of free glucose of 0.32 nmol/

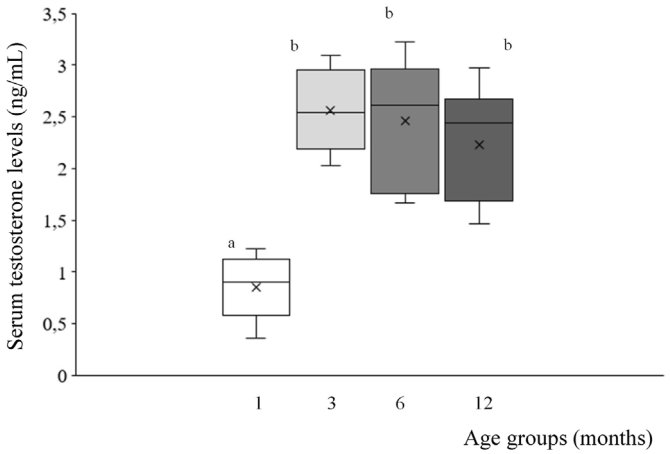

Fig. 2. Box and whisker plot representation of serum testosterone levels. White bar: 1 month-old rats. Light grey bar: 3 months-old rats. Medium grey bar: 6 months-old rats. Dark grey bar: 12 months-old rats. Results are obtained from 9 animals in each age group. Asterisks indicate the mean value for each group, whereas internal lines shown medians. Separate letters indicate significant ($P < 0.05$) differences when compared with the 1 month-old rats group.

mg protein ± 0.03 nmol/mg protein (mean ± S.E.M). A similar incubation for 2 h with either α-amylase or amyloglucosidase induced a significant ($P < 0.05$) increase in free glucose, which reached values of 1.47 nmol/mg protein ± 0.03 nmol/mg protein with α-amylase and 1.28 nmol/mg protein ± 0.04 nmol/mg protein with amyloglucosidase (means ± S.E.M.; see Fig. 5). There were no significant differences among values obtained with either α-amylase or amyloglucosidase. On the contrary, the incubation with *N*-glycosidase for 2 h induced significantly ($P < 0.05$) lower glucose levels, with values of 0.41 nmol/mg protein±0.03 nmol/mg protein (mean ± S.E.M.; see Fig. 5). Free glucose levels of samples previously digested with proteinase K without the addition of any other enzyme reached values of 0.53 nmol/mg protein±0.03 nmol/mg protein (mean ± S.E.M.; see Fig. 5). These values were significantly ($P < 0.05$) higher than those obtained in raw samples without either α-amylase or amyloglucosidase. On the other hand, incubation for 2 h with either α-amylase or amyloglucosidase of samples previously digested with proteinase K induced a significant ($P < 0.05$) increase in free glucose in both cases, which reached values of 1.52 nmol/mg protein±0.03 nmol/mg protein with α-amylase and 1.56 nmol/mg protein±0.05 nmol/mg protein with amyloglucosidase (means ± S.E.M.; see Fig. 5). Again, there were no significant differences among values obtained with either α-amylase or amyloglucosidase in these conditions, neither when compared with parallel samples incubated without a previous proteinase K digestion (Fig. 5). Finally, the incubation with *N*-glycosidase for 2 h caused significantly ($P < 0.05$) lower glucose levels, which reached values of only 0.45 nmol/mg protein±0,07 nmol/mg protein after 2 h of incubation (mean ± S.E.M.; see Fig. 5).

4. Discussion

Results obtained in this study strongly suggest that the onset of rat BPH is related to an increase in the synthesis of extracellular, ductal α-glycosidic polysaccharides with low ramification. Furthermore, these changes seem to not be related with concomitant variations in serum testosterone levels. In this regard, our results indicate that, although there was a significant increase of testosteronemia linked to puberty, there were no significant differences in post-pubertal rats, irrespectively of their age. The increase of testosteronemia linked with the onset of puberty is logical, and, in this way, has been previously reported [13,14]. On the other hand, the lack of great variations in testosteronemia in post-pubertal rats depending on age has been more controversial. Whereas older works found a noticeable drop of testosteronemia in aged rats [15], more recent publications obtained similar results to those shown in this manuscript [16–19]. This discrepancy can be due, on the one hand, to the different methodological techniques applied in each manuscript to detect testosterone and, on the other one, to animal-related differences linked to aspects such as the specific breed and genetic line or even the time of the day in which samples were taken [16–19]. In any case, taking into consideration the utilised technique to determine testosterone and the fact that testes production of testosterone has been described to be maintained in a stable manner during the entire rat lifetime [20], our results can be considered as entirely logical. In any case, the stable serum testosterone levels suggest that the onset of age-related BPH is not linked to evident changes in testosteronemia. In this way, the role of testosterone in the induction of age-related rat BPH could be more linked to a chronic action of the hormone on prostatic tissue by holding testosteronemia high and stable during all the adult life.

As stated above, results indicate that the onset of BPH was concomitant with an increase of the polysaccharide prostatic secretion. It is noteworthy that the incubation of prostate gland extracts with an enzyme media that did not contain either α-amylase or amyloglucosidase induced by themselves a small, but significant release of free glucose from tissues. This result would be a first indication that the polysaccharide content of the prostate gland is mainly composed of substances with high levels of α-glycosidic bonds. This can be deduced by the fact that α-glycosidic bonds are highly sensitive to acidic environments [21] and the utilised media had a pH around 4.8. The preponderance of α-glycosidic bonds was further confirmed by the appearance of free glucose when prostatic extracts were incubated with either α-amylase and amyloglucosidase but not with *N*-glycosidase. Furthermore, results from both PAS staining and glycosidases digestion also suggest that prostatic polysaccharides were mainly composed of compounds with low ramification levels. The low ramification was indicated by the fact that the levels of free glucose obtained after the digestion of prostate gland tissues were not significantly different when compared with non-digested ones. In this respect, it must be reminded that, whereas both α-amylase and amyloglucosidase degrade α-1,4 glycosidic bonds, only amyloglycosidase is able to hydrolyze α-1,6 ones, which are responsible for polysaccharide ramification [22–24]. A similar conclusion has been previously suggested [4,25], although in those cases, confirmation of low ramification levels was only suggested indirectly, being our results the first direct indication of this phenomenon. Likewise, the lack of an evident increase in the free glucose levels of samples previously digested with proteinase K, with the exception of control samples, suggests that most polysaccharides present in prostatic tissues are not closely linked to a protein structure or nucleus. This assertion could seem contradictory with the results obtained through the application of PAS techniques in slides previously incubated with proteinase K. One explanation for this apparent contradiction would be that the polysaccharide content observed in PAS-stained slides would be held in position with a light protein structure that, when digested, throws out the polysaccharide content from slides after the separate washings included in the PAS protocol. On the other hand, this light protein structure did not impede the lytic action of both α-amylase and amyloglucosidase, maintaining thus similar levels of free glucose in extracts regardless of their pre-incubation with proteinase K or lack of it. Our results, however, cannot completely identify what is the main

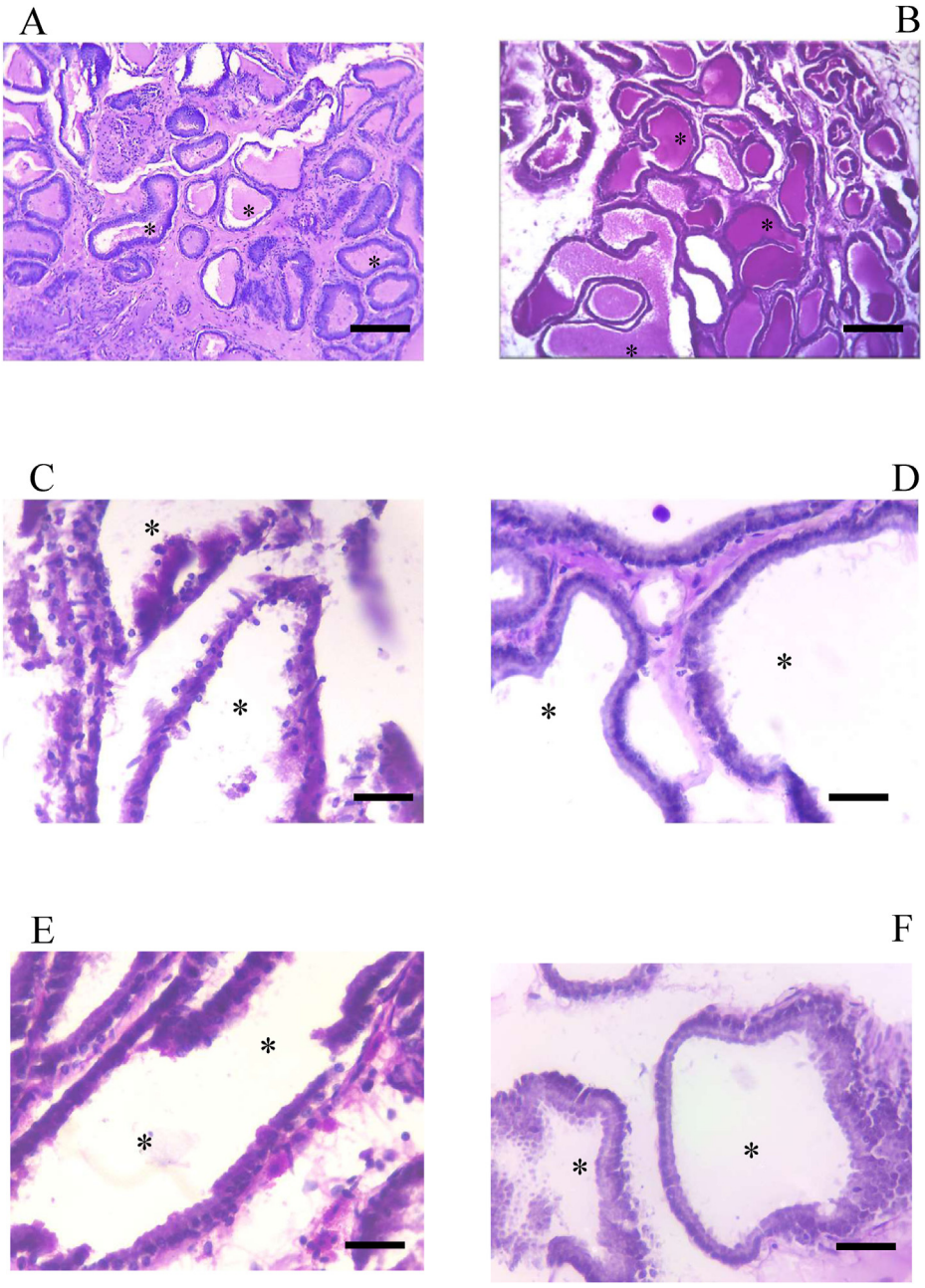

Fig. 3. Prostatic tissue from 3 months-old rats stained with the PAS technique. Prostatic samples were fixed and stained as described in the Material and Methods section. A: samples stained without incubations. B: samples incubated for 2 h with the dilution medium for both α-amylase and amyloglucosidase. C,D: samples incubated for 2 h with α-amylase. E,F: samples incubated for 2 h with amyloglucosidase. D,F: samples previously incubated with proteinase K for 8 h. All images are observed at 400X augmentations. Bars in images indicate 20 μm length. Selected images show representative results obtained in 3 months-old rats. These results are representative not only for the 3 months-old group, but for all of the other age-classified ones. Rats analysed were twenty in both 1 month-old group and 3 months-old one and nine in 6 months-old group and 12 months-old one.

polysaccharide content of prostatic tissue. Despite this, several conclusions can be reached. First, glycogen can be discarded as the main compound since it is highly ramified, containing a great number of α-1,6 glycosidic bonds [26]. In this way, results are pointing to an amylaceous polysaccharide since these compounds are characterised by low ramification levels as well as by very low or totally absent presence of *N*-glycosidic bonds [27]. Likewise, this hypothesis is concomitant with results obtained by other authors [28,29]. In this way, amylaceous compounds, mostly with a free-protein content, would be the most probable candidates to integrate the main polysaccharide component of prostatic tissue of old, BPH-affected rats.

5. Conclusions

Our results indicate that age-related benign prostate hyperplasia (BPH) in rats is concomitant with an increase in the accumulation of low-ramified polysaccharide content with predominance of α-glycosidic bonds inside of prostate ducts. This polysaccharide content was not linked with any protein structure that protects it against lithic enzymes. In summary, results suggest that the onset of rat BHP would be concomitant with local alterations of the overall polysaccharide metabolism leading to an imbalance in both polysaccharide prostate secretion and cell glycogen metabolism modulation.

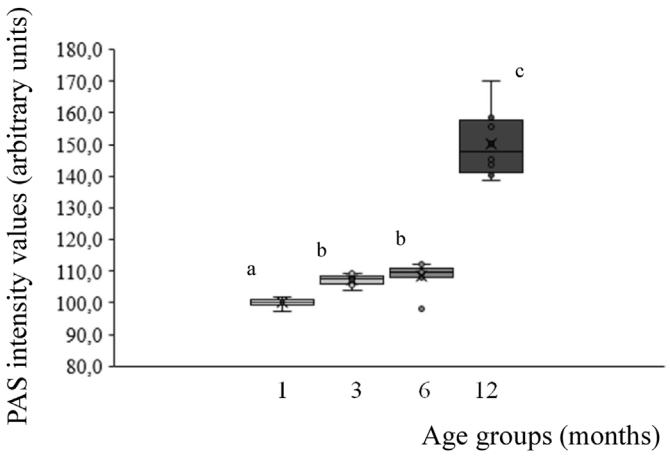

Fig. 4. Box and whisker plot representation of semiquantitative PAS intensity of prostate ductal content in rats of separate ages. The semiquantitative analysis of PAS staining intensity was conducted as explained in the Material and Methods section. White bar: 1 month-old rats. Light grey bar: 3 months-old rats. Medium grey bar: 6 months-old rats. Dark grey bar: 12 months-old rats. Results are obtained from 9 animals in each age group. Asterisks indicate the mean value for each group, whereas internal lines shown medians. Separate letters indicate significant ($P < 0.05$) differences among groups.

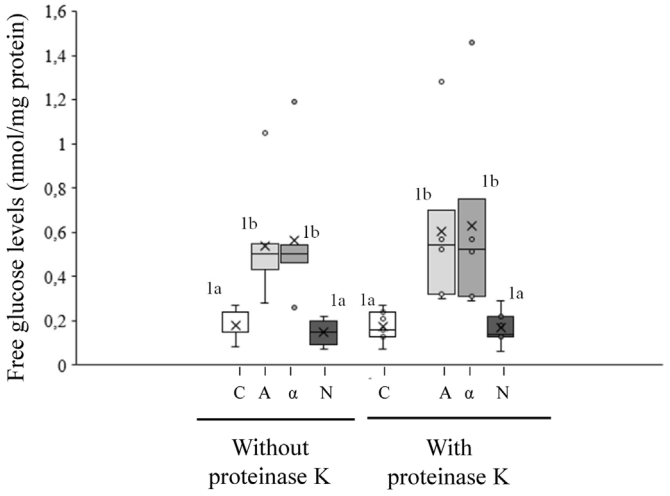

Fig. 5. Box and whisker plot representation of free glucose levels of prostatic extracts incubated with α-amylase, amyloglucosidase or *N*-glycosidase without or with a previous proteinase K treatment. Processing of samples has been described in the Materials and Methods . Samples were incubated for 2 h without any enzyme (Control group, white bars) or with α-amylase (light grey bars), amyloglucosidase (dark grey bars) or *N*-glycosidase (black bars). These incubations were performed in untreated tissues extracts (Without proteinase K group) or after a previous incubation for 1 h with proteinase K (With proteinase K group). Results are obtained from 7 samples in each group. Asterisks indicate the mean value for each group, whereas internal lines shown medians. Separate letters indicate significant ($P < 0.05$) differences when compared with the Control group within either the "Without proteinase K″ or the "With proteinase K″ ones. Separate numbers indicate significant ($P < 0.05$) differences when compared parallel groups (Control, α-amylase, amyloglucosidase and *N*-glycosidase) between the "Without proteinase K″ and the "With proteinase K″ ones.

Author contribution

L T-R: Formal analysis, performed all experimental analyses, O B-P: Formal analysis, performed all experimental analyses and B M-G: Formal analysis, performed all experimental analyses. JM F-N: Formal analysis, contributed with his advice for the correct performance of experimental analyses as well as in obtaining funding. T R: Formal analysis, contributed to the analysis of data and obtaining funding. M.M. R A: Writing – original draft, Formal analysis, contributed to the analysis and interpretation of data as well as in the final writing of the manuscript. J.E.R-G: performed the experimental design, interpretation of data and Writing – original draft, final writing of manuscript as well as contributed to obtain funding.

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