**Hyaluronan Content and Distribution in the Rat Ventral Prostate after Castration**

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Hyaluronan Content and Distribution in the Rat Ventral Prostate after Castration

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ABSTRACT

Hyaluronan (HA) has been implicated in tissue remodeling, healing, and tumor growth. This study investigated the variation in hyaluronan content, distribution, and metabolism in the rat ventral prostate in response to androgen deprivation after castration. Hyaluronan synthases (HAS 1-3) and hyaluronidases (Hyal 1-3) mRNA abundance and CD44 (a HA receptor) distribution were assessed by RT-PCR and immunohistochemistry, respectively. The results demonstrated an increased concentration, but an overall reduction of HA content. HA was located in both epithelium and stroma of the prostate of both non-castrated and castrated animals. qRT-PCR showed that Has1 and 2 are major synthases and that Hyal 1 was the predominant hydrolase expressed in the VP. qRT-PCR also showed that Has1 and Has2 mRNA increased transiently after castration, whereas Has3 mRNA declined markedly. While Hyal 1 mRNA increased slowly up to day 21 after castration, Hyal2 and Hyal3 mRNA dropped significantly. CD44 was found in the epithelial cells and in some stromal cells in both hormonal conditions. In conclusion, castration results in increased Has1 and Has2 mRNA abundance, but is associated with decreased HA absolute content, increased concentration and a predominance of short-chain HA molecules.

Key words: hyaluronan, prostate, castration, HAS, HYAL
INTRODUCTION

Hyaluronan (HA) is a major component of the extracellular matrix, and predominates whenever rapid cell proliferation, migration, and tissue repair occur (Laurent and Fraser 1992; Fraser et al. 1997). In addition, HA acts as a hydrated, space filling material and as a lubricant for tissue movement. HA also stabilizes the extracellular matrix in many tissues by binding to hyaluronan-binding proteins, such as CD44 (Knudson and Knudson 1993; Day and Prestwich 2002). CD44 is also relevant for endocytosis and intracellular degradation of HA, among other functions (Knudson et al. 2002).

Hyaluronan has been implicated in diverse aspects of prostate cancer progression and diagnosis (Lokeshwar et al. 2001). It was demonstrated that strong stromal HA expression is related to PSA recurrence, perineural infiltration, and seminal vesicle invasion (Aaltomaa et al. 2002). Hyaluronan synthesis is involved with cancer progression because HAS2 along with c-myc and 5 other genes have been located to a minimally overexpressed region (8q24) in prostate cancer (Tsuchiya et al. 2002). The demonstration by Simpson and co-workers (Simpson et al. 2002a) that inhibition of hyaluronan synthesis impairs vascularization and subcutaneous growth of implanted tumor cells reinforced the hypothesis that hyaluronan contributes to cancer progression.

In parallel to pleating and folding of the epithelial basement membrane (Carvalho and Line 1996), castration also promotes marked changes in the stroma of the rat ventral prostate (VP). We have characterized variations in elastin-associated microfibrils (Carvalho et al. 1997a), type VI collagen (Carvalho et al. 1997b), collagen fibers (Vilamaior et al. 2000), and heparan sulfate (Augusto et al. 2008). Changes in expression levels and distribution of matrix metalloproteinases and their endogenous inhibitors such as TIMPs and RECK are also involved in the remodelling of the gland (Felisbino et al. 2007; Peters et al. 2010; Justulin Jr et al. 2010). We have also discovered that epithelial shrinking includes several waves of apoptosis and a metalloproteinase-dependent peak at day 11 after castration (Bruni-Cardoso et al. 2010) and that a relevant fraction of the epithelial cells are deleted by desquamation (Rosa-Ribeiro et al. 2014).

Kofoed and co-workers (Kofoed et al. 1990) showed that uronic acid concentration increases in the rat prostate lobes after castration, and that this involves variations in different glycosaminoglycans, including HA. Terry and Clark (1996) have later demonstrated that the absolute amount of HA in the ventral prostate decreases 74% after
seven days of androgen deprivation, and concluded that glycosaminoglycans are regulated by androgens.

Given these fragmented pieces of information about hyaluronan in the rodent prostate and its involvement in human prostate cancer, we decided to investigate aspects of hyaluronan biology in the rat ventral prostate (VP) and its response to androgen deprivation promoted by castration.

The results presented here confirm previously reported changes in HA content, though using a different quantification protocol and, for the first time, localizes HA in the different histological compartments of the VP. We also quantified the variation in the expression of the three hyaluronan synthases and of three hyaluronidases mRNA levels, along with the determination of acidic hyaluronidase activity and localization of the HA receptor CD44.

MATERIALS AND METHODS

MATERIALS

All reagents were molecular biology grade, and were purchased from either Sigma Chemical Co. (Saint Louis MO, USA) or Merck (Darmstadt, Germany), unless otherwise noted.

ANIMALS

Three-month-old Wistar rats (n=76) were purchased from CEMIB-UNICAMP. The rats were subjected to orchiectomy through scrotal incision under chloral hydrate (0.3 mg/kg) anesthesia. Ventral prostates were removed 7, 14 and 21 days after castration, and sham operated, age-matched rats were employed as controls, thus forming 4 experimental groups. The ventral prostates from ten animals were used for the biochemical analyses. Those from four others were frozen and sectioned for morphology. Procedures were approved by the University's Committee for Ethics in Animal Experimentation (Protocol no. 1490-1).

HA EXTRACTION
For each group, the ventral prostates from ten rats were removed and placed in propanone overnight for delipidation. The specimens were dried and pulverized. HA was extracted using papain digestion. For 100 mg dried tissue, 2 mL digestion buffer (100 mM sodium acetate, 5 mM cysteine, 10 mM EDTA, pH 5.5), containing 1.5 mg papain/mL, was used. The digestion was carried out at 60°C for 24 h and the mixture was centrifuged (1000 x g, 20 min), and the supernatant was collected. Ice-cold ethyl alcohol was added to the supernatants (2 mL alcohol:1 mL sample) and the mixture kept overnight at -20°C. Samples were centrifuged (1000 x g) for 20 min, and the pellets re-suspended in water and heated to 60°C for 10 min. The samples were stored at 4°C.

**HA PROBE PREPARATION**

The procedure used for isolation of the HA-binding protein was described previously (Tengblad 1979). Briefly, bovine femoral cartilage was extracted with 4 M guanidine hydrochloride (GnHCl) containing protease inhibitors. The Gn-HCl extract was then dialyzed against 0.4M Gn-HCl to promote aggregate formation, and then subjected to ultracentrifugation in a cesium-chloride density gradient. The resulting A1 fraction was dialysed against 0.1M Tris, 0.03M sodium acetate, pH 4.4, and digested with trypsin (0.25 mg/mL) overnight at 37°C. The HA-binding proteins and HA were separated by chromatography on a Sepharose CL6B column (Amersham, Buckinghamshire, England) and then dialyzed against 4M GnHCl. The HA-binding protein was finally purified by Superose 6 column chromatography using an Åkta purifier system using phosphate buffered saline as eluent (Amersham).

The purified HA-binding protein (mostly aggrecan-G1 and link protein) was bioninylated using EZ-Link Sulfo-NHS-LC-Biotin (Pierce Biotechnology Inc., Rockford IL, USA). In brief, one milligram of protein was mixed with 500 µL of 50 mM sodium bicarbonate buffer, pH 8.3 and 40 µL of biotin (1 mg/mL) for 2 h in ice. Unbound biotin was separated after centrifugation with the micro-concentrator ultra-free-MC (Millipore, Billerica MA, USA).

Alternatively, the HA probe was labeled with fluorescein isothiocyanate (FITC). The probe was dialyzed against carbonate-bicarbonate buffer, pH 9.2 and then mixed with 375 µM FITC for 4 h at room temperature. Unbound FITC was removed through
chromatography on Superose 6 with PBS (5 mM phosphate, 0.15 M NaCl, pH 7.4). The eluate was monitored by the absorbance at 495 nm, and the fractions containing the FITC-conjugated proteins were concentrated and stored at 4°C in the dark.

MEASUREMENT OF HA CONTENT

The concentration of HA in ventral-prostate extracts was determined in a competitive binding assay (Lokeshwar and Rubinowicz 1999). Briefly, 96-well microtiter plates (Corning 1550) were coated with HA (Sigma Chemical Co.) at 25 µg/mL in 200 mM carbonate buffer (pH 9.6) for 4 h at 37°C. Excess HA was removed with four washes of PBS/0.05% Tween 20. Serial dilutions of HA extract were combined with bioninylated hyaluronic acid-binding protein and incubated in the HA-precoated wells at room temperature overnight. The plate was washed 4X with PBS/Tween 20, developed using avidin-HRP with OPD (o-phenylenediamine) as a substrate, and read at 490 nm. HA concentration was interpolated from a standard curve generated by plotting HA concentration against absorbance values. The results show the mean of four different experiments carried out in triplicates for each group.

DETERMINATION OF HA CHAIN SIZE VARIATION

HA extracts were subjected to gel chromatography on Sepharose CL6B (Amersham) column (120 x 0.6 cm) in 0.05 M sodium acetate buffer, pH 5.8. One-mL fractions were collected and assayed for the presence of HA (see HA content measurement above). HA chains were classified into three size groups. The void and total volume of the column were 13 mL and 36 mL, as determined by Blue Dextran 2000 and copper sulfate, respectively.

HA IN SITU DETECTION

HA was localized in the prostate-tissue sections using the FITC-labeled probe applied to 7-µm frozen sections obtained from 3 animals per experimental group. Autofluorescence was quenched with 3% H2O2 in water for 15 min, and non-specific binding sites were blocked with 3% bovine serum albumen (BSA) in TBS-T (0.05 M Tris, 0.15 M NaCl, pH 7.4 plus 0.1% Triton X-100). The sections were incubated with probe diluted in 1% BSA for 2 h at room temperature. The slides were washed with TBS-T, mounted using Vectashield (Vector Laboratories, Burlingame CA, USA), and photographed in a Zeiss Axioskop fluorescence microscope. The specificity of the staining was controlled by digesting some sections with
Streptomyces hyaluronidase prior to incubation with the probe. In this latter case, nuclei were stained with DAPI. Additional sections were reserved for immunohistochemistry.

RNA EXTRACTION AND REVERSE TRANSCRIPTION

Total RNA extraction was performed with Illustra RNAspin Mini reagent (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK), according to the manufacturer’s instructions, using 30 mg of ventral prostate. Three animals per experimental group were used in these experiments. The RNA integrity was analyzed by electrophoresis in denaturing formaldehyde 1.2% agarose gel with 10 µg of RNA, according to Sambrook and co-workers (Sambrook and W Russell 2001). Total RNA was quantified by spectrophotometry using an Ultraspec 2100 Pro spectrophotometer (Amersham Biosciences, São Paulo SP, Brazil). Five µg of the total RNA sample was reverse-transcribed with 200 U SuperScript III (Invitrogen Life Technologies) and Oligo (dT)\textsubscript{12-18} primer (Invitrogen Life Technologies) according to the supplier’s instructions. The cDNA was also quantified by spectrophotometry.

REAL-TIME POLYMERASE CHAIN REACTION

Two-step singleplex reactions were performed in a 7300 Real Time PCR System (Applied Biosystems, Foster City CA, USA) for relative quantification by the \( \Delta \Delta C_t \) method using phosphoglycerate kinase 1 (PGK) as the endogenous control. The reactions were assayed in triplicate with 20 ng cDNA, using TaqMan 2X PCr Master Mix (Applied Biosystems, Branchburg NJ, USA), and 20x TaqMan Gene Expression Assays (Rn00597231_m1 for \textit{Has1}, Rn00565774_m for \textit{Has2}, Rn00597204_m1 for \textit{Has3}, Rn02133715_s1 for \textit{Hyal1}, Rn00597038_m1 for \textit{Hyal2}, Rn01445427_g1 for \textit{Hyal3}, and Rn00821429_g1 for PGK1; Applied Biosystems, Foster City CA, USA) in a final reaction volume of 20 µL. To confirm the accuracy and reproducibility of real-time PCR, intra-assay precision was calculated according to the equation: \( E^{\left(-1/\text{slope}\right)} \), by which the PCR efficiency was calculated (Pfaffi, 2001; Meijerink et al., 2001). The endogenous control, PGK1, was chosen among ten genes tested. The control group was used as a calibrator in the first global analysis (all genes and all groups), and \textit{Has1} and \textit{Hyal1} were used as the calibrators for the other analysis (all genes in the control group). A 2-fold change in gene expression was considered significant, as suggested by Hu and co-workers (Hu et al. 2006).
CD44 IMMUNOHISTOCHEMISTRY

Frozen sections obtained as above were used. CD44 was localized using a rabbit polyclonal anti-CD44 (Santa Cruz Biotech, Santa Cruz CA, USA). Endogenous peroxidase was blocked by incubating the sections with 3% H₂O₂ (in water) for 20 min. Non-specific protein binding was blocked with 3% BSA in TBS-T. The sections were incubated with the antibody diluted in 1% BSA overnight at 4°C. The slides were washed with TBS-T and incubated with HRP-labeled anti-rabbit Ig antibody for 2 h at room temperature. The slides were washed with TBS-T and developed with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.03% hydrogen peroxide in 0.05M phosphate buffer, pH 7.4 at room temperature. The slides were counterstained with methyl green for 15 min, washed, air dried, and mounted in Entellan.
RESULTS

EFFECTS OF CASTRATION ON PROSTATE WEIGHT AND HA LEVELS

Castration resulted in marked reduction of the rat VP weight. After 7, 14, and 21 days, prostate weight was reduced to 20%, 11%, and 9% of the VP in sham-operated non-castrated control (NC on the figures) (Fig. 1A). The HA content in the rat VP was determined by a competitive binding assay using a biotinylated HA probe. Figure 1B shows the concentration (mg/g of dry tissue) of HA in prostates of sham-operated and castrated rats. Castration resulted in higher concentrations of HA in the prostate, with a peak at 14 days. There was a percent variation of 90%, 275%, and 85%, for 7, 14, and 21 days after castration. In contrast, the absolute amount of HA per VP was reduced after castration. The mean value of 20 µg HA per prostate was reduced to about 9 µg HA per prostate at days 7 and 14 after surgery (40% of the non-castrated control), and to only 5 µg per prostate at day 21 days (23.5% of the non-castrated control) (Fig. 1C).

VARIATION IN HA CHAIN LENGTH

HA chain size in the ventral prostate and the changes caused by castration was determined by gel exclusion chromatography. There was a wide range of sizes, as seen in the representative chromatograph shown in Figure 2A. According to the chromatographs, we defined three size ranges (short, medium, and long chains), and determined the relative amount of HA for each of these classes. Medium-sized chains predominated in the non-castrated group. Castration resulted in a transient increase of high-molecular-mass HA (long chains) 7 days after castration, and a later progressive accumulation of low-molecular mass (short chains) up to 21 days (Fig. 2B). Compatible with the variation in absolute content shown in Fig. 1C, we noted a decrease in all size classes, especially the medium-sized molecules (Fig. 2C).

HA LOCALIZATION

HA was localized using a FITC-labeled HA probe. HA showed a uniform distribution in the stroma, but was excluded from blood vessels and lymphatics. HA was concentrated at the base of the epithelial structures (acini and ducts) and around the smooth muscle cells. In the prostate of non-castrated rats (Fig. 3A). HA-labelling was found in the intercellular spaces of the epithelium and is constrained at the point of tight junctions, close to the apical surface of the columnar epithelial cells (Fig. 3B). Castration resulted in a more restricted distribution...
of HA (Figs. 3C-E). As the epithelium regressed, epithelial labeling was also reduced and less distinct. Some intracellular particulate staining was seen and may reflect compartmentalization within membrane-bound vesicles. In the VP of castrated animals, HA distribution was concentrated around the smooth muscle cells. Tissue sections treated with bacterial hyaluronidase before labelling abolished staining (Fig. 3F).

**HAS 1-3 AND HYAL 1-3 mRNA EXPRESSION**

qRT-PCR showed that Has1 and Has2 mRNA predominated over Has3 in the control VP. Has1 and Has2 showed equivalent expression levels (1:0.8), whereas the HAS 3 mRNA content was less than 1% (Fig. 4A). In contrast, Hyal1 mRNA predominated over Hyal2 and Hyal3 (1:0.2:0.16) in the VP of non-castrated controls (Fig. 4E). The same procedure allowed for the determination of variations in hyaluronan synthases and hyaluronidases after castration. Has1 and Has2 mRNA (Figs. 4B and C) increased after castration with a transient peak at day 14; whereas the Has3 mRNA declined conspicuously in response to androgen deprivation (Fig. 4D). Hyal1 mRNA increased by day 21 (Fig. 4E), whereas the Hyal2 and Hyal3 mRNA decreased markedly after castration (Figs. 4G and G).

**CD44 LOCALIZATION**

Polyclonal anti-CD44 was applied to frozen sections, and the signal was revealed by DAB reaction (Fig. 5). Immunohistochemistry of CD44 showed that epithelial cells were predominantly stained in the prostate of both non-castrated (Fig. 5A) and castrated rats (Figs. 5B-D). The apical surface of the epithelial cells was labeled in the prostate of non-castrated animals. Perivascular cells also showed reactivity to CD44. The reaction observed in the epithelium was apparently stronger and more uniform after castration. In the stroma, endothelial cells and mast cells were positive for CD44. Smooth muscle cells showed a weak surface reaction. The negative controls exhibited no reaction (Fig. 5E).
DISCUSSION

It has been reported that the HA concentration in the rat ventral prostate increases after castration (Kofoed et al. 1990; Terry and Clark 1996). However, because castration results in a marked reduction of prostate weight, this higher concentration does not necessarily imply increased synthesis or higher amounts of HA in the prostate. Indeed, Kofoed and co-workers (1990) reported a 74% reduction in the total amount of HA per prostate seven days after castration. Our results confirmed those published by Kofoed and co-workers (Kofoed et al. 1990), except that we noticed a 60% reduction in the total amount of HA. The discrepancy in the final figures may be due to either the use of different rat strains or the different quantification protocols employed in each case. We have also examined the variation in relative chain length by gel exclusion chromatography, and observed a transient increase in the proportion of long-chain molecules at day 7 after castration, and then a progressive increase in the short-chain molecules up to day 21 after castration. The most striking observation was the large decrease in medium-sized molecules.

We show here that HA is found in both epithelium and stroma of the VP. The presence of HA in the epithelium could be anticipated, since HA is an important product of other epithelial cells, i.e., keratinocytes. Indeed, it has been calculated that HA concentration in the intercellular space in the epidermis is extremely high as compared to other tissues (Agren et al. 1995). The presence of HA in the space above the basement membrane and below the tight junctions close to the apical surface suggests that the epithelial cells are able to synthesize HA, because stromal HA is not expected to traverse the basal lamina. It is important to notice the presence of HA in the normal epithelium of the VP, because some prostatic cancer cell lineages, including the PC3, produce a HA coat in culture (Simpson et al. 2002b), whereas others, such as LNCaP, do not. It is tempting to suggest a correlation between the epithelial disorganization observed in high-grade tumors and the increased amount of HA in the plasma. It is possible that, similarly to PSA, the plasma HA observed in cancer patients is a result of the production of HA, which is not retained by the prostate epithelial cells.

On the other hand, it seems that the distribution of HA in the stroma is not changed, although it shows a different aspect (more granular or interrupted pattern). This pattern seems to result from the rearrangement of smooth-muscle cells (which adopt a spinous outline) and collagen fibers (which become convoluted) after castration (Vilamaior et al.
2000), resulting in smaller spaces for the confinement of HA. A more-granular aspect of HA in the epithelium in castrated animals may represent endosomes at different stages. The use of specific markers for this organelle in double-labeling experiments may clarify this matter. The qRT-PCR experiments revealed that Has1 and Has2 mRNA predominate over Has3 in the ventral prostate of non-castrated control rats. Likewise, Hyal1 mRNA was at least 5-fold more abundant than Hyal2 or Hyal3. Also, the expression of Has1 and Has2 was almost equivalent (1:0.8), supporting the notion that they are components of the same enzyme complex, and that these enzymes contribute to the synthesis of HA in the VP of castrated rats, because mRNA increased after castration with a similar profile. The increased amount of hyaluronan synthase mRNA conciliates with the increased amount of hyaluronidase mRNA, to result in a smaller amount of total HA.

Real-time PCR also showed a progressively higher expression of Hyal1 after castration. The increased expression of the Hyal1 mRNA correlates with the overall decrease in HA content in the entire gland. It is also interesting to mention that Hyal1 is the plasma hyaluronidase, and its expression in the prostate may indicate a greater contribution of this organ to the increased amount of this enzyme in the plasma of cancer patients. This might indicate that HA degradation takes place predominantly in the extracellular space, but this result needs to conciliate with the presence of intracellular HA-labeling, apparently in endosomes. Again, it seems that the marked reduction in epithelial volume predominates over the increase of endosomal labeling in the epithelium, but more specific measurements are needed to confirm that extracellular degradation will surpass endosome/lysosome digestion.

CD44 immunoreactivity was dispersed in the epithelial cells of the prostate in non-castrated rats. The staining pattern changed after castration, with more-intense staining concentrated in the apical surface of the epithelium. It seems that this change in epithelial distribution of CD44 is correlated with the population of epithelial cells that survive androgen deprivation. It is well known that basal epithelial cells express CD44 (Liu et al. 1997). This suggests the predominance of basal (or at least less-differentiated epithelial cells) in the prostate of castrated animals. However, the meaning of CD44 expression on the luminal surface is unknown.
While there is no evidence for a direct regulation of HA by androgens, the present results, in particular the reduced content of HA, might be understood as an indirect effect of the overall remodelling occurring in the gland in response to androgen deprivation.

Finally, it is concluded that the total amount and length of HA chains in the rat VP are reduced after castration, and this is a result of a sustained Has1 and Has2 mRNA and an increased Hyal1 abundance. It was also shown that both epithelium and stroma contribute to the total content of HA in the prostate, but the synthesis of HA in castrated animals seems to have a major contribution from stromal cell.

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REFERENCES


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Fig. 1. Variation in the rat ventral prostate weight and HA content after castration. Fig. 1A depicts the ventral prostate weight reduction as attained by castration. After 7, 14, and 21 days, prostate weight corresponded to 20%, 11%, and 9% of the control value, respectively. Fig. 1B shows the concentration of HA in the prostate in the different experimental situations. There was an increase in the concentration of HA in the prostate of castrated rats, with a peak at 14 days after castration. The absolute amount of HA per prostate (Fig. 1C), however, decreased after castration, from the mean of 20 µg HA per prostate in the non-castrated rats, to about 9 µg at days 7 and 14 after castration, and 5 µg at day 21.

Fig. 2. Changes in HA chain size. The hydrodynamic size of the HA chains in the prostate of non-castrated and castrated rats was determined by gel exclusion chromatography in a Sepharose CL6B column. Figure 2A shows a representative chromatogram obtained for the HA extracted from the prostate of a castrated animal, 7 days after castration. The size distribution was wide. The fractions were divided into three groups, with $K_{av} = 0.1 - 0.3$ (long); $0.35 - 0.7$ (medium), and $0.75 - 1.0$ (short). HA content in each of these size groups was determined by integration of the peaks. Figure 2B reveals the percentage of HA in each size group for the different experimental points. An increase in the proportion of long-chain molecules 7 days after castration and a progressive increase in short-chain molecules were noted. Figure 2C shows the absolute amount of HA in each situation, so that the overall reduction in the amount of HA is easily seen. In this graph, one can see that the most marked change was a reduction in medium-sized chains, followed by a distinct increase in short chains.

Fig. 3. HA localization in the rat ventral prostate. HA was localized using an FITC-labeled probe. HA was distributed in both epithelial and stromal compartments of the prostate of non-castrated (Fig. 3A,B) and castrated animals (Fig. 3C-E). In the prostate of non-castrated rats, HA labeling was intense at the base of the epithelium,
and among the smooth muscle cells. In the epithelial compartment, HA was found between the epithelial cells up to the point of tight junctions. In the stroma, cells and fibers excluded HA and appeared unstained. Castration resulted in collapse of the pattern observed in the control. HA was still found in the epithelium and stroma, but showed a more particulate staining, which is thought to be due to the distribution of HA around the newly arranged cells and fibers. Some intracellular granules indicate compartmentalization within membrane-bound vesicles. The pattern observed for the castrated rats was maintained for the 7- (Fig. 3C), 14- (Fig. 3D), and 21-day (Fig. 3E) time points. Figure 3F is a control section treated with bacterial hyaluronidase before incubation with the HA probe and then stained with DAPI to reveal the cell nuclei. ep = epithelium; st = stroma. Scale bars = 25 µm (A, C-E) and 10 µm (B, F).

**Fig. 4.** Real-time PCR was employed for the determination of changes in hyaluronan synthases and hyaluronidases mRNA in response to androgen deprivation. Relative abundance of hyaluronan synthases (A) and hyaluronidases (E) mRNAs in the rat ventral prostate. *Has1* and *Has2* mRNA showed similar abundance (1:0.8), and predominated over *Has2* mRNA, which corresponded to less than 1%. *Hyal1* mRNA was at least 5-fold higher than *Hyal2* and *Hyal3*. Fold-change variation in *Has1-3* (B-D) and *Hyal1-3* (E-H) mRNA in the VP after castration. *Has1* and *Has2* (B and C) showed increased abundance after castration, with a peak at 14 days after castration. *Has3* mRNA content showed a marked decline after castration (D). *Hyal1* mRNA (F) content increased slowly after castration, whereas *Hyal2* and *Hyal3* mRNA dropped significantly (G and H).

**Fig. 5.** Localization of the hyaluronan receptor CD44 in the rat VP from non-castrated and castrated rats by immunohistochemistry. (A) Sham-operated, non-castrated control. (B-D) Castrated animals at 7, 14, and 21 days after surgery, respectively. CD44 staining is observed at the apical surfaces of the columnar epithelial cells and in endothelial cell in the stroma. Castration promotes progressive increase in labeling for CD44 in the epithelial cells, which become shorter after castration. On the other hand, stromal labeling decreases, as the diameter of blood vessels and endothelial cells become smaller. (E) Negative control. ep = epithelium; v = blood vessel; st = stroma. Scale bars = 25 µm.
Fig. 1. Variation in the rat ventral prostate weight and HA after castration. Fig. 1A depicts the ventral prostate weight reduction as attained by castration. After 7, 14, and 21 days, prostate weight corresponded to 20%, 11%, and 9% of the control value, respectively. Fig. 1B shows the concentration of HA in the prostate in the different experimental situations. There was an increase in the concentration of HA in the prostate of castrated rats, with a peak at 14 days after castration. The absolute amount of HA per prostate (Fig. 1C), however, decreased after castration, from the mean of 20 μg HA per prostate in the non-castrated rats, to about 9 μg at days 7 and 14 after castration, and 5 μg at day 21.
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Fig. 3. HA localization in the rat ventral prostate. HA was localized using an FITC-labeled probe. HA was distributed in both epithelial and stromal compartments of the prostate of non-castrated (Fig. 3A,B) and castrated animals (Fig. 3C-E). In the prostate of non-castrated rats, HA labeling was intense at the base of the epithelium, and among the smooth muscle cells. In the epithelial compartment, HA was found between the epithelial cells up to the point of tight junctions. In the stroma, cells and fibers excluded HA and appeared unstained. Castration resulted in collapse of the pattern observed in the control. HA was still found in the epithelium and stroma, but showed a more particulate staining, which is thought to be due to the distribution of HA around the newly arranged cells and fibers. Some intracellular granules indicate compartmentalization within membrane-bound vesicles. The pattern observed for the castrated rats was maintained for the 7- (Fig. 3C), 14- (Fig. 3D), and 21-day (Fig. 3E) time points. Figure 3F is a control section treated with bacterial hyaluronidase before incubation with the HA probe and then stained with DAPI to reveal the cell nuclei. ep = epithelium; st = stroma. Scale bars = 25 μm (A, C-E) and 10 μm (B, F).
Fig. 4. Real-time PCR was employed for the determination of changes in hyaluronan synthases and hyaluronidases mRNA in response to androgen deprivation. Relative abundance of hyaluronan synthases (A) and hyaluronidases (E) mRNAs in the rat ventral prostate. Has1 and Has2 mRNA showed similar expression levels (1:0.8), and predominated over Has2 mRNA, which corresponded to less than 1%. Hyal1 mRNA was at least 5-fold higher than Hyal2 and Hyal3. Fold-change variation in Has1-3 (B-D) and Hyal1-3 (E-H) mRNA in the VP after castration. Has1 and Has2 (B and C) showed increased expression after castration, with a peak at 14 days after castration. Has3 mRNA content showed a marked decline after castration (D). Hyal1 mRNA (F) content increased slowly after castration, whereas Hyal2 and Hyal3 mRNA dropped significantly (G and H).
Fig. 5. Localization of the hyaluronan receptor CD44 in the rat VP from non-castrated and castrated rats by immunohistochemistry. (A) Sham-operated, non-castrated control. (B-D) Castrated animals at 7, 14 and 21 days after surgery, respectively. CD44 staining is observed at the apical surfaces of the columnar epithelial cells and in endothelial cell in the stroma. Castration promotes progressive increase in labeling for CD44 in the epithelial cells, which become shorter after castration. On the other hand, stromal labeling decreases, as the diameter of blood vessels and endothelial cells become smaller. (E) Negative control. ep= epithelium; v= blood vessel; st= stroma. Scale bars = 25 μm.