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Castration Induces Changes in the Cation-Dependent Mannose-6-Phosphate Receptor in Rat Epididymis: Possible Implications in Secretion of Lysosomal Enzymes

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ABSTRACT

It is believed that the mammalian epididymis participates in the maturation of the sperm due to its secretory activity. High concentrations of several secreted acid hydrolases are found in the epididymal lumen. Moreover, some of these enzymes are secreted by the epididymal epithelium in an androgen-dependent fashion. In this study, we attempted to discern whether mannose-6-phosphate receptors (MPRs) regulate transport and secretion of lysosomal enzymes in the rat epididymis, and if these events are altered when the animals are subjected to hormonal manipulation. We observed that expression of cation-dependent MPR (CD-MPR) and cation-independent MPR (CI-MPR) increased significantly in caudal epididymis of castrated rats by immunoblot. This increase was corroborated by quantitation of MPRs, by binding assays. This change could be due to androgen deprivation, as a similar effect was observed after treatment with the anti-androgenic drug flutamide. Furthermore, we observed that the CD-MPR was redistributed to the apical area of the epithelium on castrated rats by immunohistochemistry, which is compatible with the redistribution of the receptors toward lighter fractions in a Percoll gradient. Consistent with a possible involvement of the CD-MPR in the secretion, we observed an increase in pro-cathepsin D levels in epididymal fluid after castration. We conclude that the CD-MPR might be regulated by hormones and that this receptor might be involved in the secretion of specific enzymes into the rat epididymis. J. Cell. Biochem. 110: 1101–1110, 2010.

KEY WORDS: MANNOSE-6-PHOSPHATE RECEPTORS; EPIDIDYMIS; LYSOSOMAL ENZYMES

The mammalian epididymis is particularly rich in acid hydrolases in concordance with a developed and active lysosomal apparatus. Some of these enzymes are highly secreted by the epithelium into the epididymal lumen, and it is thought that they participate in remodeling the sperm surface, one step in the maturation of the gamete. However, the role of these enzymes in the lumen and the mechanism by which the acid hydrolases are secreted are still poorly understood.

In most mammalian cell types, normal localization of acid hydrolases within lysosomes is specifically regulated by transmembrane type I glycoproteins, the mannose-6-phosphate receptors (MPRs), which recognize and interact with ligands bearing mannose-6-phosphate (M6P-L) for their sorting and transport to lysosomes [Hille-Rehfeld, 1995; Ghosh et al., 2003; Dahms et al., 2008]. At present, two types of MPRs have been described, the cation-dependent MPR (CD-MPR) and the cation-independent MPR (CI-MPR), which co-exist in most cell types, even though the relevance of such co-existence remains unclear [Hille-Rehfeld, 1995]. Some evidence suggests that each MPR interacts with a different set of ligands, although a redundant role for the MPRs has not been ruled out. The CI-MPR may also have additional functions, since this receptor is capable of interacting with multiple non-lysosomal ligands (e.g., IGF-II and retinoic acid) [Dahms and Hancock, 2002; Olson et al., 2002]. The two MPRs are also expressed in organs of the mammalian reproductive tract; the epididymis [Brown and Farquhar, 1984] and the testis [O'Brien et al., 1989],

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whose integrity and functionality are altered by hormonal changes [Robaire and Viger, 1995; Robaire et al., 2006, 2007]. The epididymis is critically dependent on hormones synthesized in the testis, and testosterone and its metabolites, dihydrotestosterone (DHT) and estradiol (E2), are currently accepted as the primary regulators of epididymal structure and function [Robaire et al., 2007].

Among the acid hydrolases secreted by the epididymal epithelium, several respond to hormonal changes [Mayorga and Bertini, 1982; Gupta and Setty, 1995; Abou-Haila et al., 1996; Belmonte et al., 2002]. Furthermore, some secreted enzymes bearing M6P bind to MPRs on the sperm surface during transit along the epididymal duct [Barbieri et al., 1995; Belmonte et al., 1998, 2000]. As a result, we wondered whether MPRs might somehow participate in the secretion of specific acid hydrolases and whether the expression and functionality of these receptors would be affected by hormonal changes. To assess this, we measured the expression of both the CD- and the CI-MPR in cauda epididymis of rats subjected to hormonal manipulation; either by castration or treatment with the anti-androgenic drug flutamide. Under these experimental conditions the expression of the CD-MPR and, to a lesser extent the CI-MPR, increased in the tissue of cauda epididymis with respect to controls. Moreover, changes in the distribution of the CD-MPR in cauda epididymis were also observed. To evaluate a possible relationship between the MPRs and secretion of acid hydrolases in epididymis, we have measured the activity of two glycosidases and examined cathepsin D secretion into the epididymal fluid.

MATERIALS AND METHODS

REAGENTS

The rabbit anti-rat CI-MPR polyclonal antibody was a gift from Dr. Paul Luzio (Department of Clinical Biochemistry, Cambridge University, UK). The polyclonal anti-CD-MPR antibody (raised in rabbit) was generously provided by Dr. A. Hille-Rehfeld (Stuttgart, Germany). The goat anti-cathepsin D (cat. sc-6486) antibody was purchased from Santa Cruz Biotechnology. The rabbit anti-actin antibody (cat. A 2668), rabbit anti-K/Na ATPase (cat. A 3979), biotin-conjugated anti-rabbit IgG, and horseradish peroxidase-conjugated avidin were purchased from Sigma Chemical Co. (St. Louis, MO). The 4-methylumbelliferyl substrates for α-mannosidase (α-MAN) and *N*-acetyl-β-D-glucosaminidase (β-NAG), flutamide (cat. F9397), M6P (monosodium salt, cat. M3655), and Percoll (cat. 77237, Percoll R) were also purchased from Sigma. Chemiluminescent reagents were from Pierce Biotechnology, Inc. (Rockford, IL).

CASTRATION

Adult male Sprague–Dawley rats (75–90 days old and weighting 240–270 g), maintained under standard conditions (food and water ad libitum at 20–22°C and light cycle of 12L:12D) were castrated, following the method of Mayorga and Bertini [1982]. Briefly, the animals were anesthetized by injection of ketamine hydrochloride (70 mg/kg) mixed with xylazine (5 mg/kg), according to the protocol approved by the Committee for Animal Care of the Universidad Nacional de Cuyo (Mendoza, Argentina). The testes and epididymides were exposed by abdominal incision, and the testicular vascular supply was ligated without compromising the epididymal blood supply, neither epididymal innervations. The testes were then removed, whereas the epididymides were placed back into the scrotum. After surgery, rats were housed individually and allowed to recover undisturbed for 48 h. During this post-castration period we made sure that levels of intraluminal testosterone decreased. The rats were subsequently sacrificed by ether inhalation and decapitation, in accordance within guidelines, and the epididymides were removed and processed as detailed below. The controls were handled similarly, but without surgery.

TREATMENT WITH FLUTAMIDE

The anti-androgenic drug flutamide was dissolved in sunflower oil and administered intraperitoneally at doses of 50 mg/kg day for 2 weeks. The controls were injected with oil alone at similar conditions. After this time the animals were sacrificed and the epididymides removed and processed as detailed hereinafter.

PREPARATION OF BIOLOGICAL MATERIAL FOR IMMUNOBLOTTING

Epididymides from either castrated or control rats were removed and processed according to Belmonte et al. [2002]. In this study, we have used cauda epididymis to obtain a tissue free of sperm, as it is feasible to perform retroperfusions through the vas deferens. Briefly, the epididymal fluid and spermatozoa were obtained by retrograde perfusion of the cauda with a syringe containing phosphate-buffered saline (PBS, NaCl 0.15 M, NaH_2PO_4 0.02 M, NaOH 0.017 M, pH 7.2). The spermatozoa were sedimented at 3,500*g* for 5 min and the supernatants, containing the epididymal fluid, were stored at −20°C for at least 1 month before analysis.

After perfusion, the remaining of epididymal tissue was weighed and homogenized in 1:5 (w/v) of 10 mM Tris–acetate buffer, pH 7.2, containing 0.25 M sucrose, 1% EDTA, 1 mM PMSF, 0.02% sodium azide, and 5 mM glycerophosphate (buffer H), with a Teflon/glass homogenizer and centrifuged at 800*g* for 20 min at 4°C. From the post-nuclear supernatant, a membrane-enriched fraction was obtained by pelleting at 70,000*g* for 30 min, at 4°C. The pellets (source of MPRs) were resuspended in buffer H, and the supernatants were then stored at −20°C until use.

PREPARATION OF BIOLOGICAL MATERIAL FOR BINDING ASSAYS

After perfusion, the remaining epididymal tissue was weighed and homogenized in (1:5, w/v) buffer H, with a Teflon/glass homogenizer and centrifuged at 800*g* for 20 min at 4°C as detailed above. From the post-nuclear supernatant, a membrane-enriched fraction was obtained by pelleting at 80,000*g* for 30 min at 4°C. Thereafter, the pellets were incubated with (1:5, w/v) 0.05 M Tris–HCl buffer (pH 7.2) containing 0.5% saponin, 50 mM EDTA, 1 mM PMSF, 0.02% sodium azide, and 5 mM glycerophosphate (buffer S) for 10 min on ice, and centrifuged at 80,000*g* for 30 min at 4°C. The pellets were then resuspended in buffer S (1:5, w/v) containing 0.6 M KCl, sonicated for 5 s and incubated for 10 min on ice. Finally, the samples were centrifuged at 80,000*g* for 30 min at 4°C and the resulting pellets (source of MPRs) were resuspended in buffer B (NaH_2PO_4–Na_2HPO_4 10 mM, pH 7.2). The membranes were stored at −20°C for up to 30 days before their use in binding assays.

BINDING ASSAYS

A typical binding assay was performed with membranes of cauda epididymis and β-glucuronidase purified from rat preputial gland [Tulsiani et al., 1975], known to contain M6P residues. Membrane proteins (30 μg) were incubated with 100–1,200 U β-glucuronidase, either in the presence or absence of 0.5 mM $CaCl_2$ and 0.5 mM $MnCl_2$, and in the presence or absence of 20 mM M6P, in a final volume of 0.25 ml buffer B. After 1 h incubation at 4°C, 0.75 ml of buffer B was added and the tubes were centrifuged at 13,000*g* for 30 min at 4°C. The supernatants were discarded and the pellets washed with 1 ml of binding buffer. Thereafter the pellets were resuspended in 25 μl buffer B, and assayed for β-glucuronidase activity bound to membranes. The total binding to MPRs was determined in the presence of bivalent cations and M6P. The binding to CD-MPR was calculated as the difference between the total binding and the binding in the absence of cations. For quantitation of MPRs, binding values were taken from the saturated part of each curve.

CELL FRACTIONATION IN PERCOLL GRADIENTS

In order to determine whether CD-MPR is redistributed along epidydimal cells due to castration, we studied compartmentalization of CD-MPRs by cell fractionation in Percoll gradients. This method has been widely used to corroborate any redistribution of molecules in cells or tissues. After perfusion, the epididymides were homogenized in 10 mM Tris–acetate buffer, pH 7.2, containing 1% EDTA, 1 mM PMSF, 0.02% sodium azide, and 5 mM glycerophosphate (buffer P) and centrifuged at 800*g* as detailed above. 2.5 mg of proteins from the resulting post-nuclear supernatant was adjusted to 1.15 ml with buffer P and were mixed with 4.6 ml of Percoll (15%), following the procedure of Stöckli and Rohrer [2004]. The resulting mixture (12% Percoll) was loaded on top of 0.25 ml of 2.5 M sucrose, and the tubes were centrifuged at 28,000*g* for 45 min at 4°C in a L7-80 (Beckman) Ultracentrifuge (Ti 70.1 rotor). Fractions (0.5 ml) were collected from the bottom of the gradient and each was adjusted to 6 ml with PBS. After centrifugation at 80,000*g* for 30 min at 4°C, the pellets were resuspended in PBS and processed for immunoblotting.

IMMUNOBLOTTING

All the procedures were carried out according to Romano et al. [2005]. Briefly, 45 μg of proteins from epididymal membranes (controls or castrated rats) were dissolved with Laemmli loading buffer [1970]. The proteins were run on SDS–PAGE gels (6–8% acrylamide) at 25 mA. The proteins were electrotransferred to nitrocellulose membranes (pore size 0.2 μm, Pierce Biotechnology, Inc.). CD-MPR, CI-MPR, or cathepsin D was detected using the corresponding specific antibodies, following exactly the protocol of Romano et al. [2005]. Actin or Na/K-ATPase was used as controls in some experiments, with the corresponding specific antibodies (1:1,000 diluted in PBS). The signals were detected using chemiluminescence and Kodak X-Omat film. The bands were quantified from the Kodak X-Omat films by densitometric scanning and analysis with NIH Image 1.60 and analyzed with the Scion Image Program.

IMMUNOHISTOCHEMISTRY

All the procedures were carried out according to Hermo et al. [2007]. The epididymides of either control or castrated rats were fixed with Bouin fixative for 72 h and then dehydrated and embedded in paraffin. Thin sections (5 μm) were cut and mounted on glass slides. Anti-CD-MPR was diluted 1:300 in dilution buffer from DakoCytomation (S0809; Mississauga, Canada). Immunolocalization of CD-MPR was performed using the Envision+ peroxidase diaminobenzidine (DAB) kit (K4010; DakoCytomation). Control images were obtained using normal rabbit serum at a dilution similar to that of the primary antibody. All digital images were taken with an Infinity USB 2.0 Hi-Speed camera (Lumenera Scientific, Ottawa, Canada).

HORMONE MEASUREMENTS

Estradiol, testosterone, and DHT were measured in sera by RIA, using commercial kits for the total hormones (DSL-4800, DSL-4000, and DSL-9600 double-antibody RIAs, respectively; all from Diagnostic Systems Laboratories, Webster, TX).

OTHER PROCEDURES

The activity of β-NAG and α-MAN was measured fluorometrically, using the corresponding 4-methyl-umbellyferyl substrates as described by Barrett and Heath [1977]. One unit of enzymatic activity corresponded to 1 nmol of substrate digested per hour incubation. Proteins were measured according to Lowry et al. [1951].

STATISTICAL EVALUATION

The data obtained were subjected to Student's *t*-test and the level of significance was set at $P \leq 0.05$.

RESULTS

MPRs are known to be involved in the selective transport of proteins to lysosomes. Here, we studied the expression and distribution of MPRs in rat epididymis under conditions of hormone deprivation; in castrated rats or after treatment with flutamide. As expected, after castration, levels of testosterone and DHT dropped drastically in serum (Fig. 1), whereas the estradiol concentration decreased to a lesser extent. As can be seen in Figure 2, expression of both the CD-MPR and the CI-MPR (to a lesser extent) was increased in the caudal epididymis of castrated rats. This increase may be due to other cell types invading the epithelium (e.g., fibroblasts) in response to castration. However, a similar effect was observed in the epididymis of flutamide-treated rats (Fig. 3), suggesting that the observed changes in MPRs expression was due to androgenic hormone deprivation. Furthermore, the morphological integrity of the epididymal epithelium was preserved after surgery (data not shown). We have also quantified the activity of CD- and CI-MPR in epididymal tissue by binding assays using a ligand bearing M6P, and we found that CD-MPR activity was higher in the epididymis of castrated compared to control rats (Fig. 4). However, this increase did not correlate exactly with the expression of the CD-MPR, suggesting that some of the CD-MPRs may be inactive in castrated rats. In turn, CI-MPR activity was substantially lower than that of the CD-MPRs, and an increasing trend was observed in castrated rats

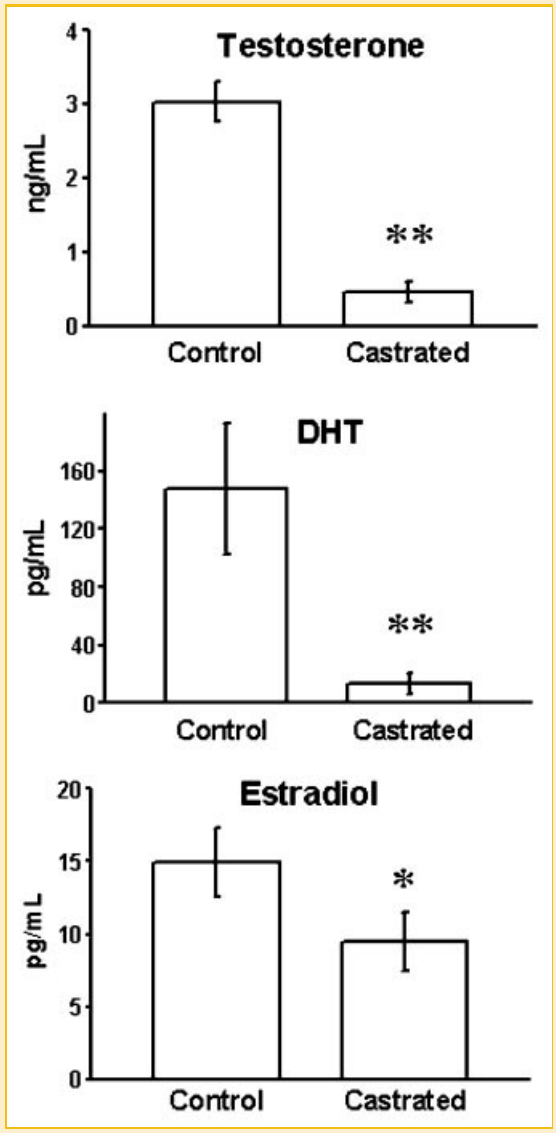

Fig. 1. Steroid hormones in serum of castrated rats (n = 12) and controls (n = 10). Values are expressed as concentrations ± SEM. **Significantly different at $P < 0.001$ and *$P < 0.05$ with respect to controls.

(Fig. 4). The CD-MPR cycles between the trans-Golgi network (TGN) and an acidic compartment, likely the late endosome. However, CD-MPR is also found at the plasma membrane and is implicated in the exocytosis of M6P-bearing ligands. In addition to the increased expression, we wondered whether the distribution of CD-MPRs is somehow altered in the epididymal epithelium after castration. We observed that epididymal CD-MPRs were redistributed toward lighter fractions of a Percoll gradient in castrated rats, possibly corresponding to the plasma membrane (Fig. 5), since this zone of the gradient was enriched in Na/K-ATPase (data not shown). In thin sections of the cauda epididymis processed for immunohistochemistry, the CD-MPR is mostly changed from a dispersed cytoplasmic distribution in the controls to the apical area of the epithelium in castrated rats (Fig. 6). Interestingly, some epithelial cells, likely "clear cells" showed low reactivity for the CD-MPR in the controls, but increased reactivity after castration in these same cells, with an apical distribution. In order to evaluate the possible implications of augmented CD-MPR activity for the secretion of epididymal hydrolases in castrated rats, we measured the activity of two enzymes, α-MAN and β-NAG in the epididymal fluid, and found that the secretion of α-MAN was significantly reduced in castrated rats while the activity of β-NAG was not altered compared to controls (Fig. 7). Similar results were observed in the rats treated with flutamide (data not shown). However, interestingly, epididymal expression and secretion of the immature form of cathepsin D (pro-cathepsin D, pCD) were increased in castrated rats, indicating that this proteolytic enzyme was not processed in lysosomes (Fig. 8). In addition, pCD was the main form found in the tissue of castrated rats while the mature forms of the enzyme were not detected (Fig. 9). It is possible that the observed effects are a consequence of androgen deprivation.

DISCUSSION

An important aspect of epididymal sperm maturation and storage seems to be the interaction of luminal proteins with the surface of spermatozoa [Brooks, 1987; Orgebin-Crist et al., 1987; Hinton et al., 1995; Kirchhoff, 1998]. Therefore, the epididymis of mammals can be studied in different angles: as an organ involved in the maturation of sperm, as a model to study secretory activity, and as an organ responsive to steroidal hormones. The main approach to understanding the effects of androgen withdrawal (or their metabolites) on the epididymis has been removal of the testes [Robaire et al., 2007]. The present study indicates that MPRs are highly expressed in rat epididymis and both the CD-MPR and to a lesser extent CI-MPR responded to castration-induced of hormone deprivation or treatment with the anti-androgenic drug flutamide. In most cell types, the MPRs mediate the transport of specific acid hydrolases to lysosomes [Hille-Rehfeld, 1995; Ghosh et al., 2003; Dahms et al., 2008]. In addition to its intracellular role in the biogenesis of the lysosome, the CI-MPR, but not the CD-MPR, participates in a number of other biological processes by interacting with various non-lysosomal molecules at the cell surface. After castration, the expression of the CD-MPR was increased in the rat epididymis, possibly as a consequence of androgenic hormone deprivation. At the same time, treatment with the anti-androgenic drug flutamide caused an increase in CD-MPR, although to a lesser extent. This supports the idea of an increase of CD-MPR due to an overexpression in epididymal epithelial cells, rather than arising from the invasion by other types of cells with an enriched lysosomal apparatus (i.e., fibroblasts or macrophages). The existence of regulated genes for CD-MPR might explain the increase observed in rat epididymis after castration [Ludwig et al., 1992]. The fact that the peak expression of CD-MPRs does not coincide with the maximum quantity of active receptors could indicate that many of them are inactive. We do not have a good explanation for this observation, although it might be that a compensatory mechanism induces high synthesis of the receptor with lower activity. In addition to changes in expression, we observed a redistribution of

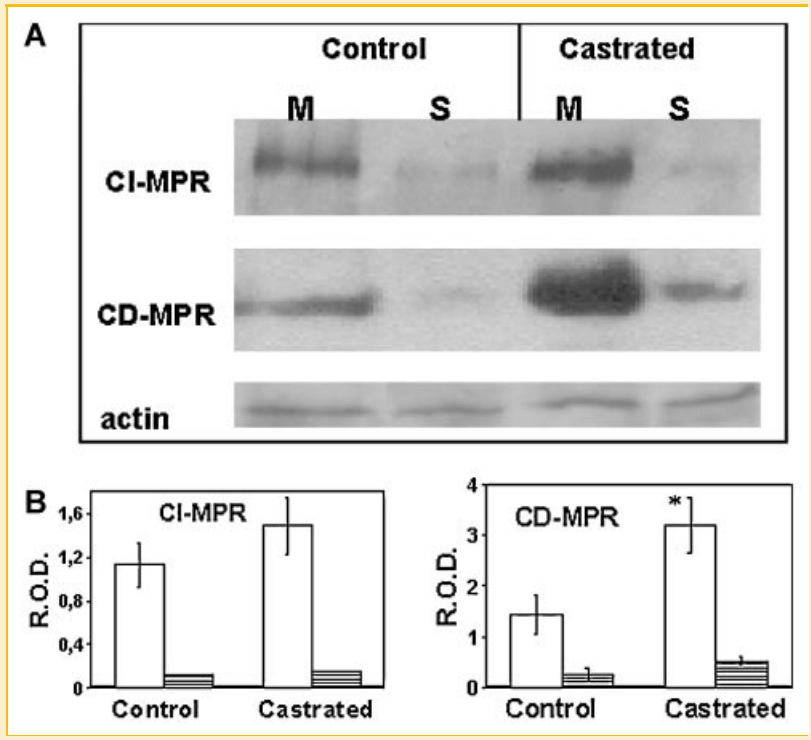

Fig. 2. A: Immunoblots of mannose-6-phosphate receptors from caudal epididymis of castrated rats and controls. The CI-MPR (upper panel) or CD-MPR (middle panel) was probed with the corresponding antibodies, as detailed in the Materials and Methods Section. Detection of actin was used as the loading control (lower panel). A membrane fraction (M), sedimented at 70,000*g*, and the supernatant (S) was assayed for each MPR. B: Quantitation of the immunoblots from four independent experiments for the CD-MPR, and two experiments for the CI-MPR, in the sedimented (white bars) and non-sedimented (shaded bars) fractions. Values are expressed as the mean of relative optical densities (ROD) ± SEM. *Significantly different at $P < 0.05$.

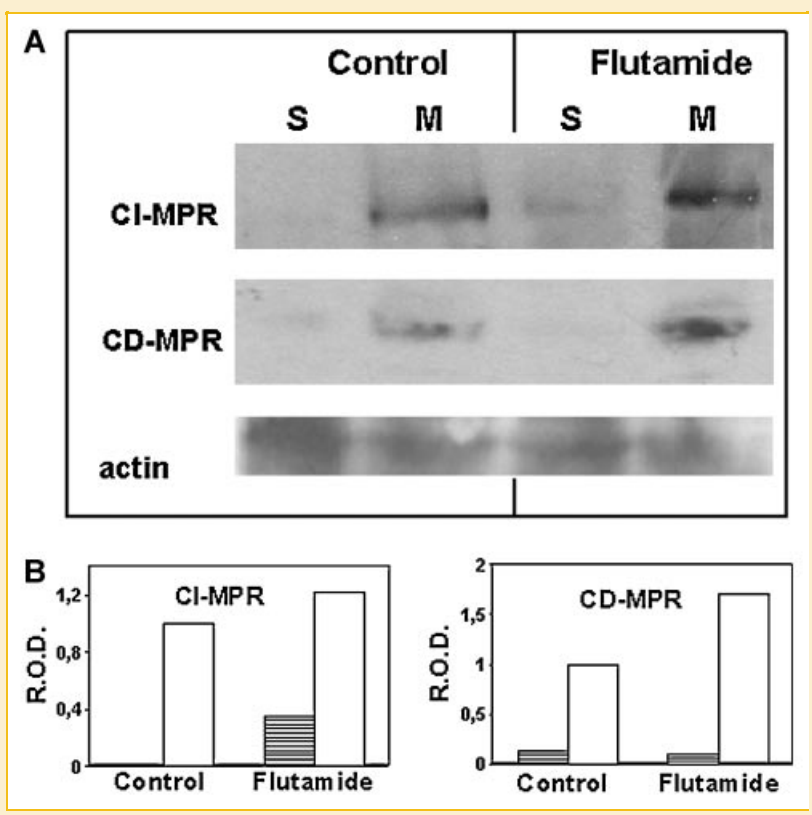

Fig. 3. A: Immunoblots for mannose-6-phosphate receptors from caudal epididymis of rats treated with flutamide and controls. The CI-MPR (upper panel) or CD-MPR (middle panel) was probed with the corresponding antibodies, as detailed in the Materials and Methods Section. Detection of actin was used as the loading control in the gels (lower panel). The membrane fraction (M), sedimented at 70,000*g*, and the supernatant (S) were assayed for each MPR. B: Quantitation of the CD-MPR and CI-MPR immunoblots from one experiment from the sedimented (white bars) and non-sedimented (shaded bars) fractions. Values are expressed as relative optical densities (ROD).

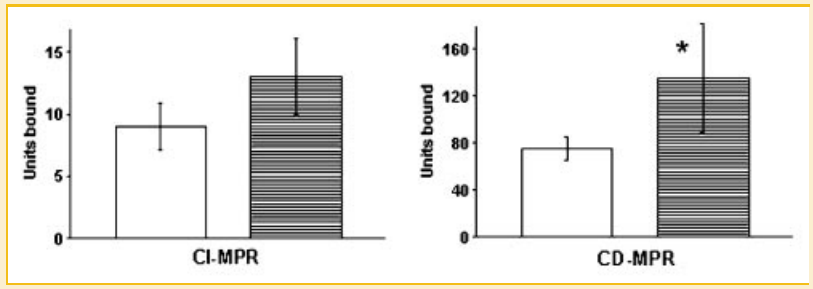

Fig. 4. Maximal binding of β-glucuronidase to CI-MPR and CD-MPR from caudal epididymal membranes from castrated (shaded bars) or control rats (white bars) (see the Materials and Methods Section). Values are expressed as the maximum units bound to the membranes ± SD from three experiments. *Significantly different at $P < 0.05$.

the CD-MPR from the epididymis of castrated rats to lighter fractions in a Percoll gradient and to the apical surface of principal cells by immunohistochemistry. This could also be coincident with the appearance of a small amount of MPR in the high-speed supernatant from castrated rat epididymal homogenates (Fig. 2). The identification of the compartment to which MPRs redistribute will be the goal of our future studies. The clear cells of the epididymal epithelium showed lower reactivity for the CD-MPR under control conditions, which could be related to a less developed lysosomal apparatus. However, this reactivity was higher in castrated rats and with a clear apical distribution. Thus, the clear cells may also respond to hormonal changes. Besides the sorting of lysosomal proteins, the CD-MPR has also been shown to mediate the secretion of lysosomal proteins in certain cell types; BHK and mouse L cells transfected with the cDNA for the human CD-MPR secrete excessive amounts of newly synthesized polypeptides bearing M6P [Chao et al., 1990]. Ligands bound to CD-MPR are transported to and released at specific sites, for example, early endosomes or the plasma membrane, from where they can exit into the medium. Thus, the redistribution of CD-MPRs that we observed in the epididymis of castrated rats could be explained by a recruitment of these glycoproteins to the plasma membrane, which would induce an increase in the secretion of certain epididymal proteins. To investigate this, we have measured the activity of two acid hydrolases, β-NAG and α-MAN in the epididymal fluid, assuming that most of the enzymes were secreted by the epithelium. Contrary to our expectations, it appeared that the secretion of α-MAN [a ligand bearing M6P; Belmonte et al., 1998] was significantly reduced in the epididymis of castrated rats, while the secretion of β-NAG did not differ between control and castrated rats. This apparent retention of α-MAN may be mediated by an increased level of the CI-MPR in the epididymis of castrated rats, as this receptor has been implicated in normal distribution of acid hydrolases to lysosomes. However, and consistent with the observed redistribution of CD-MPRs, the secretion of pCD was increased in the epididymis of castrated animals. It is thought that the intracellular trafficking of cathepsins

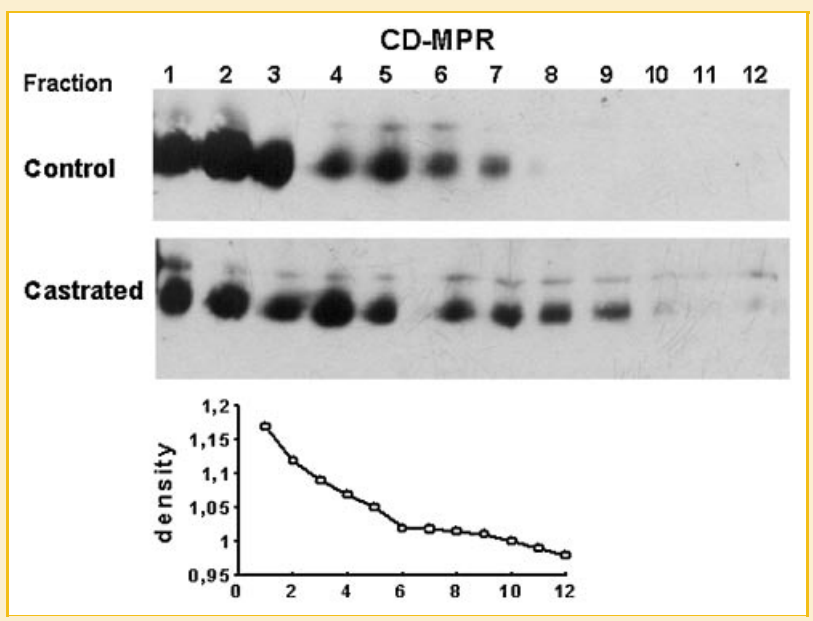

Fig. 5. Distribution of the CD-MPR in a Percoll gradient. Homogenates from cauda epididymis of castrated rats and controls were mixed with Percoll in tubes, and centrifuged as detailed in the Materials and Methods Section. CD-MPR was detected in each fraction by immunoblotting. Inset: densities of the fractions.

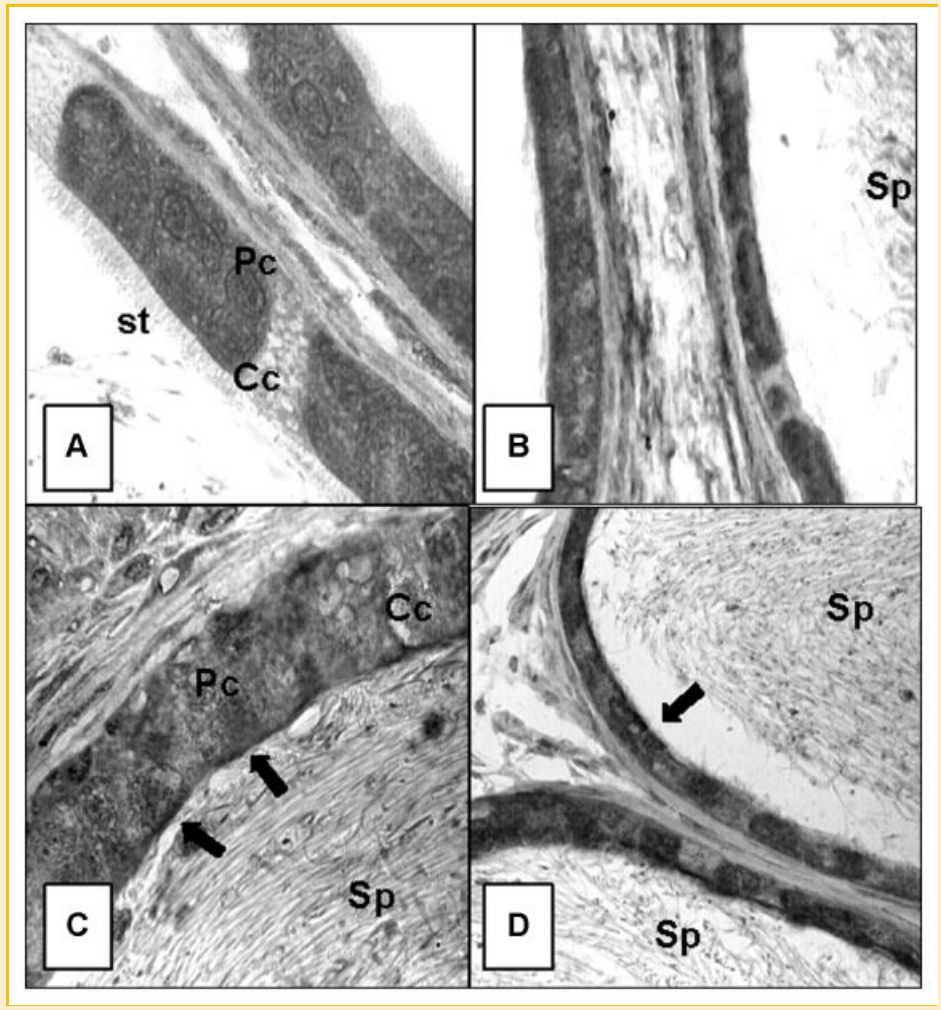

Fig. 6. Immunostaining of CD-MPR in cauda epididymis. Sections of tissue (5 μm), from control or castrated rats, were incubated with rabbit anti-sera to CD-MPR and developed with diaminobenzidine as detailed in the Materials and Methods Section, and visualized by light microscopy. A,B: Epididymis from control and (C,D) from castrated rats. Apical immunoreactivity is indicated with arrows. Pc, principal cells; Cc, clear cells; Sp, spermatozoa; st, stereocilia. Scale bars: 30 μm.

to lysosomes depends on the presence of M6P moieties in the proteinases [Vignon and Rochefort, 1992; Lorenzo et al., 2000], although alternative routes for trafficking of cathepsin D have been proposed in other cell types [Glickman and Kornfeld, 1993; Capony et al., 1994; Laurent-Matha et al., 1998; Dittmer et al., 1999; Ni et al., 2006]. The results presented here indicate that the normal transport of this enzyme to lysosomes might be interrupted in the cells of castrated rats and diverted to the secretion via the CD-MPR. In support of this, we note that pCD from the epididymis of castrated rats was enriched in the high-speed supernatant, together with the CD-MPR (Figs. 2 and 9). Moreover, secretion of pCD via CD-MPR in other cell types has been reported by other authors [Faulhaber et al., 1998]. pCD is also overexpressed and secreted by a number of tumoral cells, playing an essential role in progression of cancers [Rochefort et al., 1990; Isidoro et al., 1995; Nomura and Katunuma, 2005]. However, the secretion of pCD is not only linked to cancer cells but also plays a role in normal physiological conditions like wound healing and tissue remodeling [Vashishta et al., 2007]. Additionally, it is known that the secretion of pCD in tumor cells is influenced by estradiol, as the alpha estrogen receptor (ERalpha) is a ligand-dependent transcription factor that regulates the expression of the pCD gene through interaction with *cis*-acting estrogen response elements (EREs) [Katzenellenbogen et al., 2000]. Estrogen receptors are present in the efferent ductules from testis and in the epididymis of most species [Hess, 2003]. Therefore, the increased secretion (and expression) of pCD observed in the epididymis of castrated rats can be attributed to the influence of estrogen, although levels of this steroid in serum were reduced after castration. The role of estrogen is regulated by the cytosolic enzyme estrogen sulfotransferase (EST), which is synthesized in the liver, but is also highly expressed in testis and, in smaller amounts, in other organs including the epididymis [Song et al., 1997; Robaire et al., 2007; Frenette et al., 2009]. This enzyme can inactivate estrogens by sulfonation at the 3-hydroxyl position. Therefore, we assume that the lack of testicular contribution due to castration reduces sulfonation-dependent inactivation of estrogen in the epididymis, and thus the steroid remains active and it might be responsible for the effect observed in these experiments. In support

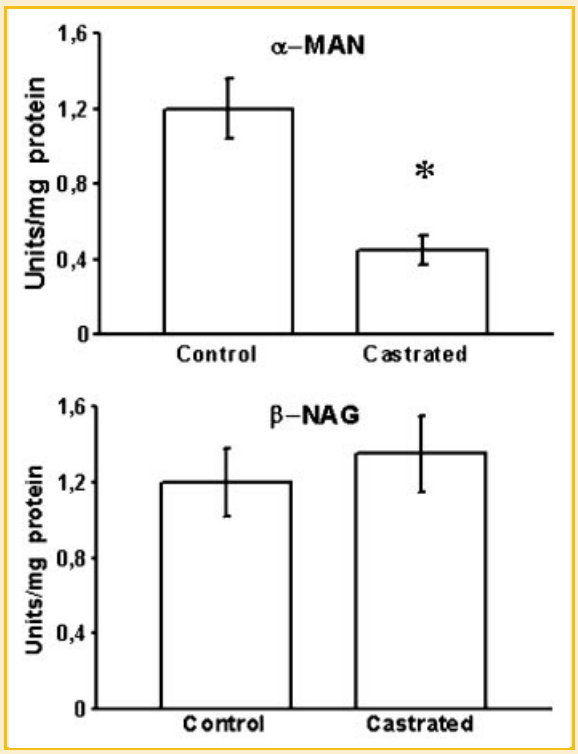

Fig. 7. Activity of α-mannosidase (α-MAN) and *N*-acetyl-β-D-glucosaminidase (β-NAG) in the fluid of cauda epididymis from castrated rats and controls, as indicated. Values are expressed as specific activity ± SD, from three experiments. *Significantly different ($P < 0.01$) compared to controls.

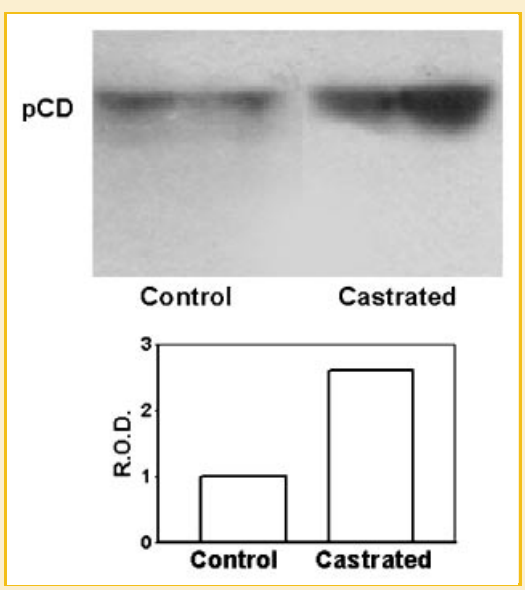

Fig. 8. Immunodetection of pro-cathepsin D (pCD) in the fluid of cauda epididymis from control or castrated rats (upper panel). The enzyme was probed with the corresponding antibody, which recognizes both mature and immature forms of the protein, as detailed in the Materials and Methods Section. Lower panel: Quantitation of the bands by densitometry. Values are expressed as relative optical density (ROD).

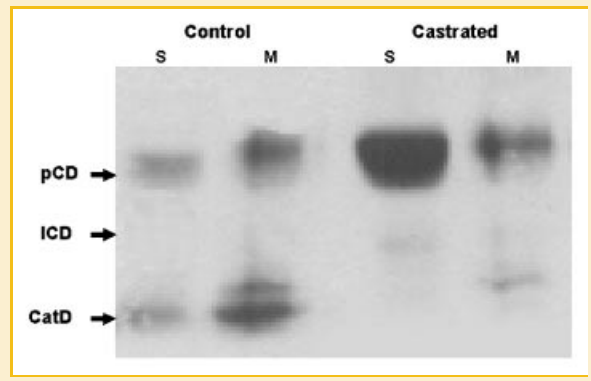

Fig. 9. Immunodetection of cathepsin D in the tissue of cauda epididymis from control or castrated rats. The fractions sedimented (M) or not sedimented (S) at 70,000*g* were assayed for cathepsin D. pCD, pro-cathepsin D (52 kDa); ICD, intermediate form of cathepsin D (44 kDa); catD, mature form of cathepsin D (30 kDa).

of this, we observed a decrease in the expression of EST in the epididymis of castrated rats (data not shown).

Why is the secretion of pCD increased in the epididymis of castrated rats? Although we do not have a concrete answer to this question, one possibility would be that the enzyme participates in the process of epididymal involution, given the strong evidence that pCD plays a dual function in apoptosis [Liaudet-Coopman et al., 2006]. In conclusion, this study shows that the expression of the MPRs in the rat epididymis, particularly the CD-MPR, are influenced by hormonal changes, which would be reflected in a change in the normal transport of lysosomal enzymes; withholding some in the epithelium (mediated by CI-MPR and/or the CD-MPR) or increasing the secretion of others (mediated by the CD-MPR). This would have enormous implications for the secretory ability and the functionality of the epididymis. It remains to determine whether regulation of the MPRs is a direct effect of testosterone, or due to an increase in the activity of metabolites, such as estradiol.

Given that castration has been accepted as a model of androgen deprivation, and moreover the lack of hormones affect sperm maturation by inducing biochemical changes [Seligman et al., 1997], immobility, infertility, and increased death of gametes [reviewed by Ezer and Robaire, 2002], our studies may help understand the role of androgens and/or their metabolites in the maturation of gametes in the epididymis, through the regulation of secretory activity in this organ. Ultimately, this may explain the molecular basis of certain dysfunctions of the male reproductive tract that result in infertility due to immaturity of sperm.

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