Effect of leuprolide acetate on urodynamic parameters of urinary bladder hyperactivity in ovariectomized rats

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Background
- The pathophysiology of the overactive bladder syndrome (OAB) is multifactorial and includes neurogenic, myogenic, urothelial and idiopathic components (De Groat, 1997; Brading, 1997; Moore & Goldman, 2006).
- Menopause is a risk factor of OAB and plays an important role in the genesis of the disease because low levels in estrogenic hormones produce changes both in muscle fibers and in innervation of bladder (Coyne et al., 2010; Aizawa et al., 2011; Kalra et al. 2009; Wobul et al., 2016).
- Gonadotropin-releasing hormone (GnRH) and its agonist Leuprolide Acetate (LA) have shown neuroregenerative properties in cellular cultures and animal models of multiple sclerosis and spinal cord injury (Calderon et al., 2012; Diaz et al., 2015).

Hypothesis
- Drugs with neuroregenerative properties may play a role in the treatment of OAB after hypogonadism.

 Aim
- The objectives of this study were to investigate the effect of LA treatment on bladder overactivity using a castrated rat model, and the plausible role of the gonadotropin-releasing hormone receptor (GnRH-R) in this pathology.

Experimental design

Figure 1. Cytometric effects of castration and LA treatment. A) Typical cytogram of an intact rat infused with saline and anesthetized with urethane; B) Characteristic cytometry from an ovariectomized rat; C) Cytometric profile from an ovariectomized animal treated with LA.

Figure 2. Changes in cytometric parameters generated by castration and effect of LA treatment in ovariectomized rats. A) ICI was lower in the OVX group compared to the SHAM group (p=0.03) without differences between the SHAM and OVX-LA groups (p=0.31); B) LUV was significantly lower in the OVX group than in SHAM (p=0.007) at the same time as there were no differences midst the SHAM and OVX-LA groups (p=0.89); C) BP had a significant increase in OVX with respect to SHAM (p=0.001) without finding differences between SHAM and OVX-LA (p=0.16); D) FH was greater in the OVX-LA group than OVX (p=0.015), without finding differences between SHAM and OVX (p=0.46) nor OVX-LA and SHAM (p=0.26).

Figure 3. Effect of leuprolide acetate on urodynamic parameters in ovariectomized rats. A) ICI was lower in the OVX group compared to the SHAM group (p=0.03) without differences between the SHAM and OVX-LA groups (p=0.31); B) LUV was significantly lower in the OVX group than in SHAM (p=0.007) at the same time as there were no differences midst the SHAM and OVX-LA groups (p=0.89); C) BP had a significant increase in OVX with respect to SHAM (p=0.001) without finding differences between SHAM and OVX-LA (p=0.16); D) FH was greater in the OVX-LA group than OVX (p=0.015), without finding differences between SHAM and OVX (p=0.46) nor OVX-LA and SHAM (p=0.26).

Results

Figure 4. GnRH-R immunoreactivity in rat bladder slides. A) Negative control from a normal rat bladder without primary antibody; B) Representative microphotograph from a bladder of SHAM group rat showing high GnRH-R immunoreactivity (green color), mainly in the urothelium (arrows); C) Image showing low immunoreactivity in the bladder of a OVX rat; D) Image showing how the treatment of LA in OVX rats presents a great immunoreactivity in the urothelium, mainly in the umbrella cells (arrows). Comparable expression and morphological patterns were observed in six more bladders. SHAM n=3, OVX n=3 and OVX-LA n=3. Lu, lumen; U, urothelium. Lp, lamina propia. Images have a 20X magnification. Nuclei were counterstained with DAPI (Blue).

Figure 5. Immunohistochemistry for neurofilaments of 68- and 200 kDa in rat bladder slides. A-C) Negative control without primary antibody; D) Arrows show representative neurofilament-68-kDa (NF68, green) in lamina propia, surprisingly high immunoreactivity was found in the urothelium; E) Neurofilament-200-kDa immunoreactivity (NF200, red) and F) Co-localization (yellow) in the sham group; G) Arrows show low NF68 immunoreactivity (green); H) Low NF200 (red) reactivity and I) Co-localization (yellow) in ovariectomized (OVX) group; J) Arrow show higher NF68 immunoreactivity (green); K) NF200 immunoreactivity (red) and L) Co-localization (yellow) in ovariectomized rats treated with Leuprolide acetate. Lu, lumen; U, urothelium; lamina propia. Images have a 20X magnification. Nuclei were counterstained with DAPI (Blue).

Discussion
- Degenerative changes in smooth muscle density and bladder innervation may explain the pathophysiological changes observed in urodynamic patterns after OVX. Our results suggest that nerve degeneration caused by hypogonadism may be reversed by the neurotrophic effect of LA administration; thus explaining the improvements on voiding patterns. Although we found a significant decrease in the number of NVCs in the OVX-LA rats, this effect correlates with previous reports showing that activation of GnRH-R increases the number of NVCs. The presence of these contractions can be explained by the fact that treatment with LA increased the extent of GnRH-R in the urothelium; and the non-voiding activation generated by the agonist on its own. We predict that the effects could be explained by one of the treatments alone, but subjective effects on bladder innervation may be of a long-lasting duration.

Conclusion
- The GnRH-R analogue LA generates significant improvements on cystometric parameters in rats with overactive bladder conditions induced by castration. Our results suggest the possibility of using agonist or antagonists of GnRH receptors as an alternative treatment for dysfunctions of the lower urinary tract associated with postmenopause.

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