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 OBS and plays an important role in the genesis of the disease because low levels in steroidal hormones produce changes both in muscle fibers and in innervation of bladder (Coyne *et al.*, 2010; Aizawa *et al.*, 2011; Kullmann *et al.*, 2009; Wróbel *et al.*, 2016).
- Gonadotropin-releasing hormone (GnRH) and his agonist Leuprolide Acetate (LA) have shown neuroregenerative properties in cellular cultures and animal models of multiple sclerosis and spinal cord injury (Calderón *et al.*, 2012; Díaz *et al.*, 2015).

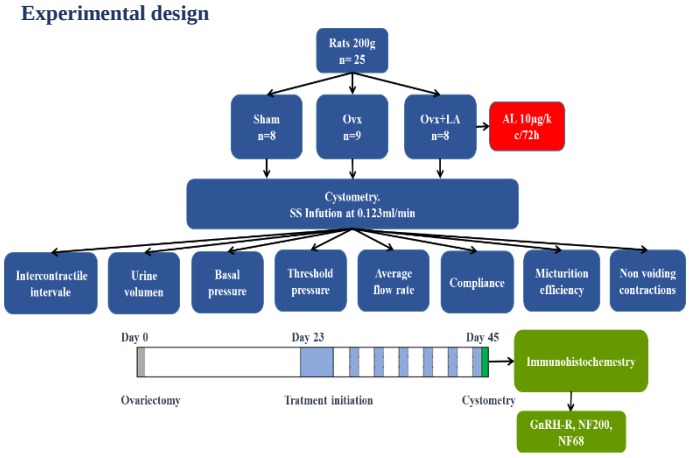
Hypothesis

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



Results

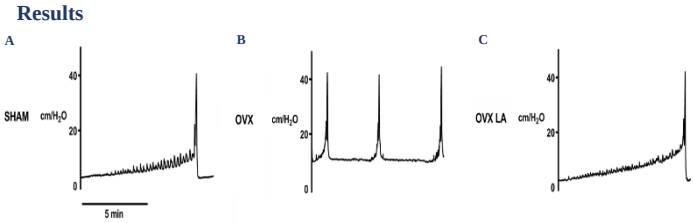


Figure 1. Cystometrical effects of castration and LA treatment. A) Typical cystometrogram of an intact rat infused with saline and anesthetized with urethane; **B)** Charactreristic cystometry from an ovarectomized rat; **C)** Cystometric profile from an ovarectomized animal treated with LA.

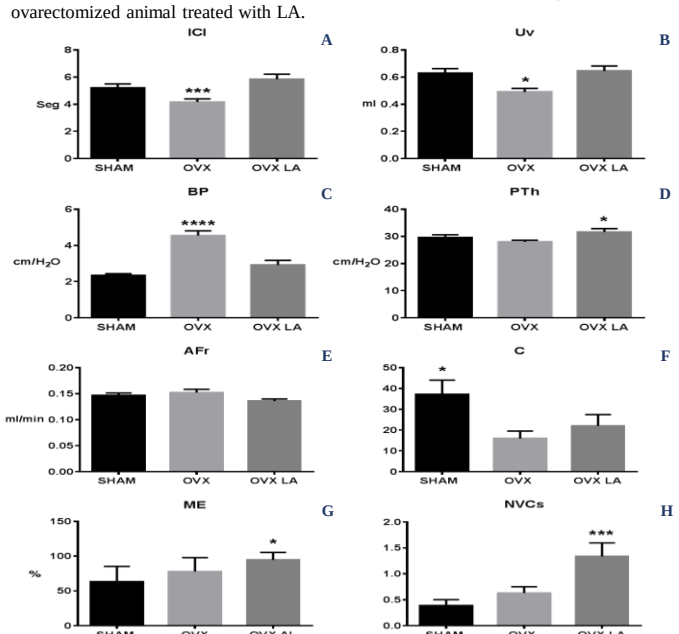


Figure 2. Changes in cystometric parameters generated by castration and effect of AL treatment in ovariectomized rats. A) ICI was lower in the OVX group compared to the SHAM group (P=0.03) without differences between the SHAM and OVX-LA groups (p=0.31); **B)** UV was significantly lower in the OVX group 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)** PTh 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) . **E)** There were no differences between groups regarding AFR.; F**)** C was reduced by ovariectomy (p = 0.02) and there were no significant difference between the groups OVX and OVX-LA (p = 0.68); **G)**; EM was greater in the OVX-LA group than SHAM (p=0.007), without finding differences between SHAM and OVX (p=0.24) nor OVX-LA and OVX (p=0.17) . **H)** A statistically significant increase in the number of NVCs was found in the OVX-LA group in comparison with the SHAM and OVX (p=0.001, p=0.01), without finding significant differences between them (p=0.5).

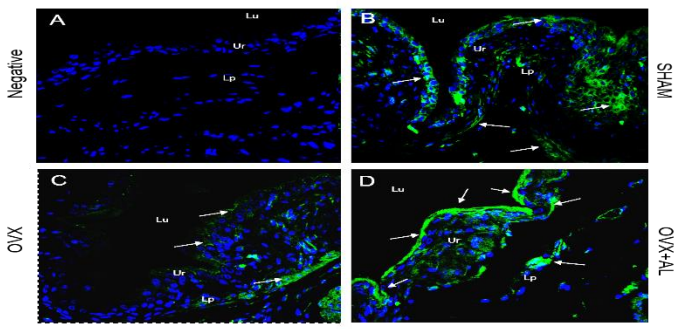


Figure 4. GHRH-R immunoreactivity in rat bladder slides. A) Negative control from a normal rat bladder without primary antibody; **B)** Representative microphotography from a bladder of SHAM group rat showing high GnRH-R immunoreactivity (green color), mainly in the urothelium (arrows); **C)** Image 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; Ur, urothelium; Lp, Lamina propria. Images have a 20X magnification. Nuclei were counterstained with DAPI (Blue).

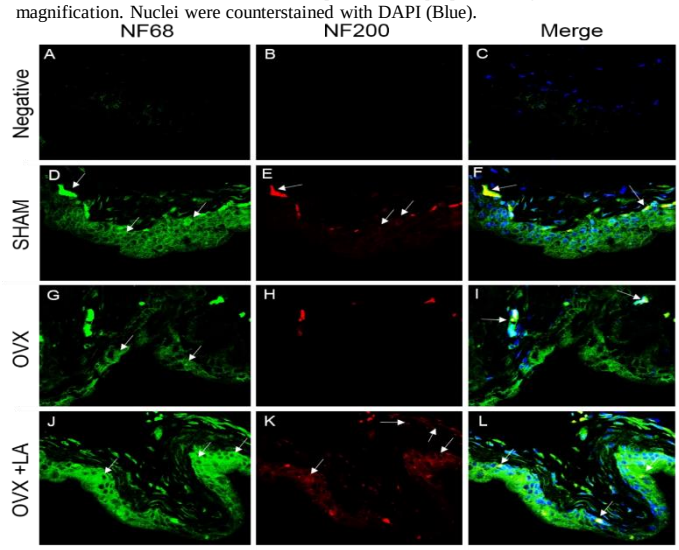


Figure 5. Immunohistochemistry for neurofilaments of 68- and 200 kDa in rat bladder slides. A-C) Negative control without primary antibody; **D)** Arrows show representative neurofilament-68-kDa (NF68; green) in lamina propria, surprisingly high immunoreactivity was found in the urothelium; **E)** Neurofilament-200-kDaimmunoreactivity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

Discussion

Degenerative changes in smooth muscle density and bladder innervation may explain the the pathological changes observed in urination patterns after OVX. Our results suggest that nerve degeneration caused by hypoestrogenism may be reversed by the neurotrophic effect of LA administration, thus explaining the improvements on voiding patterns. Although we found a significant increase 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 this pharmacological effect could disappear when ceasing the treatment, but trophic 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 agonists or antagonists of GnRH receptors as an alternative treatment for dysfunctions of the lower urinary tract associated with post-menopause.

Acknowledgments

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