Resumen

A pesar de los avances de la ciencia, las enfermedades cardiovasculares (ECV) continúan siendo la principal causa de muerte en el mundo occidental. La función vascular es regulada por diferentes agonistas incluyendo las hormonas esteroides sexuales estrógenos, progestágenos y andrógenos. La menopausia, es un período en el cual la probabilidad de contraer ECV se incrementa notablemente, hecho que ha sido atribuido a la disminución de estrógenos en circulación como consecuencia del cese de la actividad ovárica. Si bien los niveles de estradiol disminuyen marcadamente, éste no representa el único cambio hormonal de este período. La contribución ovárica de andrógenos, progesterona y estrona también se ve modificada. Los efectos vasculares de los estrógenos han sido objeto de numerosas investigaciones en las últimas décadas, mientras que la acción vascular de los andrógenos no ha recibido la misma atención. En este trabajo se propuso investigar las acciones celulares y moleculares de testosterona sobre los procesos que participan activamente en la fisiopatología vascular, con la finalidad que los conocimientos aportados por esta tesis contribuyan a elucidar el rol de esta hormona en la homeostasis y/o en la enfermedad vascular. Para tal fin, como modelo experimental se emplearon anillos de aorta torácica y cultivos de células endoteliales (CE) y células musculares lisas vasculares (CMLV) que fueron tratados in vitro con concentraciones fisiológicas del andrógeno.

Se demostró que, a tiempos muy cortos de tratamiento, testosterona estimula la producción de óxido nítrico (NO) tanto en tejido aórtico como en CE en cultivo. Esta acción se produce en forma independiente de la transcripción génica y de la síntesis proteica. El mecanismo a través del cual la hormona regula la producción del vasoactivo involucra la participación del receptor de andrógenos (RA), las vías mensajeras MAPK, PLC/PKC, PI3K/Akt y el ingreso de Ca^{2+} desde el medio extracelular. Respecto a la especificidad de la acción hormonal se demostró que el efecto es propio de testosterona, independiente de su aromatización a estradiol y debido en parte a la conversión de testosterona al andrógeno más potente dihidrotestosterona.

Sobre los procesos celulares involucrados en la lesión vascular se obtuvo evidencia que testosterona previene la adhesión de monocitos al endotelio vascular inducida por el agente proinflamatorio LPS. Si bien el tratamiento de las CE con testosterona no modifica la adhesión basal, cuando los monocitos fueron expuestos por 24 horas al andrógeno la adhesión leucocitaria se inhibió significativamente. El esteroide disminuye los niveles de expresión de los ARNm de las moléculas de adhesión ICAM-1 y VCAM-1, que median la unión firme de monocitos a las CE. En cambio el andrógeno no afectó la expresión de las integrinas CD11b y CD11c en membrana de los monocitos.

En cuanto a las funciones de testosterona sobre la interacción plaqueta - CE se demostró que la hormona inhibe la agregación plaquetaria en forma dependiente de la producción de NO por el endotelio. En un ambiente proinflamatorio, el esteroide previene la adhesión plaquetaria inducida por LPS. El andrógeno estimula la proliferación y migración de CMLV. La acción mitogénica involucra la participación del RA y es independiente de conversión de testosterona a estradiol. Si bien en condiciones basales el andrógeno no afecta la motilidad de las CMLV, en presencia de un estimulador de la migración, norepinefrina, el esteroide potencia dicho efecto.

En relación a los procesos celulares involucrados en la reparación vascular, en CE testosterona promueve la expresión del ARNm de VEGF y estimula la migración y la proliferación celular, siendo este último efecto dependiente de la producción endotelial de NO.

Los resultados obtenidos en este trabajo de tesis doctoral aportan conocimientos sobre las acciones vasculares de testosterona. A nivel molecular se obtuvo información acerca del mecanismo a través del cual el andrógeno regula la producción del principal compuesto vasoactivo que produce el lecho vascular. A nivel celular se pudo determinar el rol del esteroide en algunos de los eventos celulares implicados en la enfermedad y en la reparación vascular, encontrándose acciones deseables (en CE y su interacción con plaquetas, monocitos) y no deseables (en CMLV). Resta por determinar la relevancia fisiológica de la evidencia aportada.

Summary

Despite the advances of science, cardiovascular disease (CVD) remains the leading cause of death in the western world. Vascular function is regulated by various agonists including the sex steroid hormones estrogen, progesterone and androgen. During menopause the risk of developing CVD increases considerably. This fact has been attributed to the decrease of estrogen circulation levels due to ovarian function impairment. However, serum estradiol drop does not represent the only hormonal change of this period since ovarian synthesis of androgen, progesterone and estrone is also modified. Vascular actions of estrogen have been subject of extensive research in recent decades, while vascular actions of androgens have not received the same attention. In order to contribute to the knowledge of the role of androgens on vascular homeostasis and/or disease, in this work we investigated the cellular and molecular actions of testosterone on the regulation of cellular events involved in vascular physiology. For this purpose, rat aortic strips (RAS) and cell cultures of endothelial cells (EC) and vascular smooth muscle cells (SMVC) were used as experimental models. *In vitro* treatments were performed with physiological concentrations of testosterone.

We provide evidence that testosterone induces an acute stimulation of nitric oxide (NO) production, either in RAS and EC cultures, in a gene transcription independent manner. The mechanism of steroid action involves the participation of the androgen receptor (AR), and is dependent on MAPK, PLC/PKC and PI_3K/Akt signaling pathways activation and extracellular Ca^{2+} influx. Regarding to the specificity of the hormonal action, we demonstrated that testosterone regulates NO production “per se”, and not through its conversion to estradiol via aromatization. The enhancement in the production of the vasoactive synthesis is partially due to testosterone reduction to dihydrotestosterone.

When the effect of the androgen on cellular processes involved in vascular lesion was evaluated, we found that testosterone prevents monocytes adhesion to vascular endothelium induced by the proinflammatory agent LPS. Although testosterone treatment did not affect basal adhesion to EC, 24 hours of monocytes treatment with 1 nM testosterone significantly inhibited

leukocyte adhesion. Indeed, the steroid decreases mRNA expression of the adhesion molecules ICAM-1 and VCAM-1, proteins involved in firm monocyte adhesion to EC. On the other hand the androgen does not affect CD11b and CD11c integrins expression on monocyte surface.

Concerning platelet - EC interaction, we demonstrated that the hormone inhibits endothelium-dependent platelet aggregation through a direct action on EC via stimulation of NO production. Under inflammatory conditions the steroid prevents LPS induced platelet adhesion. The androgen stimulates proliferation and migration of SMVCs. This mitogenic action involves the participation of AR and is independent of testosterone conversion to estradiol. While under basal conditions the androgen does not affect SMVC motility, in the presence of the migration inducer agent, norepinephrine, the hormone is able to enhance the migratory effect.

Related to the cellular processes involved in vascular repair, testosterone promotes EC mRNA expression of VEGF, and stimulates cell proliferation and migration. The mitogenic action exhibited by the steroid is dependent on endothelial NO production.

The results obtained in this thesis provide knowledge about vascular actions of testosterone. At a molecular level, information was provided about the mechanism by which androgens regulate the production of one of the main biochemical factors produced by the vascular bed. At cellular level, we obtained evidence about desirable (EC-platelets, EC-monocytes interactions) and undesirable (VSMC proliferation and migration) actions of testosterone on the regulation of cellular events that play key roles on vascular remodeling. It remains to be determined the physiological relevance of our findings.

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