

Discovery of new reproductive hormones and receptors in the postgenomic era

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Recent publication of entire genomic sequences for human and multiple model organisms allows an unprecedented opportunity to elucidate the evolutionary origin of all human genes. A "molecular clock" paradigm using DNA as a timepiece can be implemented to sort out the origin of complex gene networks and pathways. For reproductive biologists, this breakthrough facilitates the analysis of reproductive genes by tracing their phylogenetic roots based on the comparison of homologs from human and lower species, including nematode, fly, fish and rodents.

1. PAIRING OF NEW LIGANDS AND RECEPTORS: Using GenBank searches, we identified new hormones and receptors based on their evolutionary origin. We have identified novel human paralogs for glycoprotein hormone subunits (alpha 2 and beta 5), relaxin/insulin-like factors, and CRH-related polypeptides. In addition, we isolated a group of five orphan LGRs (leucine-rich repeat-containing, G protein-coupled receptors) homologous to LH, FSH and TSH receptors. The matching of polypeptide hormones and receptors led to the finding of a new heterodimeric glycoprotein hormone thyrostimulin (alpha 2/beta 5) as an agonistic ligand for the TSH receptor (J Clin Invest June 2002) and two selective ligands for the type 2 CRH receptor (stresscopin and stresscopin-related peptide; Nature Med, 7:605 2000). In addition, the elusive receptors for the classic hormone relaxin were identified as LGR7 and LGR8 (Science, 295: 671 2002) whereas INSL3 was found to be the specific ligand for LGR8. The present genomic strategy of pairing polypeptide ligands and receptors will continue to provide a better understanding of the hormonal systems in the human body.

2. STUDIES BASED ON AN ORGAN-SPECIFIC DATABASE: An organ-based approach has been used to set up a database integrating existing text-based literature on individual ovarian genes and their sequence-based data in the GenBank. The Ovarian Kaleidoscope database (OKdb; <http://ovary.stanford.edu>) has accumulated near one thousand individual gene pages that are searchable based on gene function, cellular localization, chromosomal position, ovarian cell type, ovarian function, mutant phenotypes, etc. Using this database, it is possible to set up gene pathway maps for DNA array analysis, identify key gene networks essential for infertility phenotypes, compare chromosomal synteny regions for finding candidate fertility genes, categorize cell-specific and hormonally co-regulated genes for promoter analysis, and document potential ligands and receptors in the paracrine regulation of follicular development (Endocrinology 143:2041 2002). Global analysis of gene function and relationships in an organ-specific manner provides a functional genomic paradigm for the future understanding of ovarian physiology and pathophysiology.

Sex steroids and atherosclerosis: from the laboratory to the clinic**Tapan K. Mukherjee, Lauren Nathan & Gautam Chaudhuri**

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The role of estrogens and androgens in coronary artery disease (CAD) is controversial. Many epidemiologic studies indicate that estrogens are protective against CAD, whereas, others indicate that they are either ineffective or detrimental. Very little is known about the effects of androgens on endothelial function. We, therefore, assessed the effects of estrogens and androgens on endothelial function both in vitro as well as in vivo utilizing an animal model and women.

For in vitro studies to assess the effects of estrogens on vascular cell adhesion molecule-1, we utilized the human umbilical vein endothelial cells (HUVEC) as the model system. We initially utilized the natural estrogens, 17 β estradiol and 17-epiestriol as well as the synthetic estrogen, 17 α ethinyl estradiol, to assess the effects of these estrogens on TNF α induced VCAM-1 expression in HUVEC. We observed that 17-epiestriol was 400 times more potent than 17 β estradiol in suppressing TNF α induced VCAM-1 expression. Ethinyl estradiol was without effect. 17-epiestriol is a more potent agonist for the estrogen receptor β (ER β) when compared to the other estrogens studied indicating that the ER β may be more important in mediating the anti-atherogenic action of estrogens. We, therefore, selected 17-epiestriol for further studies to elucidate the mechanisms involved. We observed that the action of 17-epiestriol was biphasic, as low concentrations (100-300 pM) attenuated VCAM-1 expression, whereas, higher concentrations were less effective. This action of 17-epiestriol was mediated by nitric oxide (NO), as the effect of 17-epiestriol was attenuated in the presence of an inhibitor of NO synthesis. We further observed that TNF α induced VCAM-1 expression was mediated by NF κ B and 17-epiestriol prevented the translocation of p65 to the nucleus. This effect of 17-epiestriol was also abolished in the presence of an inhibitor of NO synthesis. On the other hand, a higher concentration of 17-epiestriol (100 nM) was ineffective in preventing the translocation of p65 to the nucleus. This biphasic effect of 17-epiestriol was also observed with relation to its actions in increasing eNOS protein. Lower concentrations (100 and 300 pM) increased eNOS protein, whereas, higher concentrations (100 and 300 nM) were less effective. In vitro studies, therefore, indicated that the beneficial effect of 17-epiestriol on endothelial function was concentration related.

We demonstrated that the aromatizable androgen testosterone, attenuated the TNF α induced VCAM-1 expression whereas dihydrotestosterone had no effect. This action of testosterone was due to conversion to estradiol as the effects of testosterone was not observed in the presence of an aromatase inhibitor.

In in vivo animal studies, we observed that 17 β estradiol was effective in attenuating initiation of atherogenesis but did not cause regression of established lesions. Similarly, we observed that estrogens were very effective in increasing myocardial blood flow in post-menopausal women with no risk factors for CAD, whereas, they were

ineffective in women known to have risk factors for CAD. In animal studies we also observed that testosterone attenuated the development of atherosclerosis and this effect was not observed in the presence of an aromatase inhibitor.

In conclusion, it therefore appears that the concentration of estrogens, the presence or absence of risk factors as well as the state of the vascular wall when estrogen replacement therapy is started may be a potential explanation for the paradoxical effects observed with estrogen on cardiovascular morbidity and mortality. It also appears that testosterone can under certain circumstances attenuate atherogenesis by conversion to estradiol by aromatase which is present in endothelial cells.