Published in final edited form as:

J Steroid Biochem Mol Biol. 2011 September ; 126(0 1): e1–e4. doi:10.1016/j.jsbmb.2011.04.011.

Integration of steroid research: Perspectives on environment factors, homeostasis in health, and disease treatment

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Keywords

Endocrine disruptors; Steroids; Breast cancer; Prostate cancer; Obesity; Diabetes; Mice model; Chemistry of synthesis

1. Aim and scope

Steroid molecules specifically control distinct aspects of cell proliferation, tissue differentiation, and normal physiological functions. Dysfunction in steroid signal transduction pathways leads to severe disorders and diminished life quality, and consequently pose an economic burden to society. Research in steroids so far has provided insights on their role in maintaining homeostasis and how to control or treat health issues such as human fertility, menopause or hypertension. However, contemporary society is facing many still unsolved issues in environmental and developmental health, and aging that are affected by steroids. These health issues include cancer, disorders of metabolism (metabolic syndrome and type 2 diabetes) and neuronal/behavioural and cognitive disorders. Many proteins in the steroid biosynthesis and signal transduction pathways are druggable targets and have elicited great interest from pharmacologists [1–5].

The aim of the Congress on Steroid Research was to bring scientists from different disciplines to one meeting. The audience was a mixture of basic and clinical scientists, researchers from the pharmaceutical industry, leaders of clinical chemistry labs and scientists from environmental protection agencies. The goals were also to provide a venue for the training of young researchers and support their networking and scientific activities.

2. Topics

The topics of the Congress on Steroid Research included:

- Environmental impact on human health
- Steroids in disease (with the focus on diabetes, obesity and cancer)
- Steroid receptor functionality
- Mechanisms of signal transduction
- Steroidogenesis and pre-receptor metabolism of steroids
- Synthesis of modulators of signal transduction pathways and steroid metabolism

The chapters that follow will provide an overview of the speakers and oral contributions. More details can be found in the corresponding abstracts in the same issue for which numbers are given in [square brackets].

3. Environmental challenge

The role of environmental exposures and their impact on epigenetics was introduced by T. Schug [K23]. Mechanisms of action include interference with epigenetic signaling (DNA methylation, chromatin remodeling or iRNA) and how some of these effects might be transmitted via the germ line for 3–4 generations. The concept of obesogens was evaluated by B. Blumberg [K22]. Tributyltin (TBT, a persistent organic pollutant) acting via PPAR γ promotes adipogenesis *in vivo* and TBT-exposure during early life alters the balance of progenitor types in the stem cell compartment to favor the production of adipocytes. As depicted by S.M. Ho [K24] the mechanism on epigenetic action of environmental estrogens may involve promoter methylation as shown for the *Nsbp1* gene. New tools for the studies of epigenetic processes were presented by U. Oppermann [K10]. New freely available chemical tools to study epigenetic modification systems were applied to the Jmjd2 (KDM4) subfamily of human H3K9 and H3K3 demethylases. A new initiative for analysing the structural requirements for endocrine disrupting chemicals (ECD) to bind to nuclear hormone receptors was presented by W. Borguet [O8]. The group discovered that some EDCs mimic the natural hormones through conserved protein–ligand contacts while others employ radically different binding mechanisms rather than simply mimicking the structures of natural ligands.

4. Diabetes and obesity

Epidemiologic observations indicate that females are less prone to metabolic syndrome and the development of insulin resistance than males. However, as presented by D.J. Clegg [K32] the onset of menopause leads to increased risk. Her studies on tissue-specific ER α -knock-out mice demonstrated the protective role of ER α . Females suffering from polycystic ovary syndrome PCOS have a high risk of type 2 diabetes (T2D) and cardiovascular disorders (CVD). A. Dunaif [K33] discovered susceptibility to PCOS marked by a genetic variant in FBN3 and further investigated association of the transcription factor 7-like 2 (TCF7L2) locus and SNPs associated with the reproductive phenotype and pancreatic β -cell

dysfunction, respectively. CVD, T2D and obesity are caused by increased levels of glucocorticoids produced by 11 β -hydroxysteroid dehydrogenases type 1 (11 β -HSD1 or HSD11B1). B.R. Walker [K31] presented possibilities of pharmacological modulation of 11 β -HSD1 and the action of selected inhibitors on the HPA axis to treat CVD. As reviewed by S.E. Bulun [K30] obesity increases risk of postmenopausal breast cancer. The group created a model of the transgenic humanized aromatase mouse. The latter reveals promoter-tissue specific (e.g., brown fat, gonads or white adipose tissue) aromatase expression and permits assays on peripheral estrogen production and its effects. Metformin is a widely used antidiabetic drug which is now used in breast cancer prevention. K.A. Brown [O6] demonstrated for the first time that metformin selectively acts on different aromatase promoters: it inhibits PII/PI.3, but does not activate PI.4 promoter and by that mechanism elicits tissue-specific effects on aromatase expression.

5. Cancer

Aromatase is the only P450 enzyme (CYP19) that converts androgens into estrogens and regulates the amount of estrogen that potentiates cellular proliferation. Aromatase inhibitors are used in the treatment of breast cancer. S. Chen [K26] described the molecular basis of de novo and acquired aromatase inhibitor resistance and presented cell lines specifically engineered for these studies. Similarly to breast cancer, a resistance to 17 α -hydroxylase/17,20-lyase (CYP17) inhibitors e.g., abiraterone acetate is observed. As depicted by T.M. Penning [K27] local (intratumoral) tissue biosynthesis of proliferatory androgens may be responsible for the emergence of castrate resistant prostate cancer (CRPC). The group has analysed functions of AKR and HSD enzymes in prostate steroid biosynthesis and developed novel N-phenylanthranilic-based lead compounds for the treatment of (CRPC). The group of H. Sasano [K28] presented results on the local impact of aromatase and estrogen receptors in non-small cell lung cancer (NSCLC), on this basis aromatase inhibitors may have a place for the treatment and prevention of NSCLC in women. B.S. Katzenellenbogen [K29] presented results on the interplay of estrogen receptors (ER α and ER β) and the extracellular signal-regulated kinase (ERK2). The group has presented data on the convergence of ER and ERK signaling pathways at the level of chromatin re-modeling and promoter action.

6. Steroid receptors

In his plenary lecture J.Å. Gustafsson [K1] addressed the similarities and differences of ER α and ER β structure and function which balance opposing proliferative and anti-proliferative activities, respectively. Present knowledge of gene regulatory networks influenced through ER β including epithelial–mesenchymal transition (EMT) and cancer were presented. As depicted by G.L. Greene [K3] the pathways of estrogen receptors ER α and ER β can be differentiated by subtype specific ligands (SERMs). Data demonstrated the importance of long-range interactions in the allosteric transmission of information from the nuclear receptor ligand-binding domain how this may contribute to the origin of tissue- and pathway-specific effects. S.A.W. Fuqua [K5] reported on a mechanisms contributing to resistance to aromatase inhibitor treatment (e.g., anastrozole) in breast cancer. The mutation K303R was found to influence the activation of phospho-S305 of ER α which mediates

signaling to the IGF/PI3K/AKT pathways. A. Nardulli [K2] analysed the transcriptional control of progesterone receptor (PR) gene expression and showed that its estrogen responsive elements (ERE) that bind ER α lie 311 kb upstream from the progesterone receptor B (PR-B) transcription start site. Using chromosome conformation capture assays the existence of further interactions in proximal and distal regions of PR-B gene were revealed. F. Labrie [K4] reviewed sensitivity and resistance to androgen deprivation therapy in prostate cancer. From clinical trials F. Labrie evaluated the treatment of CRPC with the combined use of the 17 α -hydroxylase inhibitor abiraterone and prednisone. These strategies would not only inhibit peripheral androgen formation but in addition will inhibit DHEA secretion from the adrenals. It was proposed that early blockade of DHEA production would benefit prostate cancer patients. Gender-specific incidence of thyroid cancer suggests a sex-steroid involvement. A.J. Stanley [O2] performed transcriptome profiling in human tumour and normal samples. The study demonstrated that elements responsible for gender-related incidence include changed expression of androgen receptor (AR), CBP, Sp1 or P53 and miRNA124a.

7. Signal transduction

Steroid hormones elicit rapid responses (s) via ion channels and kinases, and slow responses (hours to days) through nuclear-receptor mediated processes. These effects are referred to as the genomic and non-genomic effects, respectively. E.R. Prossnitz [K6] provided new data on the 7-transmembrane G protein-coupled receptor family (GPR30). The non-genomic role of GPR30 was demonstrated in the reproductive, nervous, immune and vascular systems, and in cancer and obesity. Thus GPR30 may represent an alternate target to ER α / β in a number of diseases. Whereas increased steroid levels are associated with cancer, the decreased levels of estrogens are associated with various neuropsychiatric disorders such as depression, anxiety and panic disorders in women. N.A. Muma [O3] reported that estrogen-mediated desensitization of serotonin 1A (5-HT_{1A}) receptor is not mediated by ER β but rather by ER α or GPR30. C.A. Lange [K7] investigated non-genomic effects mediated by the progesterone receptor. The group revealed that phospho-Ser81 PR-B mediates ck2 kinase recruitment and regulation of selected PR-B (but not PR-A) target genes such as BIRC3, HSD11B2 or HbEGF. Mechanisms of androgen-independent growth of prostate cancer were analysed by N.L. Weigel [K8] who reported effects of disruption of N-terminal coactivator binding sites (e.g., for p160) or the DNA binding domain. Furthermore N.L. Weigel presented data on MEK inhibitor (U0126) treatment causing AR down-regulation and instability. Nearly all studies involving signal transduction pathways, tumour biology, population profiling or environmental influences on steroid action require analytical methods for the accurate detection and quantification of steroids. T. Koall [K9] presented a high throughput validated method for steroid quantification. The method described is applicable not only in research laboratories but in clinical trials as well.

8. Steroidogenesis

The balance between activation and inactivation of steroid hormones drives the fate of cells and tissues. R.J. Auchus [K16] analysed the kinetics of conversions catalyzed by rat liver 3 α -HSD (an AKR) and 17 β -HSD1 (a SDR) and demonstrated that at equilibrium there was

equal conversion of keto and hydroxyl-steroids, and that cofactor availability determines the net outcome of reactions. Peripheral steroidogenesis seems to be a major source of circulating steroids in postmenopausal women. As pointed by E.R. Simpson [K14] the adipose tissue could be a significant contributor. Obesity is associated with increased breast cancer risk but the knowledge of the mechanisms responsible is limited. E.R. Simpson observed that aromatase expression in breast adipose cells is inhibited by AMPK due to sequestration of the CREB coactivator CRTC2. The data show further that leptin inhibits, and adiponectin and metformin stimulate the LKB1/AMPK pathway in these cells and at the same time aromatase expression is stimulated or inhibited, respectively. Local steroid biosynthesis in tumour tissue was a subject of presentation given by F. Stanczyk [K15]. He observed that in normal prostate tissue, levels of dihydrotestosterone (DHT) are 20–30 times higher than testosterone (T) levels. However in serum the T is 6–10 times higher than DHT. Comparison between tumour and normal tissues of the breast showed a higher intratumoral estradiol (E2) levels independent of ER status. As shown by E. Strahm [O5] the use of anabolic steroids such as nandrolone (19-nortestosterone) has a clear impact on the hypothalamic-pituitary axis. The decreased levels of FSH and LH persisted even after four months after the last exposure to nandrolone. Although animal (mouse) physiology is not a phenocopy of human, mouse models open the possibility of knocking out a gene of interest followed by a characterisation of the impact of the gene deletion. M. Poutanen [K17] presented his data on 17 β -hydroxysteroid dehydrogenases function based on knock-out models and also used transgenic models for the over expression of 17 β -HSDs. For 17 β -HSD2 a new role in placental development was shown and for the 17 β -HSD12 a participation in lipid metabolism was observed.

9. Pre-receptor metabolism

An example of an enzymemediating tissue specificity for steroid hormone action is the 11 β -HSD1. As reviewed by A. Odermatt [K11] the enzyme is coupled to hexose-6-phosphate dehydrogenase (G6PDH) and converts cortisone to cortisol. In addition to glucocorticoids 11 β -HSD1 was shown to metabolise the oxysterol 7-oxocholesterol to 7 β -hydroxycholesterol as well as the secondary bile acid 7-oxolithocholic acid to chenodeoxycholic acid. P.M. Stewart [K12] reported on the role of 11 β -HSD1 in glucocorticoid biosynthesis and the impact on hypertension, osteoporosis and obesity. The use of selective inhibitors of 11 β -HSD1 (like MK-0916) is a promising tool in the prevention of glucocorticoid excess-mediated diseases. He also presented the molecular basis for moderate cortisone reductase deficiency that was independent of G6PDH. Zebrafish is a good model for developmental biology, toxicology studies as shown by J. Tokarz [O7] for the analysis of glucocorticoid action. The group of J. Adamski identified and characterised a novel enzyme participating in cortisol metabolism. S.X. Lin [K13] reviewed the structure function–relationships of 17 β -HSD1, a key enzyme in estradiol biosynthesis. He presented a novel role of the enzyme in DHT inactivation and discussed results on the regulation of down-stream responsive genes like PCNA and BCCIP.

10. Chemistry of synthesis

Synthesis of modulators of steroid signal transduction pathways faces challenges involving specificity, similarity/differences to natural ligands and the difficulties involved in the synthesis of regio- and stereoisomers that characterizes natural product chemistry. D. Poirier [K18] reported on E2-based derivatives with C6-alkylamide and C16-bromoalkyl substituents and their further improvement with m-carbamoyl benzyl group at position 16 β of E2. The later compound was an effective 17 β -HSD type 1 inhibitor that gave an impressive IC₅₀ 68 nM in the T-47D breast cancer cell line and inhibited E1 induced growth of breast cancer xenografts in nude mouse. Next to estrone (E1) or androgens, the sulfated-estrogens are a depot for E2 release. B. Potter [K19] presented a steroid-based sulfatase inhibitor with IC₅₀ 10 pM, EMATE= E1–3-*O*-sulfamate. A further drug, a non-steroidal compound Irosustat, is at present in phases I and II clinical trials for breast-, endometrial-, prostate-cancer and the treatment of endometriosis. R.N. Hanson [O4] reported on the development of a estrogen–doxorubicin hybrid that could be delivered to the nucleus using the ER as a “Trojan horse”. Novel chemistry used 11 β -substituted steroidal antiestrogens functionalized with an azido-tetraethylene glycol moiety coupled to a complementary doxorubicin benzoyl hydrazone functionalized with a propargyl tetraethylene glycol moiety. The advantage of this approach might be reduced effects on non-estrogen target tissues. R.W. Hartmann [K20] reported on the combination of efficient steps leading to development of steroidogenic enzyme inhibitors. The strategy involved a combination of virtual screen for pharmacophores, testing compounds *in vivo/in vitro* and iteratively using this information, for the successful synthesis of inhibitors of CYP11B2 which are indicated for CVD, and the synthesis of 17 β -HSD1 inhibitors for the treatment of breast cancer and endometriosis. A revolutionary approach to generate mirror images of steroids *ent*-steroids was presented by D.F. Covey [K21]. Not present in the nature the enantiomers of naturally occurring steroids can be used to distinguish between effects of steroids that involve membrane perturbation or receptor binding. In the closing plenary lecture J.A. Katzenellenbogen [K34] presented the emerging field of steroid signal transduction pathways and how this can be dissected with chemical biology approaches: he presented methods that permit the visualization of steroid receptors *in vivo*. Non-invasive imaging with positron emission tomography (PET) can show the level of ER α or ER β with specific ligands labelled with fluorine-18. The first applications in breast cancer diagnostics are very promising.

Acknowledgments

We express our gratitude to Helmholtz Zentrum München, German Center for Environmental Health for the travel and logistics support. Funding for this conference was made possible in part by 1R13DK092108-01 grant from the National Institutes of Health. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government. We are thankful to the Elsevier for hosting the meeting and their support in the organisation and logistics. We express our gratitude to Endocrine Society USA for endorsing the Congress on Steroid Research.

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