genitalia [7]. The *Drosophila* transforming growth factor-α (TGF-α) homolog Spitz, a ligand for the epidermal growth factor receptor, functions to recruit cells into the developing retina [8]. Currently, many new signaling proteins are being identified from the *Drosophila* genome sequence [9] that include RTKs of the insulin-like growth factor-I/insulin family, a TGF-β ligand, Wnt ligands that regulate cell polarization and migration, and members of the Toll pathway which establish the embryonic dorso–ventral axis. In conclusion, signaling proteins, including VEGF, are proving instrumental in guiding developmental pathways through cell–cell communication. The emergence of new signaling factors makes this an exciting time for researchers to explore conserved developmental themes, whether they are classified as 'walks' or 'talks', employed by many species to promote appropriate developmental processes.

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Meeting Report

Sex, hormones and the cardiovascular system

Stacey A. Fannon and Sherry A. Marts

The meeting 'Sex Differences in Cardiovascular Health and Disease' was held in Madison, WI USA, on 24 July 2002.

Against the backdrop of the terminated Women's Health Initiative (WHI) trial to evaluate the benefits of estrogen plus progestin replacement therapy [1], 'Sex Differences in Cardiovascular Health and Disease' – a scientific advisory meeting organized by the Society for Women's Health Research – got underway. One of the meeting's goals was to address the molecular basis underlying hormonal effects on the cardiovascular system.

Ligands, receptors, genomes and physiology – an integrated approach

Virginia Miller (Mayo Clinic, Rochester, MN, USA) emphasized the need for an integrated approach to studying effects of sex hormones on physiology. This point was brought home only too well by the results of the WHI, which showed that a combination of estrogen and medroxyprogesterone, whilst decreasing the risk of colorectal cancer, increases the risk of breast cancer and thrombotic events. Miller stressed the need to understand fully the complex interactions between ligands, receptors and genomes before hormones or their analogs are used as potential therapeutics.

Crucial to this understanding is the fact that hormone receptors are distributed heterogeneously throughout the body. Endogenous estrogens, synthetic receptor modulators (e.g. raloxifene), and naturally occurring phytoestrogens obtained through the diet, for example, have varying effects on target cells and tissues. These variations might reflect differential expression of receptor isoforms and/or different ligand-receptor affinities. In addition, single- and multiple-nucleotide polymorphisms within the population add an extra level of complexity, and could explain, for example, why estrogen elevates plasma endothelin-1 in some recipients, but decreases it in others. 'It would be interesting,' said Miller, 'to go to the WHI and [ask] which women developed different types of cancer or cardiovascular events and what were the polymorphisms in their estrogen receptors.'

Elucidating the molecular mechanisms of hormonal action is essential to the integrated approach. Traditionally steroid effects have been viewed as genomic (i.e. mediated through transcriptional activation), but more recently evidence has grown in support of non-genomic actions.

Molecular effects on cardiovascular smooth muscle

In addition to the actions of estrogen on the endothelium, it has been known for some time that estrogen can relax denuded coronary arteries [2]. Richard White (Medical College of Georgia, Augusta, GA, USA) reported that 17β-estradiol, but not the biologically inert 17α- isoform, relaxes the smooth muscle from porcine coronary arteries, confirming that this action is not caused by some non-specific steroidal action, as had been previously suggested. So, what is the molecular basis for this action?

To answer this question, White tested if estrogen activates K^+ channels, because increasing K^+ efflux is one of the most efficient means of hyperpolarizing (and therefore relaxing) smooth muscle cells. Patch clamp recordings show that 17β-estradiol, even at concentrations as low as 1 nM, elicits a current through the cell membrane that can be inhibited by the K^+-channel blocker, IBTX. The electrophysiological properties of this estrogen-activated channel were found to be consistent with only one known K^+ channel – the Ca^{2+}-activated large conductance channel, or big conductance channel (BK_{Ca}). Similar recordings made

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on isolated coronary artery cells confirmed that estrogen also activated the BK_{Ca} in human smooth muscle.

The effects of estrogen on BK_{Ca} channels were found to be mediated by nitric oxide (NO); cells incubated with a fluorescent NO reporter glowed faintly in the absence of estrogen, but addition of the hormone caused a dramatic increase in fluorescence over tens of minutes, peaking after about one hour. The increase in NO appears to be mediated by the estrogen receptor because the antagonist ICI 182780 inhibits the response. White *et al*. found that one of the NO synthases (NOS) is present in smooth muscle cells and could be a potential target for estrogen.

What is the connection between estrogen, NO and the BK_{Ca} channel? NO can activate guanylyl cyclase, the enzyme responsible for the formation of cGMP, a known activator of protein kinase G (PKG). PKG is itself highly expressed in smooth muscle cells and plays an important role in the regulation of their activity. So one potential signal-transduction pathway would be: estrogen→NO→cGMP→PKG→BK_{Ca}, a pathway that is supported by the following experimental evidence: first, in addition to inducing NO and activating the BK_{Ca} channel, estrogen stimulates accumulation of cGMP; second, the NOS inhibitor LNMMA abolishes both the NO induction and the channel activation; third, addition of exogenous cGMP overcomes the effects of LNMMA; and fourth, the PKG inhibitor KT2583 inactivates the channel.

What remains to be determined is whether this pathway is activated by transcription; however, the rapidity of the response suggests that it is not.

Molecular bases for sex differences in ventricular arrhythmias

Torsades de Pointes (TdP), literally a twisting of the points, is a potentially lethal heart condition described after its characteristic electrocardiogram – the ECG peaks are compressed together and twisted about the baseline. TdP can easily degenerate into ventricular fibrillation, leading to sudden death. Although it occurs naturally in a small number of individuals, more often than not, it is induced by drugs. For this reason TdP can be a major impediment to the development of new medicines of all types.

In the early 1990s it was discovered that women are almost twice as susceptible to drug-induced TdP than are men [3]. Two key observations indicate that the interaction between steroid hormones and cardiac ion channels could explain the sex difference in TdP susceptibility. First, it was noted that patients with long QT intervals – representing the duration of ventricular depolarization and repolarization on an ECG – are more susceptible to TdP and that these 'long QT syndrome patients' harbor genetic polymorphisms that compromise ion-channel function [4]. Second, it was shown that prepubertal boys and girls have statistically identical QT intervals, but as boys mature, their QT gets relatively shorter [5]. This is significant because a long QT interval can increase the chances that a second ventricular action potential is initiated before the previous one has finished, potentially leading to TdP arrhythmias.

To investigate this phenomenon experimentally, Steven Ebert (Georgetown University Medical Center, Washington, DC, USA) and his colleagues examined the QT intervals of isolated, perfused rabbit hearts that were electrically paced [6]. Female rabbit hearts were found to have longer QT intervals than did males, a difference that increases as the pace of the heart is slowed. Ovariectomy had little effect on the baseline female interval, but orchiectomy causes the male interval to lengthen, equaling the female one. This latter effect can be reversed by dihydrotestosterone (DHT). The logical conclusion that androgens might be beneficial, at least for males, is born out by studies on prostate cancer patients who elect to be castrated – these men have lengthened QT intervals, an effect that could be reversed by testosterone [7].

At the molecular level, the male–female QT difference could be explained by the properties of their respective K^+ channels. The inwardly rectifying K^+ channels from male rabbit myocytes, and the delayed rectifier K^+ channel, a target for many of the TdP-inducing drugs, yielded higher current densities than did their female counterparts and might help to repolarize the cells faster, thus leading to shorter QT intervals.

Other presentations highlighted sex differences in heart failure and hypertrophic cardiomyopathy, risk factors for cardiovascular disease, risks and benefits of hormone-replacement therapy, and the historical perspective of women in cardiovasuclar clinical trials. A summary of the entire meeting will be available from the Society for Women's Health Research at http://www.womens-health.org.

Key conference outcomes

- Steroids act through both genomic and nongenomic pathways.
- The effects of estrogens on the vasculature is not confined to the endothelium or to activation of nitric oxide.
- Steroids can affect ion current densities, which could explain female–male differences in susceptibility to cardiac arrhythmias.

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