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Review

Conceptual frameworks and mouse models for studying sex differences in physiology and disease: Why compensation changes the game

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ABSTRACT

A sophisticated mechanistic understanding of physiology and disease requires knowledge of how sex-biasing factors cause sex differences in phenotype. In therian mammals, all sex differences are downstream of the unequal effects of XX vs. XY sex chromosomes. Three major categories of sex-biasing factors are activational and organizational effects of gonadal hormones, and sex chromosome effects operating outside of the gonads. These three types of effects can be discriminated from each other with established experimental designs and animal models. Two important mouse models, which allow conclusions regarding the sex-biasing effects of sex chromosome complement, interacting with gonadal hormone effects, are the Four Core Genotypes model and the XY* model. Chromosome Y consomic strains give information about the role of the Y chromosome. An important recent change in sexual differentiation theory is the increasing realization that sex-biasing factors can counteract the effects of each other, reducing rather than producing sex differences in phenotype. This change in viewpoint rationalizes a change in experimental strategies for dissecting sex chromosome effects. The overall goal is to understand the sexome, defined as the sum of effects of sex-biasing factors on gene systems and networks.

Contents

Abbreviations: A–O–S, an experimental strategy for discovering specific categories of sex-biasing factors that cause disease, explained in the text; FCG, Four Core Genotypes mouse model; *Sry*, sex-determining region of the Y chromosome, a protein-coding gene causing differentiation of testes; XXF, gonadal female mice with XX sex chromosome complement; XYF, gonadal female mice with XY sex chromosome complement; XXM, gonadal male mice with XX sex chromosome complement; XYM, gonadal male mice with XY sex chromosome complement; *Xist*, X-inactive specific transcript, an RNA gene on the X chromosome expressed from the inactive X chromosome, which initiates X inactivation.

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Introduction

Increasing interest in sex differences in physiology and disease

Within the scientific and medical community, there is increasing realization that many disease mechanisms differ in the two sexes. One sex may be affected by a specific disease much more than the other (Karastergiou et al., 2012; Miller et al., 2011; Sandberg and Ji, 2012; Voskuhl, 2011), so that even a basic appreciation of disease mechanisms requires understanding how sex-biased factors influence the disease. The majority of basic science research is performed on males (Beery and Zucker, 2011), but conclusions drawn from those studies may not apply fully to females. Importantly, if one sex is protected from disease, then study of the sex-biased protective mechanisms could lead to discovery of regulatory mechanisms that could be targeted for novel therapies. These ideas have contributed to an increase in the number and percentage of publications on sex differences in the last 15–20 years (Fig. 1).

Although the study of both sexes individually is important to establish the broad validity of scientific principles or therapeutic approaches, direct comparison of the sexes offers even greater advantages. Resolving the reasons for sex differences in disease leads to the discovery of unexpected regulatory mechanisms, and suggests new levels of protection that can be achieved in both sexes. Without reference to the other sex, it is sometimes not clear what aspects of physiology can be regulated by factors that occur already in nature. For example, the discovery that males die at greater rates at most ages across the lifespan, frames questions about what sex-specific social and biological factors are responsible for this sex difference, and whether these factors can be altered to increase lifespan of both sexes.

Fundamentally, we are asking where sex differences come from. Both phylogenetic and ontogenetic viewpoints are helpful in answering that question. Here, we discuss evolutionary reasons why sex-biasing factors might often be in opposition to each other, and review types of ontogenetic factors that can be discriminated by specific experimental designs.

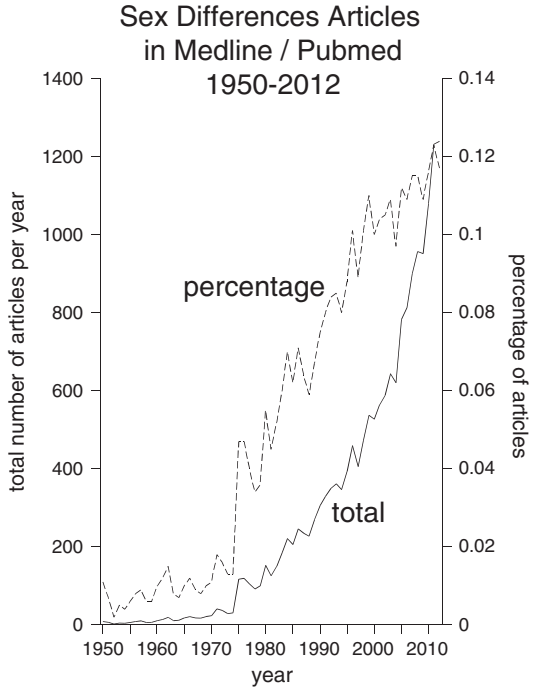

Fig. 1. Pubmed publications on sex differences. A search of Pubmed shows the increasing number of publications on sex differences since 1950. The search was for articles using the phrases "sex difference" or "gender difference" or "sexual dimorphism" or "sexually dimorphic". See http://dan.corlan.net/medline-trend.html.

The "big three" causes of sex differences in phenotype

Research between 1916 and 2010 gave rise to a relatively straightforward tripartite classification of categories of proximate (ontogenetic) causes of sex differences in phenotypes: (1) *activational effects* of gonadal steroid hormones, (2) *organizational effects* of gonadal steroid hormones, and (3) *sex chromosome effects* (Arnold, 2009b). These three classes are both conceptual and operational, because specific experimental outcomes define each class. Considering sex differences in adulthood, testicular and ovarian secretions act on many tissues to induce non-gonadal phenotypes to differ in the two sexes. These hormonal effects, predominantly of androgens, estrogens, and progestins, are reversible because they typically disappear in hours to weeks after removal of the gonads. Operationally, therefore, sex differences that are eliminated by adult gonadectomy are classified as activational effects. Some sex differences do not disappear after gonadectomy, but are caused by long-lasting, differentiating, or permanent changes caused by gonadal hormones acting at early stages of development (organizational effects of gonadal hormones, Phoenix et al., 1959). Examples include sexual differentiation of the external and internal genitals, and of specific sexual dimorphisms in the brain and behavior (Arnold and Gorski, 1984; Breedlove et al., 1999; Jost et al., 1973; McCarthy and Arnold, 2011). Classic sexual differentiation theory posits that testicular secretions, especially testosterone and Müllerian Inhibiting Hormone, act to cause masculine patterns of differentiation not found in females. Finally, some sex differences are not explained by either activational or organizational effects of gonadal hormones, but by direct effects of sex chromosome genes acting outside of the gonads. Both X and Y genes, which are differentially present in each XX vs. XY cell, act in a sex-specific or sex-biased manner to cause sex differences in non-gonadal phenotypes (Arnold, 2004, 2009b).

This conceptual framework gives rise to a relatively standard strategy (called the A–O–S approach here: activational then organizational then sex chromosome) for discovering sex-biased factors that cause sex difference in tissue function or protection from disease (Becker et al., 2005). In an animal model, the first experiment is often to remove the gonads, preferably of both sexes, to determine whether the sex difference depends on the secretion of gonadal hormones in adulthood (for simplicity we are considering adult phenotypes, and use mice as an example). Adult gonadectomy is the first choice, because the majority of sex differences appear to be caused by activational effects of gonadal hormones (e.g., Van Nas et al., 2009), although this may not always hold (Seney et al., 2013). If the sex difference is eliminated by adult gonadectomy, then the sex difference is classified as caused by activational effects of gonadal hormones, leading to further experiments to investigate which hormones are relevant, and their downstream mechanisms of action. By Occam's razor, eliminating the sex difference by adult gonadectomy means that there is no reason to invoke sex biasing factors other than activational effects. If the sex difference persists after gonadectomy, however, or is found in adult mice that have the same levels of hormones (for example, in female and male mice gonadectomized and treated with the same levels of sex steroid hormones in adulthood), then it is appropriate to test next for organizational effects. Organizational effects are discovered if females are permanently masculinized by exposure to androgens during an early development stage (in rodents just before or after birth), or if males are demasculinized or feminized when they are deprived of testosterone or androgen receptors at the same early stages of life (or later periods of organizational effects, Juraska et al., 2013; Schulz et al., 2009). If these manipulations of gonadal hormones do not explain the sex difference, then the remaining option is to consider sex chromosome effects, for example by comparing mice with different numbers of X or Y chromosome, under conditions in which the effects of gonadal hormones are similar across groups (Arnold, 2009a). Two relevant mouse models are discussed below.

The A–O–S experimental approach just outlined answers a variety of essential questions that are the first steps for finding the cellular and