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Review

Hormonal and Meta-Hormonal Determinants of Sexual Dimorphism

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A B S T R A C T

The role of hormones in the determination of sexual characteristics has been known for several decades. It has been shown, for example, that several products, including sex steroids, may influence the body development pattern, metabolic pathways, fat and muscle distribution and vocal cord anatomy, thus producing an overall outcome consistent with a masculine or feminine phenotypic pattern. These qualities are usually described as secondary sexual traits, so as to be distinguished from primary sex traits, usually referring to the gonads and external genitalia. However, it must be noted that hormonal regulation may not explain the full range of the sexual phenotype, since the central nervous system retains a significant role in the establishment of sexual identity, thus giving rise to a higher sex determination stage exclusively described in humans, namely behavioral or psychological sex. Recently, it has been suggested that differences among the sexes are not limited to brain function but they may also refer to anatomical differences and different biochemical profiles, including a distinct pattern of AR and ER distribution. This new aspect of sexual dimorphism suggests a whole system of meta-hormonal regulation, recently described as the sexual brain model. The role of local androgen and/or estrogen concentrations in the initial establishment of brain sexual dimorphism is still under evaluation, since the first results are relatively inconclusive and no direct cause and effect relationship has been proven so far. On the other hand, sex hormones have recently been found to participate in processes well beyond their initially suggested spectrum of action. For instance, ER interacts with EGFR in a number of ways, affecting development of a number of epithelial structures. Estrogen receptors have also been detected in a number of non-classic targets of steroids, such as the brain and the lungs. This observation may imply that sexual dimorphism goes a lot deeper than previously estimated, affecting virtually every organic system, suggesting, in essence, the existence of two different functional models for the whole human body, formulated and conserved throughout the evolutionary progress.

Key words: *sex steroids, steroid receptors, sexual brain, EGF receptor, sex traits*

Introduction

The determination of sex is a complicated, multistage process involving a number of genomic and biochemical determinants. Although largely dependent on inherent qualities, i.e. DNA content, environmental parameters may also influence the final outcome in terms of phenotypic variation. Since a) major environmental phenomena affecting local temperature at the hatching site have been shown to directly determine sex differentiation in a number of species, mostly reptiles, leading to the term »environmental-temperature sensitive sex determination« and b) a number of external interventions in the human fetus at the time of gestation, including drug administration and exposure to radiation, smoke or alcohol, have been associated with various distortions of sexual dimorphism observed in postnatal life, it is more than evident that sex is only the final product of a network of interactions occurring at any time throughout a large period of time, spanning from gestation to the acquisition of sexual maturity/puberty[1].

In terms of terminology, researchers in the field of developmental biology, experimental embryology and reproductive endocrinology use different terms to refer to the various phenomena associated with the acquisition of sexual traits in an individual. The term sex determination usually refers to phenomena *in utero*, based on the

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genomic determinants of sex and leading to the formation of the gonads and sex-specific genetic tracts. On the other hand, the term sex differentiation is larger, referring to the gradual appearance of all characteristics that are considered consistent with male or female sex, regardless of the developmental stage at which they are initially observed, i.e. during gestation, in perinatal life or even later in puberty. Finally, the term sexual dimorphism has attracted much attention recently, owing to the observation that sex-specific differences in macroscopic and microscopic anatomy and physiology exist in all organic systems, thus suggesting a completely distinct functional model for each sex, expanding in all reactions of the body, rather than simply the few qualities, previously considered exclusively as the sex traits[2,3].

Sex hormone-mediated reactions have been the focus of attention for many decades. Recently, the detection of hormone receptors in different organs than those traditionally viewed as the target tissues has opened the question for the possible presence of further regulatory actions also related to sexual identity, thus formulating the hypothesis of a meta-hormonal level in sex differentiation[4–6].

Regulatory Stages of Sex Differentiation

Since the time of Professor Alfred Jost's innovative work in gonadal development, sex is no longer considered as the result of a single genetic effect, but rather as a phenotypic variation regulated via a long, complicated process. Although the latter appears to be a continuum, where every previous step preconditions the available options for the next regulatory phenomenon, didactic purposes justify an attempt to facilitate relevant scientific discussion and research, achieved via the recognition of a multistage regulatory pattern. Each stage is distinct in the sense that its determinants do not seem to regulate events at a later stage (or at least not as a major contributor anymore) and the outcome is observed at a different morphological or functional level each time[2,3].

Initial sexual dimorphism is formed at a chromosomal level, since normal males will have a 46, XY and females a 46, XX DNA content. The role of X and Y as determinants of sex fate has long been recognized, leading to the distinction between autosomes (all the other chromosomes existing in homologous pairs in the nuclei of diploid cells) and gonosomes or sex chromosomes, i.e. the X and Y. This difference at chromosomal level is already installed at zygote formation, based on the content of the male contributing gamete, i.e. the spermatozoon, whereas the oocyte always bears an X sex chromosome.

The presence of a Y chromosome signifies development towards the male path. This may first be observed at a gonadal level. In the human fetus, the gonad is formed as a bipotential primordium or gonadal anlagen, dependent on the expression of major developmental genes also affecting adrenal and kidney development. This is followed by the differentiation towards an ovary or testis, i.e. gonadal dimorphism. The major determinant in this process is the *SRY* gene of the Y chromosome in males.

Downstream actions of SRY affect the subsequent development of most sex-specific structures. For instance, the *SOX9* gene family regulates local epithelial organization (spermatic cords, Seroli cells) while the *FtzF1* gene is responsible for the differentiation of steroid hormone producing cell populations (adrenals and gonads) and the *AMH* gene regulates the fate of the Muller duct products. A similar process occurs in females, with the absence of SRY action and the regulatory cooperation between *DAX1*, *FtzF1*/*SF1*, *SOX9* and *AMH*. It is interesting to note that, excluding *SRY* itself, no other major sex-related regulatory gene is actually unique to the male or female sex, the difference thus found at the time and level of gene expression[7].

Hormonal Actions in Sex Differentiation: The »Classics«

Sex-related hormones belong to two different categories. One of them includes a protein produced by the epithelial cells of the gonads, the Antimullerian Hormone or AMH (formerly known as the Muller duct inhibiting substance, or MIS). This molecule has long been studied in males as a determinant of internal genitalia. Indeed, it has been proven that AMH is produced by Sertoli cells following their differentiation in the testis via *SOX9* function. AMH may directly interact with the SRY and Sox family proteins due to the presence of a homeobox-related region. However, its receptors have also been recognized and thoroughly studied in terms of mutations that may be related to the various syndromes of resistance to AMH action. All these syndromes share the major common clinical manifestation of persistence of Muller duct products in the adult male. Indeed, the hormone is named after its most prominent quality, namely, the inhibition of further development of the Muller duct in male fetuses (i.e. 46, XY fetuses with an activated *SRY-SOX-AMH* pathway). Interestingly, AMH is also located in the female genitourinary system and experimental data suggest that its presence is necessary for normal ovarian development. The exact nature of AMH-SOX--DAX protein interactions and their role in the evolution and maintenance of the female sexual phenotype remains a debatable issue[8].

Other sex-associated hormones belong to the biochemical category of steroids. They are molecules of lipophilic nature, showing limited solubility in water--based solutions such as blood or the fluids of the extracellular space. Their circulation is based on the concomitant presence of binding agents-ligands, which form complexes with increased solubility in hydrophilic environment. For sex hormones, the major carrier protein is the sex hormone binding globulin (SHBG), a product particularly sensitive to the metabolic and hormonal equilibrium of the organism, with major differentiations of its value in extreme physiological states such as obesity and pregnancy.

The signaling pathway by which sex steroids, i.e. androgens, progesterone and estrogens mostly mediate their actions is based on intracellular receptors, named androgen, progesterone and estrogen receptors, or AR, PR and ER, respectively. It should be noted that the names suggest only the major sensitivity of each receptor and not a complete specialization. In other words, sex steroids and their receptors show some level of structural homology that results in the phenomenon of cross reaction, by which it is possible to observe actions of a sex steroid circulating in high concentrations (e.g. androgens) mediated through the receptors of a seemingly non-compatible receptor (e.g. ER or PR). In the case of androgens, it is also useful to note that a considerable number of their actions is also dependent upon their prior conversion to more active hormonal products. For testosterone, this is achieved via the activation of 5a reductase, leading to the formation of dehydrotestosterone or DHT, the most important contributor in the process of genital tract masculinization, including phenomena such as prostate development (morphological and functional maturity)[4,7,9].

It is also true that estrogens are also products of androgen metabolism, through the action of the P450 aromatase enzyme complex. This reaction is crucial for normal ovarian development and it requires the combined action of both of the gonad's major cell populations, i.e. the thecal cells, responsible for initial androgen production and the granulosa cells, sites of local aromatization and estrogen production. Modern data has shown that aromatization is maintained to some extend throughout female life and its limitation may result in masculinization phenomena even after menopause. In this case, the major site of aromatization is shifted from the aging ovary to the peripheral tissues. In particular, the adipose tissue has been shown to have extensive synthetic properties, some of which refer to tissue-specific hormones and paracrine messengers (adipokines) and others being homologous to hormones and cytokins also produced in other systems (e.g. resistin, TGFb, TNFa). In the case of sex steroids, the adipose tissue is considered a major site of production following menopause and ovarian follicle atresia. Aromatase activity is particularly increased locally, thus maintaing an adequate estrogen flow for the normal maintanance of a female phenotype (constant androgen/estrogen ratio). A recent finding of great potential implications refers to the possible existence of aromatization and sex steroid production sites in the central nervous system. If this is indeed so, then research will be faced with the challenge of the study of intracranial sex steroid metabolism and its potential in the establishment of sexual dimorphism throughout life[8].

Regardless of the target tissue, binding of a sex steroid to its receptor results in the formation of a complex, which is then shifted to the nucleus. Genomic actions of sex steroids are mediated via specific compatible regions in the DNA sequence named »hormone response elements or HREs« (e.g. androgen response element).

New Aspects in Sex Steroid Physiology

Until recently, genomic action was the main concept used to explain all sex steroid-mediated actions. Following the steroid: receptor complex binding to its special HRE, subsequent DNA manipulations was considered responsible for all sex steroid-associated actions affecting sex differentiation. These include formation of external genitalia, adipose tissue distribution, hair, bone and muscle development and distribution, testis descend in males and breast maturation in females[10–15].

Interestingly, recent studies revealed possible alternatives to this regulatory pathway. It has been suggested that estrogen actions are not necessarily mediated via the nucleus but also in an extragenomic sequence of events, which might involve a membrane receptor.

In addition, it has been shown that estrogens and epidermal growth factor (EGF) may share common messengers in their signaling pathway. This observation has been particularly useful to explain cases of cross-reaction in breast cancer patients treated with selective estrogen receptor modulators (SERMs). In this case, the expected/desired outcome would be a decrease in tissue growth, since estrogens, as previously mentioned, are a major contributor in breast development, bearing qualities similar to those expected of a growth factor. However, most patients show a gradual resistance to SERMs. It seems that this may partially be due to an increase in EGF and EGF receptor (EGFR) expression in tumor cells. The latter process implicates several molecules beyond the initial tyrosine kinase, leading to a different equilibrium in intracellular reactions that may actually eventually promote instead of inhibit tumor progress, in the presence of SERMs[16].

Moreover, significant conclusions can be reached via studies of androgen and estrogen receptor distributions in men and women of various age groups. Localization data have proven a much vaster presence and expression of AR and ER than previously assumed. Among the newly detected target-tissues for sex steroid action, one may distinguish, the lung, the liver and the central and peripheral nervous system (CNS and PNS, respectively), to name but a few characteristic examples[17].

In the case of the lung, ER type a presence has been described several years ago. In 1996, ER type 2 were also found in the tissue. Despite suggestions for a possible secondary effect and thus, limited importance for the organ's integrity, relevant research bloomed. More recent experimental data in this field from Erb knockout mice showed that absence of ER is directly responsible for abnormal lung development, with a large-scale distortion of alveolar micro-architecture. An analysis of local gene expression suggests that this phenomenon must be attributed to a generalized deregulation of several genes' action normally activated downstream the estrogen-ER-HRE pathway. These observations may also be useful in future advances in lung cancer treatment. In this case, EGF has already been studied as a potential target for oncological targeted treatment and some products are already avail-

able or under evaluation as therapeutic options. However, these recent findings for ER expression in the normal lung raise discussion for possible implications, such as the ability to attempt a combined chemotherapy strategy[18].

In the case of the liver, macroscopic and microscopic differences between the sexes have been known for decades. Recently, researchers attempted to explain these differences studying regional differences in the expression of hormones and growth factors. It seems that differences may include several target genes and receptors, including somatostatin (SS), a major inhibitory regulator already detected in the hypothalamus, the pancreas and the gastrointestinal tract. A local antagonist of SS action is the hepatocyte growth factor or HGF. It might also be possible to include other products in this multifactorial analysis of sex-associated differences in liver development. These might include insulin-like growth factors (IGFs) and ER expression. The exact manner by which all these different signals cooperate together and with external metabolic factors to achieve the dynamic equilibrium of liver function remains a challenge for current research in the field of Endocrinology[19].

Dimorphism in brain function has been a controversy for Neurosciences since the 19th century. Many observations have attempted to characterize specific modes of behavior as typically masculine or feminine, respectively, however, most of them were limited in few patient observations, largely biased by personal beliefs and mostly of subjective nature, thus considered inadequate for a modern scientific discussion. However, recent series have filled this gap, offering trustworthy data that prove statistically significant differences among the sexes in a behavioral level. These differences in cognitive functions are numerous, referring to arithmetic, positional and lexical skills, oral or written expression, synthetic and analytic procedures, determination and positioning in time and space and general state of memory. Overall assessment of this dimorphism in brain function leads to the conclusion of a general sex-specific organization of its function, described by the term »the sexual brain«.

Sexual Dimorphism in the Neural System: »Behavioral Sex« Revisited

Until recently, reproductive biology recognized a behavioral level in sex determination as a meta-hormonal regulatory phenomenon. By definition, scientists referred to the way in which each and every individual chooses to determine his/her sex and accepted the fact that it constitutes a final, distinct level in sexual dimorphism. This process was attributed to psychological, rather than organic factors. Recent data challenge this belief, claiming that at least some of the differences observed may also be explained via sex hormone activity in the CNS[20].

Furthermore, hormonal differences may constitute some kind of predisposition to homo- or heterosexuality, although there is no data suggesting that this is ultimately predetermined and emphasis is still placed on environmental influences and personal choice. Among the different parts of the CNS showing anatomical and functional dimorphism the most characteristic ones are the hypothalamus, the amygdala and the bulbocavernosus nucleus.

In terms of AR and ER expression in the CNS, immunohistochemical and *in situ* hybridization techniques have detected their presence in different parts of the system, depending on both the sex of the individual and the age at the time of study. Perinatally, estrogen receptors have been detected in the preoptic area and the anteroventral periventricular nuclei of the hypothalamus. On the other hand, throughout life, the septal AVP is positive for both androgen and estrogen receptors. The same is true in animal models for the nucleus robustus archistriatum. Contrary to these examples, the spinal bulbocavernosus nucleus appears to exclusively include androgen receptors. In adults, the posterodorsal medial amygdala is another sex-hormone sensitive region, with the expression of both ARs and ERs.

The role of progesterone in CNS has also been studied. It seems that PRs are not expressed locally. Nevertheless, progesterone may still mediate reactions in the area. This is attributed to its capacity to bind directly to a subunit of GABAnergic neurons, which are amply distributed in the CNS. Thus, progesterone may actually be a major player in sexual dimorphism, affecting both peripheral organ function and maturation in the female (e.g. breast, endometrium) and central regulation of dimorphic behavior in both sexes[21].

The sex brain model becomes even more attractive by observations of local steroid metabolism in the CNS. Indeed, aromatization has been detected within the brain. Several masculinizing actions of androgens in the brain seem to result from aromatization of testosterone to estrogen, further enhancing the theory of steroid hormone cross-reactions as the basis of sex-specific variation in humans. An interesting biochemical observation refers to the interaction between estradiol and a-fetoprotein (AFP). The two molecules form a complex, which, in the female, may inhibit estrogen access to the CNS and brain masculinization. To which extend such a process may be important for the pathophysiology of syndromes with increased AFP production remains a question. In any case, such an inhibitory effect would limit estrogen activity in the female considerably, allowing only reactions that mediated via local aromatization within the CNS. Ectopic androgen action would also be possible, through the previously presented cross-reaction model for steroid hormones.

It has been suggested that the maintenance of an optimal androgen: estrogen and AR: ER activity ratio in the brain is crucial for micro-environmental stability and prolonged neuronal survival. Distortions in this equilibrium may promote local cytotoxic effects, leading to apoptotic phenomena. Such a procedure may also justify differences between the gross anatomy of the CNS in the two sexes, as well as between hetero- and homosexual individuals. Although limited in few, very specific nuclei and regions of the brain, these differences must be an

adaptive developmental phenomenon, since they are not related to any obvious functional disadvantage and they are not extended to any other parts of the CNS or the rest of the body. Among the theories suggested for these cases of anatomical dimorphism, it is interesting to note the suggestion for a possible history of *in utero* exposure to high concentrations of sex steroids[21].

Naturally, all homosexual individuals do not have a history of exposure to androgens or estrogens, respectively. Additionally, differences in behavior and practice between the sexes, as well as between homosexual and heterosexual individuals extend beyond those affected by the few brain regions discussed above.

In effect, this implies that hormonal regulation of sex behavior may not be the only factor responsible for all the differences observed[22]. Further research will be necessary to clarify the exact role of hormones in sex determination and differentiation. Understanding the complexity of the regulatory patterns involved in this process is a necessary tool for the explanation of various pathophysiological phenomena affecting sex physiology as part of their clinical manifestations. This goal promises to allow the development of new therapeutic agents for sex-related diseases and the targeted, aetiological treatment of all affected individuals in the near future.

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HORMONSKE I METAHORMONSKE DETERMINANTE SPOLNOG DIMORFIZMA

SAŽETAK

Uloga hormona u utvrđivanju spolnih karakteristika poznata je već nekoliko desetljeća. Dokazano je, na primjer, da pojedini produkti, uključujući spolne steroide, mogu utjecati na obrazac tjelesnog razvoja, metaboličke putove, distribuciju mišićnog i masnog tkiva te anatomiju glasnica, stvarajući sveobuhvatni ishod u skladu s muškim ili ženskim fenotipskim obrascem. Takve odlike obično se opisuju kao sekundarne spolne osobine, a kako bi se razlikovale od primarnih spolnih osobina, obično se odnose na gonade i vanjske genitalije. No, mora se spomenuti da hormonska regulacija ne može objasniti cjelokupni opseg spolnog fenotipa jer središnji živčani sustav zadržava značajnu ulogu u uspostavljanju spolnog identiteta, dajući povoda višem stupnju spolne determinacije, posebno opisane kod ljudi kao bihevioralni ili psihološki spol. U novije vrijeme predloženo je da razlike među spolovima nisu ograničene na moždanu funkciju, nego se mogu odnositi i na anatomske razlike te biokemijske profile, uključujući različite obrasce AR i ER distribucije. To novo stajalište o spolnom dimorfizmu predlaže čitav sustav metahormonske regulacije, u novije vrijeme opisane kao model spolnog mozga (engl. the sexual brain model). Uloga koncentracije lokalnog androgena i/ili estrogena u početnoj uspostavi spolnog dimorfizma još se proučava jer su prvi rezultati relativno neuvjerljivi te do danas nije dokazan direktna poveznica. S druge strane, nađeno je da spolni hormoni sudjeluju u procesu koji je daleko od njihovog početno predloženog spektra djelovanja. Na primjer, ER međusobno djeluje s EGFR-om na puno načina, utječući na razvoj određenoga broja epitelnih struktura. Estrogenski receptori također su detektirani u organima kao što su mozak i pluća. Ovo istraživanje može implicirati na to da spolni dimorfizam ide još dublje nego što je prethodno utvrđeno, utječući praktično na sve organske sustave, predlažući, u osnovi, postojanje dvaju različitih funkcionalnih modela za čitavo ljudsko tijelo, izraženih i očuvanih kroz proces evolucije.