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Review

Sex Steroids: Beyond Conventional Dimorphism

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A B S T R A C T

Sexual dimorphism is a characteristic of a large number of species, ranging from lower invertebrates to mammals and, last but not least, humans. Recognition of the various factors regulating sexual dimorphism initial establishment (i.e. sex determination and differentiation) and subsequent life-long adaptation to distinct functional and behavioural patterns has remained a hot topic for several decades. As our understanding of the various molecular pathways involved in this process increases, the significant role of sex steroids becomes more evident. At the same time, the recognition of new sites of steroid production (e.g. parts of the brain) and aromatization, as well as new target cells (owing to the proposed presence of additional receptors to those classically considered as primary steroid receptors) has lead to the need to revisit their spectrum of actions within a novel, multifactorial context. Thus, anthropology and medicine are presented with the challenge to unravel a major mystery, i.e. that of sexual orientation and differentiation and its potential contribution in human evolution and civilization development, taking advantage of the high-tech research tools provided by modern biotechnology. This short review summarizes the basic principles of sex determination and sex steroid function as they have been classically described in the literature and then proceeds to present examples of how modern research methods have started to offer a new insight on the more subtle details of this process, stressing that it is extending to virtually every single part and system of the body.

***Key words**: androgens, aromatization, dimorphism, estrogens, sex determination, sex differentiation*

Introduction

Sex, as defined in biology, is a structured model of functional adaptation that is characterized by unique reproductive qualities. In this essence, a species (i.e. the total population of a specific life form that may mate and produce fertile offspring) is subdivided in a number of sexual subgroups (most commonly two in vertebrates), which differ in their reproductive system anatomy and physiology. Only very primitive lower organisms lack this evolutionary characteristic, thus being forced to reproduce via a simple dichotomy of the initial / paternal individual. For all other life forms, sex has been a successful way to achieve biological variety and pluralism within the species' population, a critical advantage in the struggle of survival via the physical selection process. Within the context of Anthropology, sex has been in the centre of cultural and physical development, associated with major historical breakthroughs and significant socioeconomical achievements. Thus, it is not an exaggeration to consider sex a central force in human activity, participating in and/or affecting virtually every evolutionary advance.

Although sex is a very largely distributed behavioral system in the biosphere, it doesn't seem to bear the same significance throughout the various species. In some cases, it only refers to the specific organization of the genome and the gonads, while relevantly few differences can be observed in the phenotype (e.g. in invertebrate nematodes, such as *Caenorhabditis elegans*). On the other hand, there are animals whose sexual dimorphism is clearer and extends to non-strictly reproductive aspects. This phenomenon may be associated with secondary characteristics necessary to attract a partner and, thus, achieve reproductive success (and, ultimately, species survival). On the other hand, these extensions of sexual dimorphism may also include functional adaptations that have been naturally selected, due to their association with desired qualities for the socially approved role of each sex. In the case of birds, for instance, sex-specific differences are observed in feather layout, length, thickness and color as well as voice production, frequency and intensity. According to ornithology, these are not random qualities, but rather a result of natural selection as well,

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since they are thought to provide a reproductive advantage, by both attracting the attention of members of the opposite sex in the pre-mating period of the year and providing protection from natural enemies. The latter may be accomplished via natural camouflage or alarming (i.e. sound warning produced by one member of the species to be heard by nearby members of the flock in case a predator is detected).

As far as humans are concerned, sexual dimorphism extends to virtually every function of the body and every organ. The inter-sexual differences start from a molecular level (enzyme/metabolic pathway variations) and proceed to higher hierarchical levels, causing more massive effects. The latter may be easily visible (e.g. fat and body hair distribution) or macroscopically less evident, but still highly influential (e.g. liver size and metabolic capacity, brain nuclei size and interconnection). At least part of this vast heterogeneity is directly or indirectly attributed to sex steroid action, making them the key (but definitely not the only) players in sexual dimorphism[1–2].

Sex Steroid Production and Action

Hormones are by definition molecules of various size and biochemical structure that are produced by extremely specialized epithelial (and neuroepithelial – neuroendocrine) cells in the body and act via the blood circulation in equally specialized target cells, which are recognized by the fact that they contain receptors to which the hormone may bind. The formation of the hormone-receptor complex follows the general principles of the external messenger-ligand model for signal transduction at a cellular level and is followed by a cascade of events at cytoplasmic and/or nuclear/genomic level, which produce the various effects associated with the activity of each hormone.

By biochemical categorization, hormones are placed in three distinct major groups: 1) peptide / protein hydrophilic hormones, produced by protein-secreting epithelial cells and acting via receptors located on the cellular membrane of the target cells, 2) endogenous amines (e.g. thyroid hormones, serotonin, melatonin) and 3) steroid hormones. The latter are clearly hydrophobic substances that cannot remain soluble in the plasma and are thus transferred only after being bound to special carrier proteins (e.g. sex hormone binding globulin – SHBG, corticosteroid binding globulin – CBG and albumin). Although nomenclature has been chosen to show the primary action of every steroid, today it is known that all members of the group influence development, metabolic and reproductive functions alike, the difference only being the relevant sensitivity of each molecule to the various cell targets. For instance, cortisol is the main representative of the glucocorticoids, i.e. steroids influencing glucose equilibrium and metabolic balance. However, androgens and estrogens also have an established role in glycemic control. Similarly, aldosterone is the most typical member of the alatocorticoid group. However, hypertension is also common in Cushing's syndrome (the clinical effect of retained long-term hypercortisolemia) and part of the androgen-associated effects of the metabolic syndrome in elderly males. Finally, androgens are named after their supposed role as creators of the male sex, as opposed to estrogens, the cause of female reproductive behavior. This simplistic approach is no longer considered applicable, since both kinds of hormones are produced in both sexes and act simultaneously via androgen and estrogen receptors, as well as additional receptors still under evaluation. The latter are the centre of attention in modern Reproductive Endocrinology, since they seem to explain numerous steroid actions that appear shortly after the hormone is localized in the specific target-tissue, making it unlikely to attribute such phenomena to genomic effects induced via the classic nuclear receptor pathways. To complete an already highly-challenging pattern, one must also mention that the other, »non-sex« steroids are now also known to contribute to the reproductive phenotype (e.g. hirsutism observed in Cushing's syndrome)[3].

The location of steroid hormone production has also been a hot topic in the international literature lately. Although it has been known for centuries that androgens in the male are primarily produced in the testis (hence the name testosterone, i.e. steroid of the testis) and estrogens in the ovary (causing seasonal sexual desire or »estros« in females), it soon became evident that this may not necessarily be an organ-exclusive effect. The first challenge in this theorem came when it became clear that androgens and estrogens are in fact present in both sexes and it is in fact their ratio and not their unique expression that characterizes (rather than determines) each sex. Moreover, subsequent research has shown that steroid production is possible in several extra-gonadal sites. For instance, a number of research groups, including that of the authors, have shown that the result of surgical and/or pharmaceutical castration in male rats is a considerable, but not complete reduction in androgen concentration. This phenomenon is partially explained by the continued production of androgens (although not testosterone itself) by the adrenal glands. However, it is interesting that while some effects of androgen depletion are clearly visible (e.g. prostate gland massive apoptosis) other masculine-associated features (e.g. sexual conduct-libido) may be less affected. This may be due to the presence of randomly distributed hormone-secreting cells in all the body. The initial discovery of such cells in the gastrointestinal tract was viewed as a major surprise (since it was the first clear challenge to the principle that hormones should always be associated with a specific glandular source that forms a distinct anatomical structure), but soon this was followed by similar findings in the respiratory system and the skin (neuroendocrine cells, products of the neural crest), leading to the »diffuse endocrine system« model. According to the latter, hormones may be produced by specialized cells present either in clusters/glands or in random assortments in the body.

At a molecular level, it is now possible to detect even single-standing hormone-secreting cells anywhere in the body, using specific markers against the mRNA or protein of the relevant enzymes or their stimuli (immunohistochemistry or in situ hybridization). In the case of sex steroids, scientists may attempt to locate the product of the primary developmental/embryological stimulus for the differentiation of steroid-producing cells, i.e. SF1 (steroidogenic factor 1) or some of the various enzymes of the steroidogenesis pathway[2,4]. It is reminded that some of the latter are tissue specific, such as aldosterone synthase, located only in a specific layer of the adrenal gland, while others are more diffuse, such as aromatase, the enzyme catalyzing androgen conversion to estrogens. By this process, it is now clearly established that the adipose tissue (and especially abdominal fat) is a huge source of aromatase activity and, thus, scientists may justify some »feminization« – type phenomena observed in overweight/obese males (especially in elderly individuals, who also exhibit decreased androgen production from the testis – late onset hypogonadism/LOH or andropause). Unfortunately, physical anthropology largely deals with skeletal material, depriving it of the ability to directly observe these effects of sex steroids in soft tissues and internal organs. However, social anthropology allows study of variations in living populations, making it a continuous natural experiment of the ways in which many biological determinants, including sex, may influence the structure and function of humans as both independent units and members of communities.

In recent years, the definition of steroid production sites has also been expanded to include two additional mechanisms, i.e. hormones produced by deregulated cancer cells (ectopic hormonal production – paraneoplastic endocrine syndromes) and medically prescribed hormonal supplementation[1,4]. A latest addition in steroid production is the relevantly recent discovery of cells retaining the potency to produce steroids within the nervous system, in which case their function is that of a neurotrophin and neuromediator, rather than that of a conventional hormone.

Sex Steroids and Brain Function: Are Thoughts and Feelings Dimorphic?

The question of sexual dimorphism at the central nervous system is central in the attempt to unveil the subtle details that form a mature man or woman as they are observed in the modern world. Naturally, part of the differences in behavior between men and women are more socially driven and less strictly biological-hormonal. This is exactly the reason why different populations may adopt extremely different habits in terms of daily activities, such as clothing, use of ornaments, kind of employment and contribution to household activities, to name but a few examples. Reproductive behavior is also variable, with every civilization placing a different threshold of tolerance/acceptable behavior, taking religious, philosophical and socioeconomical-demographic concerns into account. For instance, in some societies polygamy is commonplace whereas it is strictly forbidden (as a both legal and moral offence) in most developed countries. Furthermore, strict heterosexuality is considered the »straight«/most socially acceptable sexual orientation for the majority of the population in typical western communities, while in the developing world, several local societies accept bisexuality as normal, mainstream behavior (this approach is also strongly related to religious and philosophical influences of the Jewish and Christian faith, which has gradually shifted the social behavior standard, since, in fact, in ancient Greece, homosexual sexual intercourse was considered as an integral part of the young citizen's training to become a mature person, fully integrated in the city's social circle).

Nevertheless, it remains true that hormones must also play a significant role in the establishment of sexual identity, since large-scale interventions in their levels, either spontaneous/accidental or intended, result in various degrees of distortion in libido (sexual desire) and attraction towards the opposite or the same sex. In recognition of the above situation concerning brain sexual dimorphism, reproductive biology has accepted the presence of a discreet additional level of sex determination above chromosomal, gonadal and hormonal sex, referring to »behavioral sex« or »the sexual brain«. Examples of the various dimensions that the latter principle may take have been reported by the authors in previous contributions on sexual dimorphism[1,5]. The current scientific understanding of brain dimorphism is unfortunately too limited to allow the matching of the numerous sex-specific differences in behavior detected by psychiatrists and psychologists to the few structural differences observed so far[6,7]. However, these findings stress the need to reconsider the role of sex steroids within a much larger frame than the traditional one, especially when discussing proposed therapeutic applications (e.g. sex shifting, hormonal replacement therapy, testosterone replacement in late-onset hypogonadism). This skeptical tone is further demanded when examining the growing evidence for sex-specific (and sex-steroid-related) differences in various peripheral organs.

»Less Evident« Dimorphic Organs: Liver, Lung, Skin and Bone

As has been previously stated, the analysis of sex steroid receptor expression via modern techniques, including immunohistochemistry, reverse-transcript polymerase chain reaction (RT-PCR), *in situ* hybridization and microarrays, has revealed that such receptors may be present in almost every human tissue, thus extending the spectrum of steroid functions, compared to already well-established sexually dimorphic phenotype qualities. Moreover, the current understanding of molecular endocrinology has made it possible to comprehend that a one-to-one association between hormone and receptor is a huge over-simplification, far from the true situation observed *in vivo*. What is actually happening is that andro-

gens, estrogens and progesterone may act via androgen, estrogen receptor a and b and progesterone receptors, the difference only being found at the relative ease (affinity) with which every complex is formed and dissolved. Interestingly, some of the most important androgen actions (e.g. effects at the central and autonomous nervous system level) are not in fact associated with local increase in androgen-androgen receptor complexes and, therefore, should be attributed to cross reaction via other receptors, especially estrogen receptor type a. All the above cases are examples of nuclear or genomic actions of steroids and their common feature is a delayed response, since they result in the alteration of gene expression, which in turn takes some time to shift the protein production of the affected cells. However, at the same time, steroids also seem to cause faster adaptive effects which shouldn't reasonably be associated with nuclear receptors. The explanation available today for this phenomenon is that apart from the above process, a secondary pathway is also present, causing immediate responses at a cytoplasmic level. For instance, such a circuit has been suggested between estrogens and the epidermal growth factor receptor, causing various complications in breast cancer hormonal treatment. Therefore, there is growing evidence for the presence of the so-called non-genomic effects of sex steroids, the exact physiological significance of which remains to be determined. To make matters even more complicated, it has been proven that testosterone itself may act via the androgen receptor in some cases, via non-genomic pathways in others and via enzyme catabolism to estrogens or dihydrotestosterone (DHT) in the rest, resulting in a very complex network of interactions, which is still very hard to grasp, let alone alter via medical intervention, to the benefit of the community[1–2].

In the case of the liver, even early anatomists have noted the gross differences in mean organ weight among males and females. However, at a functional analysis level, few differences had been described, largely attributed to age and ethnic specialties rather than sex alone, as well as normal variation among individuals. In recent years, this simplistic belief has been questioned, as research has now proven that specific enzymes involved in liver activity are differentially expressed among the sexes (e.g. isoforms of the P450 cytochrome)[8,9]. Moreover, exposure to the growth hormone results in different biochemical reactions in the liver of males and females, an effect that only proves the long-standing hypothesis that human sexual dimorphism goes very deep, much more than meets the eye at first. The presence of sex steroid receptors in the liver is now well established, as well as that of transcriptional factors that could be some of the second messengers used to mediate the hormonal message. KRAB zinc finger repressors may be important in this liver regulatory circuit[10]. This example is useful to illustrate not only how diverse sex steroid actions can be, but also how interactions with other hormones, such as the growth hormone, may produce distinct effects compared to those seen when examining every hormone separately. The latter observation is very significant to differentiate endocrinological phenomena studied in controlled environments *in vitro* with more complicated problems faced when attempting to transfer this experience to the *in vivo* systems[11].

As far as the lungs are concerned, knowledge of endocrine activity is much more limited, both in terms of local production as well as the effect of circulating hormones on lung cells. Still, some data is available, leading to the understanding that the respiratory epithelium is also a target of sex steroid action, expressing both progesterone and estrogen receptors. Furthermore, the distribution pattern of the above is clearly distinct between males and females, as relevant research in rodents has revealed. Although there is insufficient data to determine the exact significance of this variation at a clinical level, it should be reasonable to assume that some inter-sexual differences, such as susceptibility to bronchial asthma and lung cancer and efficacy of pharmaceutical treatments for the respiratory system (bronchodilators, anti-smoking measures, chemotherapy), could be a result of basic differential sensitivity of the tissue to hormonal stimuli. If this is indeed so, then respiratory drug pharmacogenomics may have to look into this matter with greater attention[12].

Sexual dimorphism also involves visible organ structures, such as the skin and the underlying elements of the musculoskeletal system. This has been observed from ancient times and should therefore be of little interest at present. What is more interesting, though, is the justification of these evident differences within an evolutionary perspective. In other words, one should attempt to explain the reason why the particular skin colors have been originally placed in their positions around the world as well as the exact benefit from the differences in body composition and muscle volume among the sexes[1].

The second part of this dilemma appears simpler to explain, since the female has always been adapted in the best possible way to preserve fetal human life (as depicted by thicker abdominal fat, large milk-producing breasts and wider pelvic bones to allow displacement during labor) while the male has been selected to withstand harsh weather conditions and dominate over hostile animals and other individuals, in the struggle for shelter and food supply (social impact on sex differentiation). Even today, differences in particular bone sensitivity to estrogens can be seen not only macroscopically (e.g. in physical anthropology/anthropometric evaluation of various bones), but also microscopically (different distribution of estrogen receptor isoforms in bones/osteoblasts)[13]. Interestingly, differences also extend to vascular smooth muscle cells (prevalence of estrogen receptor b mRNA), a finding which may partially explain the different susceptibility of the two sexes to ischemic heart disease, stroke, peripheral vascular disease and several forms of systemic vasculitis, although of course immunological factors and lifestyle trends are of equal, if not higher significance to genetic predisposition in the etiology of these diseases[14].

As far as the first question is concerned, several theories have been proposed to explain skin color, including the natural and sexual selection hypothesis[15–17]. In one such example, skin color is associated with the relative risk of exposure to ultra violet sun radiation (UVR), which is true for black color in the tropics but fails to explain the rest of the world. From another point of view Darwin's sexual selection refers to an inherent tendency to prefer whiter individuals as sexual partners. It is possible that both of these factors act at the same time antagonistically. On the other hand, a role for sex hormones in this field may also be present, hiding some survival benefit that remains to be detected.

Conclusions

Sexual dimorphism is a highly delicate process, involving numerous genes and corresponding to the advancement of every species in the evolutionary process[18]. It is, in effect, yet another application of the general natural rule of economy/minimal energy consumption in biology, since few regulatory elements (in this case, sex steroids) are combined in different manners to contribute to the huge phenotype variation observed in the biosphere. The lesson for anthropology is, that sexual characteristics must be seen as both a determinant and a product of every civilization and nothing less. The message for medicine, on the other hand, is that steroid physiology is a huge chapter in the process of understanding human function and disease in detail[19,20] and, after decades of research, we are only just past the introduction.

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SPOLNI STEROIDI – ONKRAJ KONVENCIONALNIH DIMORFIZAMA

SAŽETAK

Spolni dimorfizam je obilježje velikog broja vrsta, od nižih beskralježnjaka do sisavaca pa na kraju i ljudi. Prepoznavanje različitih čimbenika koji reguliraju inicijalno uspostavljanje seksualnog dimorfizma (tj. spolno određivanje i diferencijacija) te naknadna dugoročna prilagodba na različite funkcionalne i bihevioralne obrasce već je nekoliko desetljeća jedno od gorućih pitanja. Kako se naše razumijevanje različitih molekularnih putova koji su uključeni u ovaj proces povećava tako značajna uloga spolnih steroida postaje sve očitija. U isto vrijeme, prepoznavanje novih mjesta proizvodnje steroida (npr. neki dijelovi mozga) i aromatizacije, kao i novih ciljnih stanica doveli su do potrebe za preispitivanjem spektra njihovog djelovanja unutar novog složenijeg konteksta. Antropologija i medicina suočeni su sa izazovom otkrića velike tajne, o seksualnoj orijentaciji i diferencijaciji te njihov mogući doprinos na ljudsku evoluciju i razvoj civilizacije, uz pomoć visokotehnoloških istraživačkih alata koje nudi suvremena biotehnologija. Ovaj kratki osvrt sažima osnovne principe spolnog određivanja i spolne funkcije steroida kao što su opisani u literaturi, a zatim nastavlja sa primjerima kako moderne metode istraživanja daju novi uvid u suptilnije detalje ovog procesa, pokazujući kako on obuhvaća gotovo svaki dio tjelesnih sustava.