MOLECULAR ASPECTS OF STEROID ACTION IN MARINE FISHES

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Contents

Summary

Steroids impinge upon every physiological process, and the maintenance of their proper actions are therefore critical to the health and reproductive success of all vertebrates. The fact that steroids can readily pass through cell membranes has important consequences for the activity and degradation of these hormones. As they cannot be sequestered within steroidogenic cells, steroids must be made upon demand. Because they are not readily soluble in water, they are transported in bulk by plasma proteins and must be modified before they can be efficiently excreted. Once made, steroids elicit many of their actions by entering the nucleus to alter gene expression. Our understanding of the molecular mechanisms behind steroidogenesis, steroid action and steroid metabolism in fishes has lagged behind that of other vertebrates, despite the fact that fishes are the most abundant vertebrates, are very amenable experimental models and are the source of protein for millions of humans. However, studies in fishes have sometimes pioneered new avenues of research, as illustrated by recent findings regarding the molecular mechanisms behind novel steroid actions elicited from target cell membranes. This paper will review the molecules involved in the synthesis, actions and degradation of steroid hormones, with an emphasis on the current understanding of the role of steroids in physiological processes in fishes.

1. Introduction

The many cell types within individual animals must communicate with each other to coordinate the different physiological processes they conduct. Two main pathways of intercellular communication exist in vertebrates. There is a network of specialized cells (the nervous system), which can rapidly communicate information to specific cells via electrical conductance. A more generalized form of communication is through the production of chemical messengers (hormones) which are transported though the circulatory system and received by cells that possess hormone-specific receptor proteins. Steroids are a class of hormones that are synthesized from cholesterol, an abundant component of cell membranes. Steroids are lipophilic and can pass readily through plasma membranes, which influences how these hormones are synthesized and elicit their actions. Due to their lipophilicity, steroids cannot be sequestered within steroidogenic cells and therefore must be made on demand. Active steroids retain four carbon-ring structures found in cholesterol (designated rings A-D) (Figure 1). The activity of a given steroid is determined by the number and chemical nature of carbon molecules that make up the steroid nucleus and of the oxygen molecules that decorate it. Each steroid is therefore the product of a combination of different steroidogenic proteins that must be regulated so that the right type of steroid is made in the correct amount at the correct time.

Steroids have a low solubility in aqueous solutions such as blood, which hinders the ability of the circulatory system to deliver steroids to target cells. However, many animals have special steroid-binding proteins dissolved in blood that bind and transport

steroids and may also act as a buffer against excess hormone. It has also been hypothesized that the binding protein-steroid complex might also represent an alternate pathway through which these hormones can communicate with target cells.

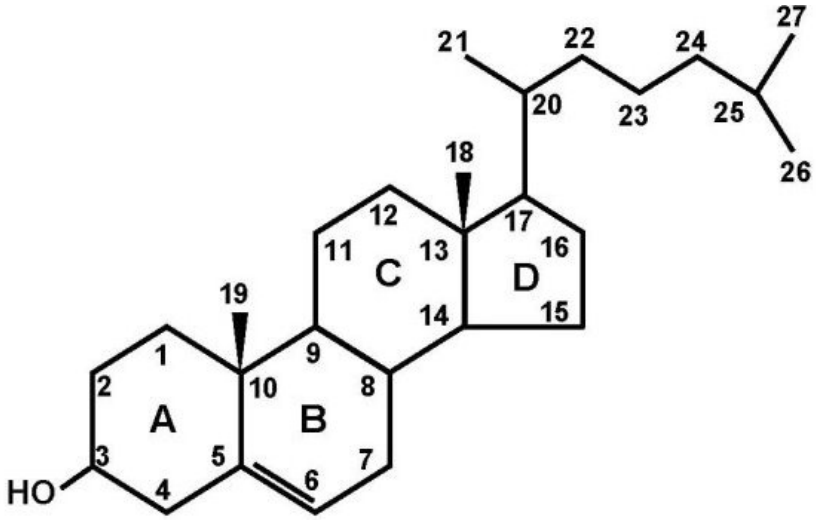


Figure 1. Cholesterol. The core structure of all sterols is comprised of four carbon rings, which are labeled "A" through "D" by convention. Likewise, the carbon molecules that forms the backbone of these molecules are numbered beginning with the A ring. Cholesterol has twenty-seven carbons including a side chain attached to the "D" ring that is removed in the initial steps of steroid formation.

The actions of steroids are mediated primarily through intracellular proteins called nuclear receptors; the ability of steroids to pass through plasma membranes allows direct access to such receptors. In general, nuclear receptors act to alter the transcription of target genes by binding to receptor-specific sequences in gene promoters. Because they elicit changes in gene transcription, the nuclear receptor-mediated effects of steroids typically occur over several hours and can be long-lasting. However, a second class of steroid receptors that mediate more rapid effects within target cells has recently been characterized. These proteins are found in the plasma membrane of target cells and when bound by steroid immediately activate intracellular second messenger systems, which can elicit a plethora of rapid cellular responses.

Finally, as with all hormones, steroids must be eliminated once their message has been delivered. The lipophilicity of steroids demands that these compounds be metabolized into more soluble forms before they can be efficiently excreted. Most vertebrates express a suite of specific enzymes whose activity increases the solubility of steroids so that they can be more readily excreted in urine.

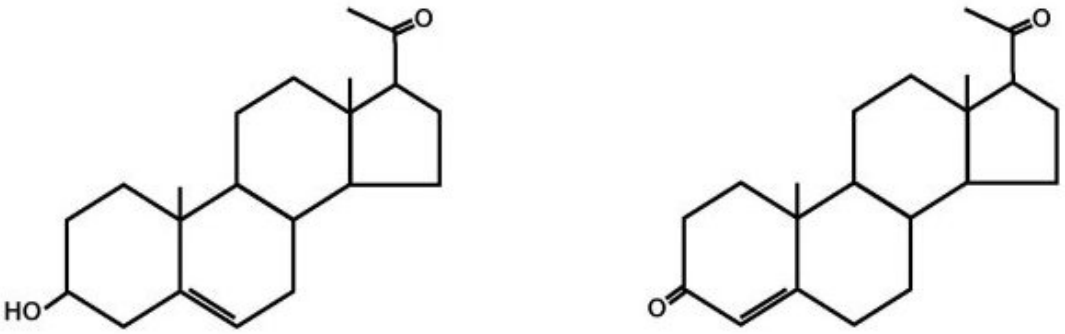
Because steroids impinge upon every physiological process in vertebrates, disruption of their actions can have serious health implications. Therefore, much effort has been placed towards understanding the synthesis, metabolism and action of steroids in humans. Due to the success of targeted medical research and the human genome project, the genes encoding most human steroidogenic enzymes, steroid-metabolizing enzymes,

steroid-binding proteins and steroid receptors have been isolated and well-characterized. These reagents have allowed comprehensive studies of steroid action in mammals. Unfortunately, our understanding of the role of steroid hormones in less derived vertebrates such as fishes has progressed much slower. This is despite the fact that fishes are the most abundant of all vertebrates and occupy virtually all aquatic environments. They therefore represent a unique opportunity to examine how physiological systems (such as those involved in steroid action) have evolved to meet the disparate challenges of different habitats. In addition, because of their abundance, ease of maintenance in captivity and the presence of specialized tissues/organs in many species, fishes are ideal models for the study of specific physiological systems. Finally, billions of humans rely heavily on fish for food; millions of tons of fish are obtained from the wild and through mariculture, providing great incentive to understand these animals at several biological levels. This review will therefore focus on the current state of understanding of the molecular mechanisms involved in the synthesis, metabolism, transport and actions of steroid hormones in marine fishes.

2. Steroid Classes

The physiological activity of a steroid is defined by the number and orientation of carbon molecules in the steroid nucleus and the presence, location and chemical nature of oxygen molecules on those carbons. Cholesterol, the ultimate precursor of all steroids, contains twenty-seven carbon molecules. Some steroid-like molecules, such as Vitamin D_3, retain the long hydrophobic side-chain present in cholesterol; however, true steroids are formed after the removal of six carbons from this side chain.

2.1. Progestins



5-PREGNEN-3β-OL-20-ONE
PREGNENOLONE
P5

4-PREGNEN-3,-20-DIONE
PROGESTERONE
P4

Figure 2. Progestins. Progestins are twenty-one carbon steroids that can serve as neurosteroids or reproductive steroids and also serve as substrates for the synthesis of other steroids. The chemical and trivial names, as well as frequently used abbreviations, are given below each structure.

The removal of the six-carbon side chain from cholesterol creates the simplest steroids, the progestins, which have twenty-one carbon molecules. These steroids include pregnenolone, progesterone, corticosteroids and maturation inducing steroids (MIS). Although pregnenolone and progesterone (Figure 2) serve as precursors for most other bioactive steroids, they are also important hormones in their own right. For example, pregnenolone is synthesized in the brain and may be important in regulating specific behaviors. Progesterone has a critical reproductive role in mammals, as it acts in the maintenance of developing embryos, and may also serve as a neurosteroid (see below).

2.1.1. Corticosteroids

Corticosteroids include glucocorticoids (GCs) and mineralocorticoids (MCs), produced by the adrenal cortex of mammals and interrenal cells of less-derived vertebrates (Figure 3). In mammals, the glucocorticoid receptor (GR) is ubiquitously expressed; because GCs are always present in blood, the GC/GR complex may act as a basal transcription factor.

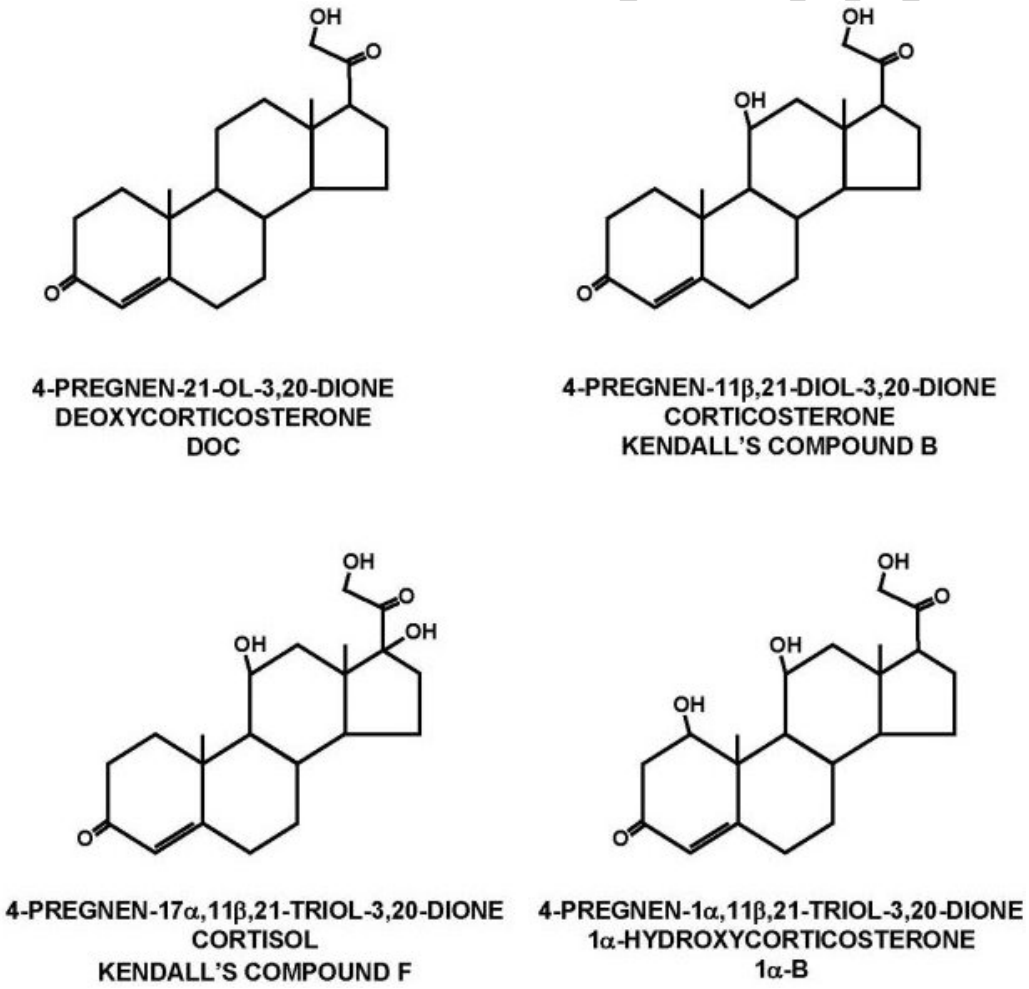


Figure 3. Corticosteroids. Corticosteroids are twenty-one carbon steroids that regulate metabolism, hydromineral balance and serve in the stress response.

At elevated concentrations, GCs can induce hyperglycemia by stimulating glucose synthesis and mobilization of glucose from glycogen stores. GCs regulate lymphocyte and immunoglobulin concentrations, thereby helping to avoid detrimental autoimmune responses. GCs are also involved in the "fight or flight" stress response. This physiological response to stress includes activation of the sympathetic nervous system and the release of catecholamines (adrenaline), which are dependent upon GCs for full activity, an example of the permissive effects of some steroids.

Aldosterone is the primary MC in lungfish, amphibians, reptiles, birds and mammals and is responsible for the regulation of blood sodium and potassium. Most fishes do not synthesize aldosterone, and a single steroid (cortisol in teleost fishes; 1α-hydroxycorticosterone {1α-B} in elasmobranch fishes) is thought to serve as both a mineralocorticoid and a glucocorticoid in these animals. There is evidence that other steroids, such as 11-deoxycorticosterone (DOC), may serve as MCs. Cortisol apparently plays a role in the adaptation to both hypo- and hyperosmotic challenges in teleosts, while the role of 1α-B in elasmobranch fishes is still being defined.

2.1.2. Maturation Inducing Steroids

During gametogenesis, eggs and sperm are arrested at specific developmental stages and must be induced to proceed before becoming viable through a process called gamete maturation. In vertebrates, maturation inducing steroids (MIS) reinitiate the developmental process. The mammalian MIS is thought to be progesterone, however very different progestins serve this role in fishes: 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) is the most potent inducer of maturation in several families of marine fishes, while 17α,20β,21-trihydroxy-4-pregnen-3-one (17α,20β-S) is the MIS in sciaenids (e.g. Atlantic croaker, spotted seatrout) (Figure 4).

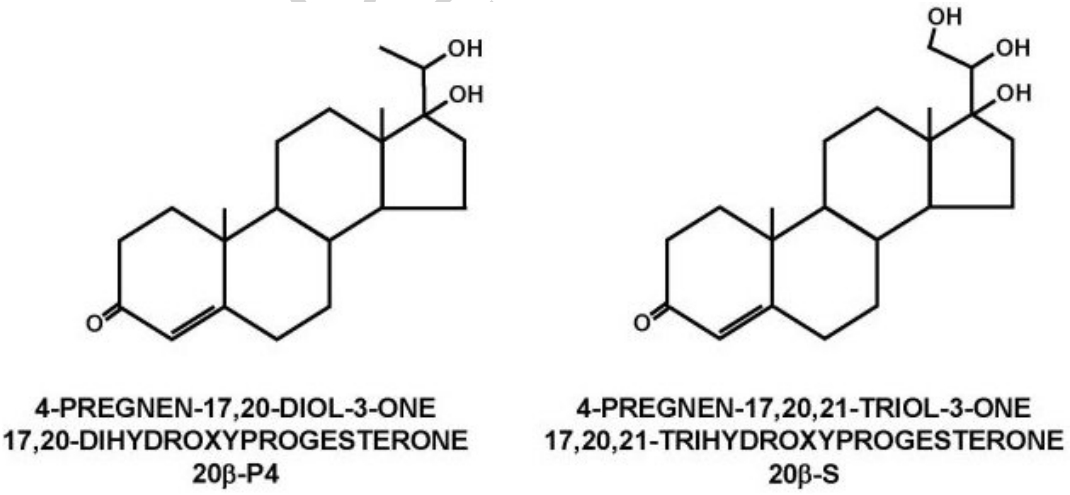


Figure 4. Maturation inducing steroids. Maturation inducing steroids (MIS) are twenty-one carbon steroids that regulate the final maturational processes that produce fertilizable oocytes in fishes. Two MIS have been well characterized, 17,20-dihydroxyprogesterone (produced in salmonids) and 17,20,21-trihydroxyprogesterone (produced in sciaenids).

2.2. Androgens

Androgens are sex steroids containing nineteen carbons formed by the removal of the remaining two carbons of the original cholesterol side chain (Figure 5). Androgens are typically thought of as products of the testis, however they are also made by other tissues in many vertebrates (including adrenal cortex/interrenal tissue and brain). Androgens are important to testicular development, the production of male gametes and the regulation of many male behaviors. Because they are the direct precursors of estrogens (discussed below) females also synthesize androgens but it is apparent that androgens have important roles in female vertebrates in addition to estrogen biosynthesis.

2.3. Estrogens

Estrogens are formed from androgens through the activity of CYP19 (aromatase). This enzyme removes carbon nineteen from androgen precursors, while also converting the "A-ring" into a phenolic structure (Figure 6). Estrogens are necessary for ovarian development, the production of female gametes and regulating female reproductive behaviors. Usually thought of as a female hormone, endogenously produced estrogens also play very important roles in males.

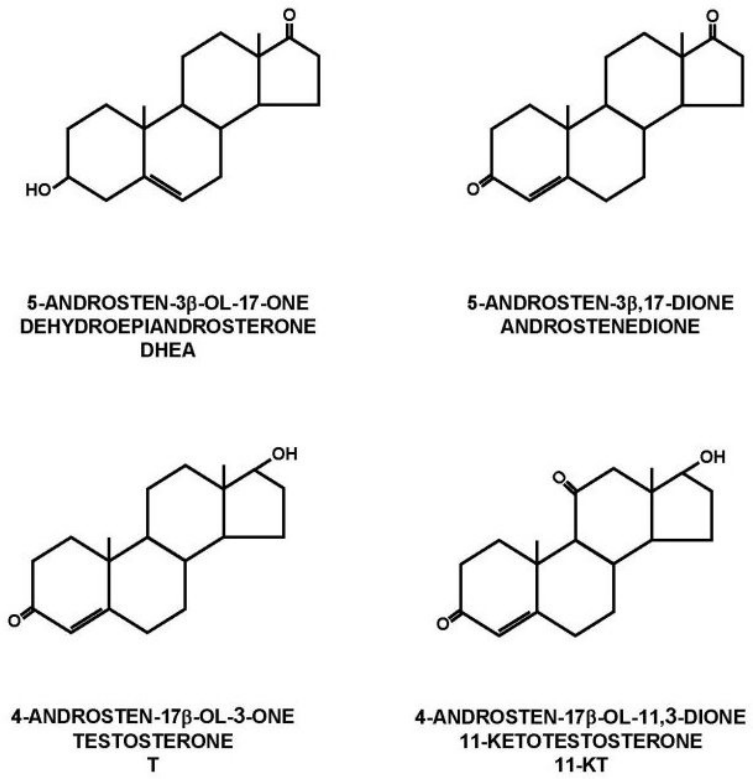


Figure 5. Androgens. Androgens are nineteen carbon sex steroids which regulate reproductive processes in both male and female vertebrates. The most potent androgen in fishes is 11-ketotestosterone.

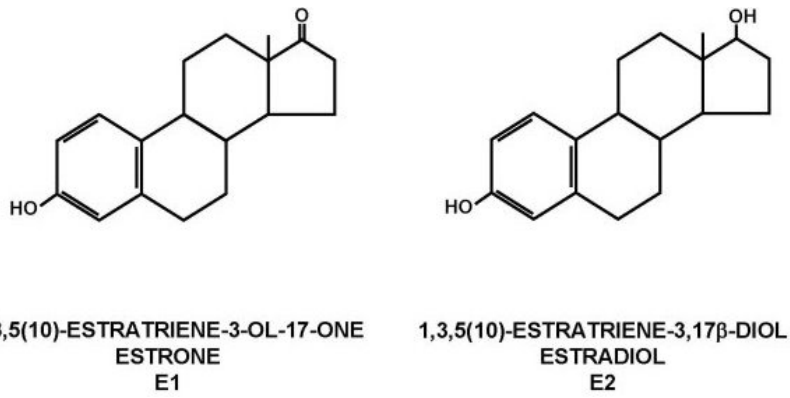


Figure 6. Estrogens. Estrogens are eighteen carbon sex steroids which regulate reproductive processes in both male and female vertebrates. The predominant estrogen in fishes is estradiol.

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Biographical Sketches

Brian Scott Nunez, Ph.D., is a molecular endocrinologist who studies the role of steroid hormones in the physiological response to environmental change in fishes. He received his M.S. in Oceanography from Old Dominion University in 1992 and his Ph.D. in Physiology from Louisiana State University in 1996. He is currently an assistant professor at the University of Texas at Austin Marine Science Institute,

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