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Review

A brief history of the search for the protein(s) involved in the acute regulation of steroidogenesis

Douglas M. Stocco [a, *], Amy H. Zhao [b], Lan N. Tu [b], Kanako Morohaku [b, 1], Vimal Selvaraj [b]

[a] Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA
[b] Department of Animal Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA

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ABSTRACT

The synthesis of steroid hormones occurs in specific cells and tissues in the body in response to trophic hormones and other signals. In order to synthesize steroids *de novo*, cholesterol, the precursor of all steroid hormones, must be mobilized from cellular stores to the inner mitochondrial membrane (IMM) to be converted into the first steroid formed, pregnenolone. This delivery of cholesterol to the IMM is the rate-limiting step in this process, and has long been known to require the rapid synthesis of a new protein(s) in response to stimulation. Although several possibilities for this protein have arisen over the past few decades, most of the recent attention to fill this role has centered on the candidacies of the proteins the Translocator Protein (TSPO) and the Steroidogenic Acute Regulatory Protein (StAR). In this review, the process of regulating steroidogenesis is briefly described, the characteristics of the candidate proteins and the data supporting their candidacies summarized, and some recent findings that propose a serious challenge for the role of TSPO in this process are discussed.

1. Introduction

Steroidogenesis is the process by which important steroid hormones are synthesized by specific tissues and cells in the body. Examples of these important steroids are the glucocorticoids that are synthesized in the adrenal cortex, the mineralocorticoids that are synthesized in the adrenal glomerulosa, the ovarian and placental progestins and estrogens, the testicular androgens and several neurosteroids such as pregnenolone, progesterone, 5α-dihydroprogesterone, allopregnanolone and DHEA, that are synthesized in the brain. The adrenal glucocorticoids serve to regulate carbohydrate metabolism and manage stress and the mineralocorticoids are involved in salt balance and the maintenance of blood pressure. Ovarian progesterone and estrogen are involved in the maintenance of female secondary sex characteristics and reproductive function while testicular testosterone is involved in maintaining male secondary sex characteristics and is essential for male fertility. Neurosteroid functions include stimulation of GABAergic responses, modulation of the response of Purkinje cells to excitatory amino acids and the enhancement of memory function. Other tissues and cells have also been reported to have the capacity for *de novo* steroid synthesis, but the localization of where steroids are synthesized and their respective functions are not the main focus of this review. Rather, this review will concentrate on the manner in which the synthesis of steroid hormones are regulated and the history of the efforts that have been made to uncover the components and the mechanisms involved in this regulation. This history dates back approximately six decades when it was first observed that the synthesis of steroid hormones *in vitro* could be stimulated with trophic hormones and that this synthesis required the production of a new protein(s), as will be described later in this review. This singular observation formed the basis for what has been a long and most interesting search for the putative regulatory protein(s). We will briefly summarize the early studies that were performed in the search for this regulatory protein(s), the necessary characteristics of the candidates required to perform this function and some of the controversies that have arisen along the way, and indeed, remain to the present time. To be sure, this has been an interesting undertaking by a number of investigators in the field and it would seem safe to say that at this juncture in time, the entire story of what factors are involved in the acute regulation of steroid

* Corresponding author.
E-mail address: doug.stocco@ttuhsc.edu (D.M. Stocco).
[1] Present address: Department of Bioscience, Tokyo University of Agriculture, Tokyo 156-8502, Japan.

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hormone biosynthesis and how they function is not yet completely understood.

2. Characteristics of the regulation of steroid hormone biosynthesis

The initial step in steroidogenesis is the conversion of cholesterol to the first steroid formed, pregnenolone, which occurs in all steroidogenic tissues (Miller, 1988, Pescador et al., 1997). This conversion is a result of the action of the cytochrome P450 side-chain cleavage enzyme (P450scc; CYP11A1), that is part of the cholesterol side chain cleavage system that resides on the matrix side of the inner mitochondrial membrane (Farkash et al., 1986). Pregnenolone then exits the mitochondria and is converted to progesterone and other steroids in the microsomal compartment and, in some cases, downstream steroids re-enter the mitochondrial compartment to be converted to the final product dependent upon the complement of steroidogenic enzymes present within specialized cells in those tissues (Miller, 1998). Understanding the enzymes involved in steroidgenesis and, most importantly, their intracellular location, is key to understanding how the synthesis of steroid hormones is regulated.

The biosynthesis of steroid hormones are mostly regulated by pituitary trophic hormones such as adrenocorticotropin (ACTH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) and this regulation occurs in two phases. The acute phase occurs very rapidly (within minutes) and is responsible for the production of steroids in response to immediate needs (Stocco and Clark, 1996). In addition, chronic regulation (on the order of hours) also occurs and consists of the longer-term expression of the mRNAs and proteins for the steroidogenic pathway enzymes to provide for continuing steroid synthesis following the acute phase (Miller, 1988, Simpson et al., 1992, Simpson and Waterman, 1988, Waterman and Simpson, 1985). This review will focus only on elements involved in the acute phase of steroid hormone biosynthesis. Like most biosynthetic pathways, the steroidogenic pathway has a rate-limiting step and following a number of years of speculation, it was experimentally determined that the regulated and rate-limiting step in steroidogenesis was the delivery of the substrate cholesterol from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) where the P450scc enzyme is located (Black et al., 1994, Farkash et al., 1986). This step was rate-limiting because the hydrophobic cholesterol could not cross the aqueous mitochondrial intermembrane space to the relatively cholesterol poor IMM within the time frame that was observed for the acute synthesis of steroids. There followed a significant period of investigation to determine the nature of the acutely regulated step. Early investigations were performed using adrenal perfusions *in vitro* and it was observed that ACTH could stimulate the biosynthesis of steroids (Stone and Hechter, 1954) and importantly, that acute steroid production had an absolute requirement for the synthesis of new proteins (Ferguson, 1962, 1963, Garren et al., 1965, Garren et al., 1966, Garren, 1968). Subsequent studies demonstrated that the putative regulator protein in all likelihood functioned at the level of the delivery of cholesterol to the P450scc enzyme in the IMM, the regulated step (Arthur and Boyd, 1974, Davis and Garren, 1968, Ohno et al., 1983, Privalle et al., 1983, Simpson and Boyd, 1966). The overall significance of all these early observations was that the search for the putative regulator now had a specific target on which to focus, namely, a newly synthesized protein.

The discovery and characterization of the putative protein regulator of steroidogenesis was the goal of several laboratories and over the ensuing decades candidate proteins for the acute regulator emerged. These candidates included the Sterol Carrier Protein 2 (SCP2) (Vahouny et al., 1987), the Steroidogenesis-Activator Polypeptide (SAP) (Pedersen and Brownie, 1983, 1987), the Peripheral Benzodiazepine Receptor/Translocator Protein (PBR/TSPO) and the Steroidogenic Acute Regulatory protein (StAR), and all were placed in contention for being the protein responsible for the acute regulation of cholesterol transfer to the IMM and thus, steroidogenesis.

Experimental evidence revealed that each of these proteins appeared to have characteristics that rendered them as viable candidates. However, after the initial studies demonstrating that SCP2 could enhance steroid synthesis in isolated mitochondria, further research on this candidate based on observations in knockout mice ruled out an involvement for SCP2 in steroidogenesis (Seedorf et al., 1998). Also, while first isolated as a small molecular weight peptide (Pedersen and Brownie, 1983, 1987), SAP was later identified as a fragment of the much larger glucose-regulated protein 78 (GRP78) (Li et al., 1989). GRP78 is a regulator of the unfolded protein response (UPR) (Wang et al., 2009), and it was demonstrated that GRP78 knockout mice died at an early embryonic stage (Luo et al., 2006). Later studies examining GRP78 conditional knockouts indicated a phenotype in oncogenic signaling (Wey et al., 2012), and examination of GRP78 structure showed that it contained a nucleotide-binding domain and a peptide substrate-binding domain (Wisniewska et al., 2010), but had no characteristics that indicated the potential for lipid transport. Therefore, a function for GRP78 in the acute regulation of cholesterol transport appeared unlikely. In contrast to SCP2 and SAP, the candidate proteins TSPO and StAR have continued to be studied in great detail.

3. The Steroidogenic Acute Regulatory Protein

A candidate protein for the acute regulator of steroidogenesis was first described by Orme-Johnson and her colleagues as an ACTH-induced 30 kDa phosphoprotein in hormone-treated rat and mouse adrenocortical cells, and as an LH-induced protein in rat corpus luteum cells and mouse Leydig cells (Alberta et al., 1989, Epstein and Orme-Johnson, 1991a,b, Krueger and Orme-Johnson, 1983, Pon et al., 1986a,b, Pon and Orme-Johnson, 1988). These studies demonstrated that a close relationship existed between the appearance of the 30 kDa proteins and steroid hormone biosynthesis and that their synthesis was sensitive to cycloheximide. Our laboratory was engaged in similar studies in hormone-stimulated MA-10 mouse Leydig tumor cells and described a family of proteins which were identical to those described by Orme-Johnson (Stocco and Kilgore, 1988, Stocco and Chaudhary, 1990, Stocco and Chen, 1991, Stocco and Sodeman, 1991, Stocco, 1992, Stocco and Ascoli, 1993, Stocco et al., 1995). These proteins were found localized to the mitochondria and consisted of several forms of a newly synthesized 30 kDa protein. The proteins, as identified by 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE), are shown in Fig. 1. Later studies determined that the 30 kDa mitochondrial proteins were processed from a 37 kDa precursor protein that contained a mitochondrial signaling sequence in its N-terminus (Epstein and Orme-Johnson, 1991a, b, Stocco and Sodeman, 1991). In general, all of the studies that were performed demonstrated tight correlations between the synthesis of steroids and the synthesis of the 30 kDa mitochondrial proteins and thus, they represented good candidates for the regulatory protein (Alberta et al., 1989, Epstein and Orme-Johnson, 1991a, b, Krueger and Orme-Johnson, 1983, Pon et al., 1986a, b, Pon and Orme-Johnson, 1988, Stocco and Kilgore, 1988, Stocco and Chaudhary, 1990, Stocco and Chen, 1991, Stocco and Sodeman, 1991, Stocco, 1992, Stocco and Ascoli, 1993, Stocco et al., 1995). The purification of the 30 kDa protein, the cloning of the cDNA for the 37 kDa protein