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Review

The spatial and temporal regulation of the hormonal signal. Role of mitochondria in the formation of a protein complex required for the activation of cholesterol transport and steroids synthesis

Cecilia Poderoso [a], Alejandra Duarte [a], Mariana Cooke [a], Ulises Orlando [a], Vanesa Gottifredi [b], Angela R. Solano [a], Jose R. Lemos [c], Ernesto J. Podestá [a,*]

[a] Instituto de Investigaciones Biomedicas (INBIOMED UBA-CONICET), Department of Biochemistry, School of Medicine, University of Buenos Aires, Paraguay 2155, C1121ABG Buenos Aires, Argentina
[b] Fundación Instituto Leloir, Av. Patricias Argentinas 435, Buenos Aires CI1405WE, Argentina
[c] University of Massachusetts Medical School, Department of Microbiology and Physiological Systems, 55 Lake Avenue North S4-424, Worcester, MA 01655, USA

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ABSTRACT

The mitochondria are critical for steroidogenesis since the ability of cholesterol to move into mitochondria to be available for cytochrome P450, CYP11A1, determines the efficacy of steroid production. Several proteins kinases, such as PKA, MEK and ERK which are essential to complete steroidogenesis, form a mitochondria-associated complex. The protein–protein interactions between kinases and key factors during the transport of cholesterol takes place in the contact sites between the two mitochondrial membranes; however, no mitochondrial targeting sequence has been described for these kinases. Here we discuss the possibility that mitochondrial reorganization may be mediating a compartmentalized cellular response. This reorganization could allow the physical interaction between the hormone-receptor complex and the enzymatic and lipidic machinery necessary for the complete steroid synthesis and release. The movement of organelles in specialized cells could impact on biological processes that include, but are not limited to, steroid synthesis.

Contents

Abbreviations: CYP11A1, cytochrome P450, family 11, subfamily A, polypeptide 1; PKA, cyclic-AMP-dependent protein kinase; ACTH, adrenocorticotropin hormone; LH, luteinizing hormone; IMM, inner mitochondrial membrane; P5, pregnenolone; ER, the endoplasmic reticulum; OMM, outer mitochondrial membrane; ERK1/2, extracellular signal-regulated kinases; AKAPs, the A-kinase anchor proteins; mt-YFP, mitochondria-targeted YFP; hCG, human chorionic gonadotropin; cAMP, 8Br-cAMP; Mito-GFP, mitochondria-targeted GFP; P4, progesterone; EGF, epidermal growth factor; Mfn, Mitofusin; OPA1, optic atrophy; Drp1, dynamin related protein 1; MAM, mitochondria associated membrane; Acsl4, acyl-CoA synthetase-4; PTP, protein tyrosine phosphatase; StAR, steroidogenic acute regulatory; AII, angiotensin II; K^+, potassium; TSPO, translocator protein; VDAC, voltage dependent anion channel.

* Corresponding author. Address: Department of Biochemistry, School of Medicine, University of Buenos Aires, Paraguay 2155, 5th C1121ABG Buenos Aires, Argentina. Tel.: +54 11 45083672x36; fax: +54 11 45083672x31.
E-mail address: biohrdc@fmed.uba.ar (E.J. Podestá).

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1. Introduction

It is well recognized that the interaction of a peptide hormone with its receptors on the surface of a cell and the initiation of signal transduction are the most important steps in its mechanism of action. There has been a great amount of data describing the kinetics of the hormone-receptor interaction as well as the activation of second messenger formation. However, several important questions regarding the mechanism of peptide hormone action still remain elusive.

Steroid hormones are synthesized in steroidogenic cells of the adrenal gland, ovary, testis, placenta, and brain and are required for normal reproductive function and body homeostasis. Steroid synthesis is regulated by trophic hormones, specifically, adrenocorticotropin hormone (ACTH) in adrenocortical cells, luteinizing hormone (LH) in testicular Leydig cells and LH and follicle-stimulating hormone (FSH) in ovarian cells, respectively. These hormones activate G protein-coupled receptors resulting in the activation of adenylyl cyclase and an increase in intracellular cyclic-AMP levels (Dufau et al., 1980). This increase promotes the activation of cyclic-AMP-dependent protein kinase (PKA), protein synthesis and protein phosphorylation (Dufau et al., 1977) (Podesta et al., 1979a, b; Rae et al., 1979). All these processes contribute to the delivery of cholesterol from the outer to the inner mitochondrial membrane, the rate-limiting step in steroid production.

Steroid synthesis is initiated at the inner mitochondrial membrane (IMM), where the cytochrome P450 cholesterol side chain cleavage enzyme (CYP11A1) catalyzes the conversion of cholesterol to pregnenolone (P5) (Hall, 1985). Then, P5 enters the endoplasmic reticulum (ER) where further enzymatic reactions occur. Afterwards, the steroid formed returns to the mitochondrion to produce the final steroid hormone being the case of glucocorticoids and mineralocorticoids while sex steroids do not require a second round of processing inside the mitochondria.

In the complex process of steroidogenesis, the mitochondrion is the site where the rate limiting step-cholesterol transport across the mitochondrial membranes – occurs (Crivello and Jefcoate, 1980; Privalle et al., 1983).

Cholesterol transport requires specific interactions at the mitochondria between several proteins including the voltage-dependent anion channel (VDAC) (McEnery et al., 1992), the peripheral benzodiazepine receptor (PBR, currently named translocator protein or TSPO) (Papadopoulos et al., 2006), the PBR-associated protein (PAP7) (Liu et al., 2006), and the steroidogenic acute regulatory protein (StAR) (Clark et al., 1994; Krueger and Orme-Johnson, 1983; Liu et al., 2006). The StAR protein, which has the mitochondrial target sequence at the N terminus, is synthesized as a 37 kDa precursor protein in the cytoplasm, which is cleaved in the mitochondrial matrix to form a 30 kDa protein (Bose et al., 2002; Epstein and Orme-Johnson, 1991; Stocco and Sodeman, 1991). The N terminal 47 or 62 aminoacid-truncated murine or human forms of StAR protein stimulates cholesterol transport outside the mitochondria, indicating that the 30 kDa form is active at the outer mitochondrial membrane (Arakane et al., 1996; Miller, 2007).

The concept of "compartmentalization" has been utilized to explain some inconsistencies in second-messenger action (Greengard, 1978). One example is the fact that the resting intracellular cyclic-AMP concentration is three orders of magnitude higher than the measurable K_a of PKA; which is, nevertheless, not fully activated under these conditions (Rubin and Rosen, 1975). Is the PKA compartmentalized or "protected" from resting cyclic-AMP levels and thus only responds to de novo synthesis of cyclic-AMP localized to the vicinity of the receptor-activated cyclase?

Several proteins, such as PKA regulatory subunit Iα (Rone et al., 2009), MEK and extracellular signal-regulated kinases (ERK1/2), (Poderoso et al., 2008), which are essential to complete steroidogenesis form a mitochondria-associated complex. The physical protein–protein interactions between protein kinases and key factors during the transport of cholesterol takes place in the contact sites between the two mitochondrial membranes, (Rone et al., 2009) however, no mitochondrial targeting sequence has been described for these protein kinases.

In previous work, the relevance in PKA compartmentalization has been supported by the findings that an A-kinase anchoring protein, AKAP121, retains PKA in the mitochondria of steroidogenic cells allowing the recruitment of both StAR mRNA and PKA to the mitochondria (Dyson et al., 2008).

In the last few years, evidence has been presented suggesting that the specificity of the hormonal signal can be achieved through the spatial and temporal regulation of the response. For example, the phosphorylation of proteins in the different intracellular compartments of the cell which are induced by PKA and the MAP-kinase family is critical for the regulation of multiple functions. This activation of kinases in different intracellular compartments changes according to the specific stimulus. In the steroidogenic system that uses cyclic-AMP as the second messenger, the signal originates in the plasma membrane producing a translocation of PKA and ERK1/2 to the mitochondria (Poderoso et al., 2008). This translocation is essential for the normal regulation of steroid synthesis (Poderoso et al., 2008). This phenomenon can be observed also in other physiological systems. In the case of epidermal growth factor stimulation, for example, ERK is also translocated to the nucleus. The striking features are that neither PKA nor ERK have any peptide signal that directs them to either the mitochondria or the nucleus. Neither do transport proteins for these kinases exist in these cells.

During the last few years the majority of studies on the location of kinases in the different compartments of cells were directed towards the mechanism of retention of these kinases in the different organelles (Carlucci et al., 2008; Feliciello et al., 2005). However, no studies have attempted to understand how proteins without specific signal peptides are able to travel to their respective sites of action. In this manuscript we will go through several published results attempting to answer these important questions regarding the mechanisms of hormone action.

2. Hormone stimulation at the single cell level

It is known that certain responses generated by cyclic-AMP are much more efficient when the messenger is produced through receptor activation, suggesting a compartmentalization between the site of cyclic-AMP production and the PKA system. Unfortunately, direct evidence for such compartmentalization has been scarce.

The regulation of steroid synthesis is one of the systems where the compartmentalization of the hormonal signal may be important for the correct functioning of the steroidogenic cells. Using single cells and limiting stimulation to a "patch" of membrane has made possible understanding how one form of signal transduction proceeds, i.e. the mechanism by which neurotransmitter occupied-receptors lead to the gating of individual ion channels (Camardo et al., 1983; Siegelbaum and Tsien, 1980).

Using this same approach it has been possible to stimulate a single steroidogenic cell (Podesta et al., 1991). These studies have contributed in understanding another form of membrane signal transduction at the single cell level and the role of compartmentalized responses in the mechanism of peptide hormone action in steroid synthesis.

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