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Review

Hormonal activation of a kinase cascade localized at the mitochondria is required for StAR protein activity

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ABSTRACT

It is known that ERK1/2 and MEK1/2 participate in the regulation of Star gene transcription. However, their role in StAR protein post-transcriptional regulation is not described yet. In this study we analyzed the relationship between the MAPK cascade and StAR protein phosphorylation and function. We have demonstrated that (a) steroidogenesis in MA-10 Leydig cells depends on the specific of ERK1/2 activation at the mitochondria; (b) ERK1/2 phosphorylation is driven by mitochondrial PKA and constitutive MEK1/2 in this organelle; (c) active ERK1/2 interacts with StAR protein, leads to StAR protein phosphorylation at Ser^{232} only in the presence of cholesterol; (d) directed mutagenesis of Ser^{232} (S232A) inhibited *in vitro* StAR protein phosphorylation by ERK1; (e) transient transfection of MA-10 cells with StAR S232A cDNA markedly reduced the yield of progesterone production. We show that StAR protein is a substrate of ERK1/2, and that mitochondrial ERK1/2 is part of a multimeric complex that regulates cholesterol transport.

Contents

1. Introduction

Many human mitochondrial disorders are related to abnormalities in mitochondrial proteins. In steroidogenic tissues, truncation of the Steroidogenic Acute regulatory protein (StAR protein) is associated with the steroid deficiency disease, congenital lipoid adrenal hyperplasia (Lin et al., 1995). StAR protein is a component of a protein complex that functions in the rate limiting step of steroidogenesis (Stocco, 2000), by transporting cholesterol from the outer to the inner mitochondrial membranes. In all steroidogenic tissues, phosphorylation-dependent events are required for the acute stimulation of steroid biosynthesis through the activation of protein kinases (Dufau et al., 1977; Le and Schimmer, 2001; Pezzi et al., 1996; Podesta et al., 1978, 1979; Sala et al., 1979; Schimmer et al., 1977). Among those are the cAMP dependent protein kinase (PKA), the protein kinase C (PKC), the calcium/calmodulin-dependent protein kinase and the mitogen activated protein kinases (MAPKs). Thus hormones, ions or growth factors modulate steroid biosynthesis by the post-translational phosphorylation of proteins. The question still remains as to how phosphorylation events can transmit a specific signal to its mitochondrial site of action.

Although it is known that StAR is a phosphoprotein and that it is involved in the mechanism of action of steroidogenic hormones, the role of StAR protein phosphorylation is not completely understood. The transcription of the Star gene increases in a cAMP (cAMP)-PKA-dependent manner (Stocco, 2000). In addition, the non-genomic

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post-translational effects of PKA have been reported in relationship to StAR protein (Arakane et al., 1997). PKA phosphorylates murine and human StAR proteins at specific motifs like Ser 56/57 and Ser 194/195, respectively (Arakane et al., 1997; Fleury et al., 2004).

In addition to the well-documented role of PKA activation in trophic hormone-stimulated steroid biosynthesis, ERK1/2 and its upstream activator MEK1/2 also participate in the regulation of steroidogenesis through genomic and non-genomic actions (Gyles et al., 2001; Manna et al., 2007; Martinat et al., 2005; Martinelle et al., 2004).

In this paper we will discuss the role of ERK in the regulation of steroid biosynthesis, the phosphorylation of StAR protein by ERK1/2 at the mitochondria and the role of this phosphorylation in StAR protein function and activity.

2. Role of MAPKs in the regulation of steroid biosynthesis

There are several reports studying the role of the members of the MAPKs in the regulation of steroid synthesis both at the levels of genomic and not genomic regulation.

One of the first reports (Gyles et al., 2001) indicated that cAMP-induced steroid synthesis is dependent upon the phosphorylation and activation of ERKs. ERK activation results in enhanced phosphorylation of SF-1 and increased steroid production through increased transcription of the Star gene. They also showed that adenylate cyclase activation with forskolin caused a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus. These findings correlate with an increase in StAR mRNA levels, StAR protein accumulation, and steroidogenesis. Similarly, ERK inhibition led to a reduction in the levels of forskolin-stimulated StAR mRNA, StAR protein, and steroid secretion.

In agreement with Gyles et al., Martinelle et al. (2004) described the involvement of the ERK cascade in human chorionic gonadotropin (hCG)-induced steroidogenesis by primary cultures of immature rat Leydig cell. Their findings indicate that PKA and PKC function as upstream kinases in connection with transduction of the signal from the gonadotropin receptor to the ERK cascade. These MAPKs enhance the stimulatory effects of hCG on the novo synthesis of StAR protein and the activity of protein phosphatase 2A, which are associated with increased androgen production by Leydig cells. Inhibition of MEK1/2 by U0126 suppressed all of these cellular responses to hCG. Martinat et al. (2005) showed also that preincubation of rat primary Leydig cells and the mouse tumoral Leydig cell line MLTC-1 with the MEK1/2 inhibitors U0126 and PD98059 reduced LH-induced steroidogenesis. However they showed that these inhibitors tonically enhance the expression of the StAR protein. In a recent paper Manna et al. (2007) also showed that inhibition of ERK1/2 activity by U0126 increased Star gene expression and decreased progesterone production in MA-10 Leydig cells stimulated with dibutyryl cAMP.

Otis et al. (2007) showed that the inhibition of proliferation and stimulation of hypertrophy induced by Angiotensin II (AII) involve both ERK1/2 and p38 MAPK activation. The increase in cell protein content induced by AII entails formation of cortical actin ring and Rac-dependent activation of ERK1/2 and p38. They also showed that AII-induced activation of ERK1/2 and p38 are implicated in aldosterone secretion by enhancing expression of Star gene and 3β-hydroxysteroid dehydrogenase (Otis et al., 2007). According to Casal et al. (2007), AII activated ERK1/2 within 10 min in bovine adrenocortical glomerulosa cells, a maximal activation was achieved within 30 min and ERK1/2 phosphorylation levels decreased thereafter.

In adrenal zona fasciculata cells, using the Y1 cell line as a system, Le and Schimmer (2001), showed that ACTH increases MEK phosphorylation and MEK activity in wild-type Y-1 cells and in the protein kinase deficient mutant Kin-8. The effect of ACTH on MEK and ERK (5 min incubation) is evidenced in growth arrested cells but not in logarithmically growing cells. In agreement with these results, Lotfi et al. (2000) showed that FGF2 elicits a strong mitogenic response in G_0/G_1-arrested cells with a rapid and transient activation of ERK. Using rats exposed to different ACTH dosage as well as variable duration, Ferreira et al. (2007) showed that ACTH increased adrenal weight and corticosterone levels when compared with control or dexamethasone-treated animals. They also showed that ACTH increases ERKs activation in a time and dose dependent manner. They conclude that chronic ACTH induces ERKs activation and that this plays an important role in the induction of cell proliferation as well as in steroidogenesis (Ferreira et al., 2007).

It is interesting that treatment of MA-10 Leydig cells with phorbol-12-myristate-13-acetate (PMA) to activate PKC enhances Star gene expression (associated with a slight increase in progesterone synthesis) but not its phosphorylation, detected by the use of an antibody that recognizes a PKA-dependent phosphorylation. Inhibition of ERK1/2 activity by U0126 decreased PMA-stimulated Star gene expression (Manna et al., 2007).

It appears that the activation of the MEK1/2–ERK1/2 cascade enhances steroid synthesis. In contrast, whether the MEK1/2, ERK1/2 cascade is necessary for the induction of Star gene expression is less obvious. In addition, although it is known that the activity of StAR protein can be regulated by post-translational phosphorylation, it was not clear until recently that StAR protein is a target for the MEK1/2, ERK1/2 cascade.

In this regard, we recently described the role of MEK1/2–ERK1/2 cascade in the hCG/LH stimulation of StAR protein activity and steroid synthesis (Poderoso et al., 2008). As described by Manna et al. (2007) and Martinelle et al. (2004), we showed that PKA is an upstream kinase in the stimulation of MEK and ERK activities. The effect observed with the inhibitors of MEK1/2 on progesterone synthesis induced by hCG or cAMP were not mediated by inhibition of PKA, since this enzyme remained fully active in the presence of both inhibitors U0126 and PD98095 (Poderoso et al., 2008). These inhibitors had not effect on the activity of the P450scc, since 22R-OH-cholesterol, a freely diffusible analogue of cholesterol, initiated steroid production even when the inhibitors were in the culture medium (Martinelle et al., 2004; Poderoso et al., 2008).

We also showed that active ERK1/2 is necessary for steroidogenesis using a different approach. The overexpression of a wild-type form of ERK2 in MA-10 Leydig cells produced an increase in steroid production stimulated by submaximal concentration of cAMP (Poderoso et al., 2008). An inactive form of ERK2, the H230R variant, which fails to interact with MEK1, but retains the ability to interact with MEK2 in a weakened fashion, did not produce the effect of wild-type ERK2 (Poderoso et al., 2008).

3. MEK1/2 and ERK1/2 at the mitochondria

It is interesting that the inhibitors appear to act at a site in the regulatory pathway after PKA activation and before the transport of cholesterol. Thus, one of the targets may be located at the mitochondria site. Gyles et al. (2001) have observed that activation of adenylate cyclase by forskolin caused a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus. We demonstrated by western blot analysis and confocal studies that in addition to a time-dependent increase in ERK activity and translocation from cytoplasm to nucleus there is a temporal mitochondrial ERK1/2 activation which is obligatory for PKA-mediated steroidogenesis in Leydig cells (Poderoso et al., 2008). It is important to note that the phosphorylation of mitochondrial ERK precedes the