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Review

Tyrosine phosphatases in steroidogenic cells: Regulation and function

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Abstract

In adrenocortical and Leydig cells PKA activation by trophic hormones increases the activity of protein tyrosine phosphatases and also induces the expression of MAP kinase phosphatase 1 (MKP-1), a dual activity protein phosphatase (serine/threonine and tyrosine). This work summarizes the knowledge on the regulation and the role played by cAMP-activated tyrosine phosphatases as well as MKP-1 in the hormonal activation of the acute and chronic phases of steroidogenesis.

Keywords: Tyrosine phosphatases; MAP kinase phosphatase; StAR; Acyl-CoA-synthetase; Hydroxylase

Contents

1. Introduction

Protein phosphorylation is an integral component of signal transduction pathways within eukaryotic cells and it is regulated by the fine interplay of protein kinases and phosphatases. Whereas until 1980 only phosphoserine and phosphothreonine had been identified as phosphoamino acids naturally present in the cellular proteins, today the role of tyrosine phosphorylation in important cellular processes as proliferation, differentiation and migration is well recognized (Walton and Dixon, 1993; Bourdeau et al., 2005).

Proteins with activity of tyrosine phosphatase include "classical" protein tyrosine phosphatases (PTPs) able to dephosphorylate phosphotyrosine residues and "dual-specificity" phosphatases (DSPs) which act on phosphoserine/phosphothreonine and on phosphotyrosine residues (Walton and Dixon, 1993; Stoker, 2005). Important members of DSPs group are the phosphatases acting on the regulation of MAP kinases.

This work attempts to summarize the knowledge about the hormonal action on tyrosine phosphatases and the role of these enzymes in steroid-producing cells.

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2. Activation of tyrosine phosphatases by PKA action in steroid-producing cells

In steroidogenic cells, the hormonal regulation of members of both families of tyrosine phosphatases has been analyzed. One of the early works about tyrosine dephosphorylation in steroid producing cells was reported by Han and Rubin (1996). They observed that increased cAMP content in Y1 adrenocortical cells triggers the rapid and selective dephosphorylation of tyrosine residues on paxillin, a focal adhesion protein (Burridge et al., 1992) which is maximally dephosphorylated before cAMP-induced changes in cell shape are detected. Notably, protein tyrosine phosphatase inhibitors abrogate all such effects of cAMP. Those investigators then postulated that cAMP may regulate the paxillin phosphorylation state by eliciting an increase in tyrosine phosphatase activity rather than by an inhibitory action on tyrosine kinases. Later studies showed the induction of paxillin dephosphorylation by ACTH also in bovine adrenocortical cells (Vilgrain et al., 1998). Nevertheless, none of these studies included a direct determination of an ACTH-dependent tyrosine phosphatase activity. We demonstrated that in rat adrenal zona fasciculata (ZF) *in vivo* ACTH treatment reduces phosphotyrosine content in endogenous proteins, one of them identified as paxillin, and produces a transient increase of tyrosine phosphatase activity in the cytosolic fraction reaching a maximum (two-fold) after 15 min (Paz et al., 1999). *In vitro* incubation of adrenal ZF with 8Br-cAMP also produces tyrosine phosphatase activation, suggesting that it is mediated by PKA-dependent phosphorylation events.

Using an in gel tyrosine phosphatase assay, we showed that ACTH promotes the rapid activation of more than one tyrosine phosphatase in rat adrenal ZF (Paz et al., 1999). These ACTH-activated enzymes include a protein of 120 kDa whose activity is increased by *in vitro* phosphorylation with PKA (Paz et al., 2000). To date our studies are focused on the identification of the ACTH-activated tyrosine phosphatases from ZF. The fact that ACTH promotes the PKA-dependent phosphorylation and activation of the phosphatase PTP1D in bovine adrenocortical cells (Rocchi et al., 2000) strongly supports the notion that this enzyme could be a target of ACTH action also in rat adrenal ZF.

In Leydig cells, the effects of cAMP on tyrosine phosphatase activity seem to be similar to those observed in adrenocortical cells. Indeed, we demonstrated the cAMP-dependent activation of tyrosine phosphatases of 120 and 80 kDa in MA-10 cells (Cornejo Maciel et al., 2001).

3. Transcriptional regulation of tyrosine phosphatases

3.1. MAP kinases and MAP kinase phosphatases in ACTH- and LH-signaling pathway

MAP kinases (MAPKs: ERKs, JNKs/SAPKs and p38) constitute a family of cytoplasmic serine/threonine kinases ubiquitously expressed that play a crucial role in transmitting transmembrane signals required for cell growth, differentiation and apoptosis (Kelly and Chu, 2000; Pearson et al., 2001). A common feature of MAP kinases is the requirement of dual threonine and tyrosine phosphorylation to display maximal activity (Kelly and Chu, 2000). Upon stimulation, MAP kinases translocate to the nucleus where they phosphorylate transcription factors and thus, regulate gene transcription.

Although the acute and chronic regulation of adrenal function by ACTH (Rae et al., 1979; Wong et al., 1992; Schimmer, 1995) is primarily mediated by PKA-phosphorylated proteins (Podesta et al., 1979; Rae et al., 1979; Sala et al., 1979), ACTH also prompts the activation of members of MAP kinases (Watanabe et al., 1997; Le and Schimmer, 2001). A similar set of protein kinases is also activated in Leydig cells by LH/CG (Dufau et al., 1977; Hirakawa and Ascoli, 2003).

The hormonal action on MAP kinases family members in steroidogenic cells has been analyzed using several experimental models. Watanabe et al. demonstrated that ACTH stimulates JNK activity three- to four-fold both in the adrenal cortex *in vivo* and in the Y1 mouse adrenocortical cell line through a PKC and Ca^{2+}-dependent pathway (Watanabe et al., 1997). In Y1 cells, ACTH also stimulates the phosphorylation and activation of ERK1/2 involving MAPK kinase MEK (Le and Schimmer, 2001). Given that the ability of ACTH to activate MAPK pathway is not disrupted in the protein kinase A-defective Y1 mutant Kin-8, the authors concluded that ACTH action on ERK1/2 activation is independent on PKA activity. A different mechanism seems to be involved in the activation of ERK1/2 by LH in Leydig cells. In fact, the phosphorylation of ERK1/2 in MA-10 Leydig cells is mediated by a PKA-dependent activation of RAS, a component upstream of ERK1/2 in the MAPK cascade (Hirakawa and Ascoli, 2003).

Since MAPK activation depends on phosphorylation processes, the magnitude and duration of their activity are linked to the activity of phosphatases capable to prompt MAPK dephosphorylation. MAPK phosphatases (MKPs) constitute a family of dual specificity phosphatases specifically involved in the dephosphorylation of all MAPK family members (Kelly and Chu, 2000; Keyse, 2000; Theodosiou and Ashworth, 2002). Several distinct mammalian MKP family members have been identified and characterized and they can be divided in two broad classes. One group, typified by MKP-1, comprises nuclear enzymes rapidly induced by growth factors or stress signals (Rohan et al., 1993; Keyse, 2000). The second group, typified by MKP-3, includes predominantly cytosolic enzymes induced with delayed kinetics by specific stimuli, but not by environmental stress (Groom et al., 1996; Dowd et al., 1998). Some aspects of the expression of a MKP family member in steroid-producing cells are presented in the following sections.

3.2. ACTH action on MKP-1 gene expression in adrenocortical cells

The fact that ACTH increases MAPK activities suggests that MKPs could be also regulated by this hormone. Then, we analyzed its effect on the dual phosphatase MKP-1 in Y1 cells (Bey et al., 2003). This study showed that ACTH transiently increases both MKP-1 mRNA and protein levels. ACTH-mediated mRNA increase is blunted by actinomycin D, denoting a hormonal action on MKP-1 gene transcription and not on mRNA stability.

Temporal profile of MKP-1 mRNA levels shows that the increase is evident after 30 min, peaks at 1 h (six-fold) and returns to basal levels thereafter. Whereas the temporal profile of MKP-1 mRNA amounts induced by 8Br-cAMP resembles the effect elicited by ACTH, 8Br-cAMP produces a weaker effect (four-fold). Based on this difference and on the results that show that PKA inhibition abrogates the effect of 8Br-cAMP on MKP-1 messenger but only partially reduces the induction produced by ACTH, we suggested that both, PKA-dependent and independent mechanisms participate in the rapid increase of MKP-1 (Bey et al., 2003).

Sewer and Waterman analyzed the hormonal regulation of MKP-1 also in human adrenocortical cells. They described that in H295R human adrenocortical cells ACTH induces MKP-1 protein and mRNA levels and that MKP-1 can be phosphorylated by PKA action in both, *in vivo* and *in vitro* assays (Sewer and Waterman, 2003).

3.3. Effects of cAMP and heat stress on MKP-1 gene expression in MA-10 Leydig cells

Given that ACTH and LH/CG share common events in their signaling cascades, PKA being one of the most characterized components, the induction of MKP-1 by cAMP in Leydig cells is expected. We demonstrated that in MA-10 Leydig cells 30 min of stimulation with 8Br-cAMP are enough to increase MKP-1 mRNA levels and that after 60 min the stimulation is maximal (four-fold) (Fig. 1Panel A).

In several systems, stimuli which prompt MAPK activation also induce MKP-1 (Kelly and Chu, 2000). Moreover, several reports depict the action of MAPKs as component of the signaling cascade that lead to MKP-1 induction (Cook et al., 1997; Schliess et al., 1998). Our studies indicated that in MA-10 Leydig cells the induction of MKP-1 triggered by cAMP is independent of ERK1/2 since a Mek inhibitor (PD98059) has no effect on MPK-1 mRNA accumulation (Fig. 1Panel B). These results are in agreement with our current studies in Y1 cells, where ACTH induces MKP-1 by a mechanism independent of ERK1/2 pathway.

Our finding showing that cAMP is able to induce MKP-1 not only in adrenocortical cells, but also in Leydig cells strengthens the putative participation of this phosphatase in cAMP-stimulated steroid production.

As previously mentioned, stress signals also are able to induce MKP-1. Leydig cells are highly sensitive to heat stress and steroid production is severely but transiently reduced by this stressor (Liu and Stocco, 1997; Murphy et al., 2001). With this in mind, we demonstrated that in MA-10 cells, heat shock (HS, 10 min, 45 °C) induces not only the rapid and transient activation of MAPKs involved in the cell survival and cell death, such as ERK1/2 and JNK1/2, respectively, but also the induction of MKP-1 (Gorostizaga et al., 2005). HS-induced MKP-1 mRNA level was significant at 30 min, reached a maximum at 60 min (three-fold) and declined thereafter. Moreover, the kinetics of MKP-1 protein induction is congruent with the kinetics of MAPK dephosphorylation, suggesting that MKP-1 recognizes both ERK1/2 and JNK1/2 as targets (Gorostizaga et al., 2005).

While 10 min at 45 °C blocks steroid production in MA-10 cells, this stress condition produces little or no effect on steroidogenesis in Y1 cells (Murphy et al., 2001). This well documented difference suggests that the signal cascades triggered by HS in MA-10 and Y1 cells are different. However, HS promotes ERK1/2 and JNK1/2 activation and MKP-1 induction in Y1 cells in a similar way as detected in MA-10 Leydig cells (Gorostizaga et al., 2004). Therefore, the minor sensitivity of adrenocortical cells to HS could not be due to a differential expression of MKP-1. Whether or not this phosphatase is involved in the recovery of the steroidogenic capability after HS remains undetermined.

As it was mentioned above, MKP-1 induction by cAMP in Leydig cells is independent of ERK1/2 activities. In contrast, the induction triggered by HS requires ERK1/2 action (Gorostizaga et al., 2005). Therefore, the regulation of MKP-1 mRNA levels appears to be regulated by ERK1/2-dependent or independent pathways in a stimulus dependent fashion.

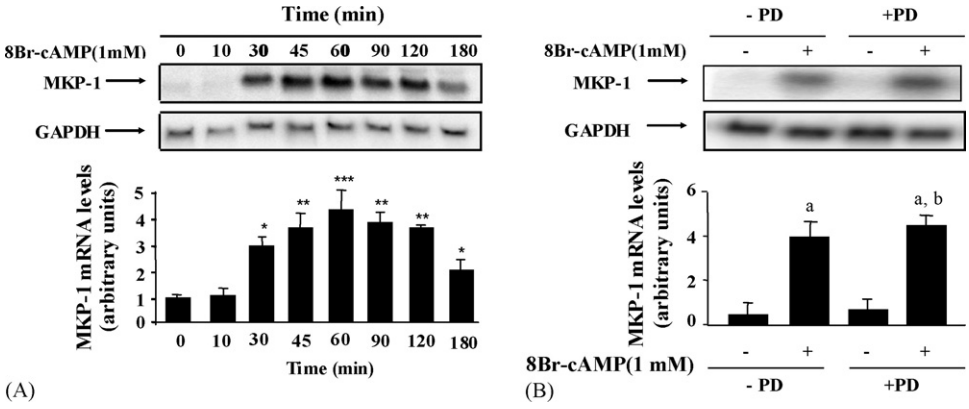

Fig. 1. 8Br-cAMP induces MKP-1 mRNA by an ERK independent mechanism in MA-10 cells. Serum starved cells were stimulated with 8Br-cAMP (1 mM) in the absence (Panel A) or in the presence of PD98059 (Panel B). MKP-1 mRNA was analyzed by Northern blot using specific probes. The figure shows autoradiograms of a representative experiment. The autoradiograms were quantitated by scanning densitometry and MKP-1 signal was normalized against the signal of GAPDH mRNA. The graphs represent the results obtained from three independent experiments (mean ± S.D., in arbitrary units). *Panel A*: Time course of MKP-1 induction triggered by 8Br-cAMP: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, vs. time 0. *Panel B*: Effect of PD98059 on MKP-1 induction after 60 min of stimulation with 8Br-cAMP: a, $P < 0.001$ vs. non-stimulated cells; b, ns vs. non-PD treated cells.

4. Functional analysis of tyrosine phosphatases in the hormonal regulation of steroidogenesis

4.1. Potential targets of phosphatase activity

It is well established that ACTH and LH exert their stimulatory action on steroid biosynthesis by evoking acute and chronic responses stimulated by PKA. The chronic response of ACTH and LH includes the induction of genes encoding enzymes involved in steroid synthesis, mainly steroid hydroxylases (CYPs) (Simpson et al., 1990). In contrast, in the acute phase of steroidogenesis the levels of hydroxylases remain unchanged (Koritz and Kumar, 1970).

Regarding the acute response to steroidogenic hormones, the PKA-mediated induction of the steroidogenic acute regulatory (StAR) protein is a well characterized early event in the hormonal action. StAR protein has a key role on the stimulation of steroidogenesis (Clark et al., 1994; Stocco and Clark, 1996). In fact, this protein increases cholesterol transport to the inner mitochondrial membrane, the rate limiting step in the stimulation of steroidogenesis (Crivello and Jefcoate, 1980), where steroid synthesis is initiated.

Arachidonic acid (AA) release is another event triggered by PKA and involved in the delivery of cholesterol into the mitochondria. Our group has characterized a system that acts in a concerted fashion to release AA in steroidogenic cells (Maloberti et al., 2002). This system is composed by an acyl-CoA synthetase (ACS4) and a thioesterase (Acot2) (Paz et al., 1994; Finkielstein et al., 1998). In turn, this AA and/or its metabolites stimulate the access of cholesterol to the mitochondria by an action on StAR induction (Maloberti et al., 2005). PKA regulates this pathway increasing Acot2 activity and up-regulating ACS4 protein levels (Cornejo Maciel et al., 2005). The crucial role of these enzymes in steroidogenesis is certainly demonstrated by the fact that StAR expression and steroidogenesis are reduced in cells where Acot2 or ACS4 expression is blocked (Maloberti et al., 2005).

The knowledge summarized above indicates that StAR and ACS4 are key proteins of the acute hormonal response whereas steroid hydroxylases are key proteins in the chronic response to the hormone. A common feature of all these proteins is the fact that the hormonal action increases their levels by a PKA-dependent mechanism. Moreover, several reports indicate that protein phosphatase activity could be another common event in the mechanism of induction of these proteins.

4.2. Role of cAMP-activated tyrosine phosphatases in steroidogenesis

The role played by tyrosine phosphatase activity in the acute steroidogenic response to cAMP was determined using tyrosine phosphatase cell permeant inhibitors: pervanadate (PV) and phenylarsine oxide (PAO). The results indicate that the action of tyrosine phosphatase(s) in a site located downstream of PKA and upstream of the access of cholesterol to the inner mitochondrial membrane is a requirement for adrenal and Leydig cell steroidogenesis (Paz et al., 1999; Cornejo Maciel et al., 2001). Moreover, our results indicate that the action of tyrosine phosphatases on steroidogenesis is due to an action on StAR gene expression (Paz et al., 2002).

Recently, we demonstrated that inhibition of tyrosine phosphatase activity abrogates the hormonal effect on ACS4 and StAR protein levels (Cornejo Maciel et al., 2005; Cano et al., 2006). Since exogenous AA is able to overcome the effect of a tyrosine phosphatase inhibitor on StAR protein level, we proposed a model where the sequence of events triggered by ACTH/LH/cAMP includes the PKA-mediated activation of tyrosine phosphatase(s), the action of these enzymes on ACS4 induction, the AA release mediated by the system ACS4/Acot2 and the action of AA (or its metabolites) on StAR induction.

As it was mentioned, our works suggest the action of tyrosine phosphatases on StAR mRNA induction mediated by cAMP. Besides, the cAMP-mediated activation of tyrosine phosphatases in adrenocortical and Leydig cells is a rapid event. Moreover, PTP1D activation in bovine adrenocortical cells is detected after a few minutes of ACTH stimulation. Thus, it is proposed that the activation of a tyrosine phosphatase, instead of the induction of a tyrosine phosphatase is responsible of both the increase of tyrosine phosphatase activity and the activation of StAR induction. In this context, ACS4 induction and AA release may be a link between tyrosine phosphatase activity and StAR induction, as we already proposed (Cornejo Maciel et al., 2005).

Another possible site of action in the acute stimulation of steroidogenesis could be on the subcellular structures since cytoskeleton components appear to have a role in steroidogenesis (Hall and Almahbobi, 1997). It was already mentioned that ACTH promotes tyrosine dephosphorylation of paxillin, a cytoskeletal protein. Thus, it is plausible that at least one tyrosine phosphatase activated by hormone action causes paxillin-associated changes in the cellular architecture that impair the movements of cholesterol-containing lipid droplets towards the mitochondria.

4.3. Role of hormone-induced MKP-1 in steroidogenesis

The findings on MKP-1 induction by ACTH reveal a role of ACTH to counteract MAP kinase activation. Indeed, we observed that the rate of ERK dephosphorylation in ACTH-stimulated Y1 cells is slower than in cells stimulated in the presence of a protein synthesis inhibitor (Bey et al., 2003). It is possible to attribute these differences to the lack of MKP-1 in cells stimulated under conditions where MKP-1 synthesis is blocked. On the other hand, the fact that MKP-1 gene expression is increased by a steroidogenic hormone such as ACTH raises the question whether MKP-1 function is linked to the regulation of steroid synthesis. Moreover, whereas MKP-1 induction by ACTH could be related to the adrenocortical stress response, the induction of MKP-1 by cAMP also in Leydig cells further supports a common functional role of this enzyme in steroid producing cells rather than a specific role in adrenocortical cells. In this regard, recent works highlight the relevance of protein phosphatase activity in steroid hormone biosynthesis by its action on the expression of steroidogenic enzymes.

SF-1 is an orphan nuclear hormone receptor which participates in the expression of genes encoding proteins involved in steroidogenesis: cytochrome P450 side-chain cleavage enzyme, several steroid hydroxylases (CYPs) (Lee et al., 1996; Hu et al., 2001), StAR (Sugawara et al., 1996) and melanocortin 2 receptor (Mc2R) (Naville et al., 1999). Thus, the action of SF-1 is a common component in early and delayed transcriptional events triggered by hormonal action in steroidogenic systems. It was demonstrated that serine/threonine and tyrosine phosphatase inhibitors block the cAMP-inducible binding of SF-1 to the promoter of the human CYP17 (hCYP17) gene and also the expression and transcriptional activity of the hCYP17 (Sewer and Waterman, 2002), suggesting that a dual activity phosphatase could be involved in this process. Later, Sewer and Waterman (2003) demonstrated that ACTH and cAMP increase MKP-1 expression in H295R human adrenocortical cells and that the overexpression of this enzyme increases the transcriptional activity of a human CYP17 promoter-reporter construct. These results, in addition to the facts that cAMP induces SF1 dephosphorylation and that cAMP-stimulated hCYP17 expression is attenuated by silencing MKP-1 expression, highlight the role of MKP-1 on cAMP-dependent transcriptional activation of hCYP17. Moreover, all this summarized knowledge strongly suggests that in addition of CYP17 other SF-1-regulated genes could be targets of MKP-1.

StAR gene expression is another process involving SF-1 participation. In Y1 adrenocortical cells and MA-10 Leydig cells hormone-dependent ERK activation results in enhanced phosphorylation of SF-1 and increased steroid production through increased transcription of StAR gene (Gyles et al., 2001). Then, MKP-1 could impair StAR gene expression by its action on ERK activity, revealing opposite effects of MKP-1 on CYPs and StAR expression. However, it is unlikely that MKP-1 interferes in StAR gene expression since ERK activation and SF-1 binding to StAR promoter precede the increase in MKP-1 protein levels. Rather, it is possible that a coordination of the phosphorylation and dephosphorylation of SF-1 leads to the temporal activation of specific sets of SF-1-regulated genes. Indeed, a kinetic model of SF-1 transcriptional activation has been recently proposed (Winnay and Hammer, 2006).

5. Conclusions, remarks and perspectives

All recent works summarized in this review reveal that steroidogenic hormones can modulate tyrosine phosphatase activity and also the level of expression of these enzymes. One tyrosine phosphatase which expression is induced by hormonal action is MKP-1; however, we cannot rule out that other tyrosine phosphatases – "classical" PTPs or DSPs – could be also target of hormonal modulation at transcriptional level. Regarding tyrosine phosphatases activated by hormonal action, the knowledge is more limited. Indeed, whereas in bovine adrenocortical cells it was determined that PTP1D is activated by ACTH/cAMP, we have demonstrated the activation of tyrosine phosphatases of 120, 80 and 50 kDa in rat ZF whose identification remains elusive. The most important challenge for our future work is to identify these proteins – as well as the corresponding substrate – since this is the only way to determine the true role of each hormone-activated tyrosine phosphatase in the biology of the steroid-producing cells. In this context, the knowledge on MKP-1 expression triggered by cAMP has contributed to elucidate the mechanism by which the second messenger regulates the expression of steroid hydroxylases. Moreover, important advances in the knowledge surrounding the relationship between MKP-1 and SF-1 highlight the participation of this enzyme in determining the dynamic character of SF-1 transcriptional activity and also on its potential action on the expression of other genes related to the steroidogenic function. In this regard, even merely speculative, ACS4 could be target of SF-1 and consequently, subjected to MKP-1 modulation.

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