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AN ACTH-ACTIVATED PROTEIN TYROSINE PHOSPHATASE (PTP) IS MODULATED BY PKA-MEDIATED PHOSPHORYLATION

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ABSTRACT

In adrenal cortex, ACTH regulation of steroidogenesis depends on PKA-dependent serine/threonine phosphorylation and also on the activity of protein tyrosine phosphatases (PTPs). In addition, ACTH increases total PTPs involving at least three soluble PTPs (50, 82 and 115 kDa). Serine/threonine phosphorylation of these enzymes themselves could be a regulatory mechanism of their activity since the increase of total PTP activity is dependent on PKA-activation. In this report we analyzed the effect of *in vitro* phospho-dephosphorylation processes on the activity displayed by the ACTH-activated PTP of 115 kDa. Using an in-gel PTP assay we demonstrate that dephosphorylation catalyzed by potato acid phosphatase (PAP) reduces the activity of the 115 kDa PTP present in ZF from ACTH-treated animals and PKA-mediated phosphorylation reverses this effect.

INTRODUCTION

In adrenal zona fasciculata (ZF), corticotropin (ACTH) activates PKA-dependent serine/threonine phosphorylation leading ultimately to increased biosynthesis of steroids (1).

Our recent reports indicate that, in addition to the well characterized serine/threonine phosphorylation events, ACTH signaling pathway also includes

tyrosine dephosphorylation. We have demonstrated that, in rat adrenal ZF, protein tyrosine phosphatase (PTP) activity is required for ACTH-stimulated steroidogenesis and that this hormone produces a rapid a transient increase of total PTP activity in the cytosolic fraction through a cAMP-dependent pathway (2,3). PTPs comprise a structurally diverse family of enzymes, which includes both transmembrane receptor-like and non-transmembrane forms (4,5). Several mechanisms are involved in the regulation of these enzymes, including serine/threonine phosphorylation. Such mechanism would be implicated in the regulation of ACTH-activated PTP(s), since a cAMP analogue mimics the effects of the hormone on the activity of these enzymes.

As an approach to determine the role of serine/threonine phosphorylation on the activities displayed by the ACTH-activated PTPs, we analyzed the PTP activity profile of adrenal ZF soluble proteins, following incubation with potato acid phosphatase (PAP). Here, we present evidence for the activation by serine/threonine phosphorylation of the PTP with a mass of 115 kDa.

MATERIALS AND METHODS

Adult male Wistar rats were supplied with dexamethasone (10 μg/ml, *ad libitum*) in the drinking water for 16 h before sacrifice. Following sacrifice, adrenal glands were decapsulated, homogenized and subjected to cellular fractionation. Cytosolic ZF proteins from control or ACTH-treated rats (200 μg/kg) were incubated with potato acid phosphatase (PAP). In other experiments, samples were subjected to PKA phosphorylation after PAP action. Finally, all samples were subjected to the in-gel PTP assay, following described procedures (3,6). PTP activity is evidenced in the autoradiogram as clear bands on a dark background.

RESULTS AND DISCUSSION

ACTH administration (15 min) increases PTP activity corresponding to proteins of 115, 82 and 50 kDa, as shown in Fig. 1. In order to determine if phospho-dephosphorylation processes are involved in the regulation of the 115 kDa PTP, we

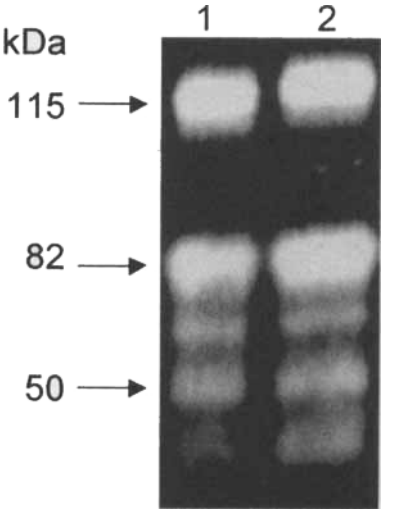

FIGURE 1
Partial identification of corticotropin-activated PTPs in adrenal ZF. Twenty micrograms of cytosolic proteins of adrenal ZF obtained from control (lane 1) and ACTH-treated rats (15 min) (lane 2) were analyzed by in-gel PTP assay. The figure shows the autoradiogram of the gel.

first incubated adrenal ZF proteins from control and ACTH-treated rats with PAP. Incubation with PAP partially reduced the activity of 115 kDa PTP present in the cytosolic fraction derived from ACTH-treated rats (Fig. 2). This result suggests that the 115 kDa PTP activity is modulated by phosphorylation. Therefore, we attempted to reverse the action of PAP with catalytic subunit of PKA. The activity of 115 kDa PTP present in ZF soluble proteins obtained from ACTH-treated animals was reduced by incubation with PAP and this effect was reversed by the subsequent incubation with PKA as evidenced in Fig. 3. PTPs corresponding to 82 and 50 kDa proteins apparently did not change their activity by phosphate removal by PAP.

In this work we set out to test the hypothesis that an ACTH-activated PTP of 115 kDa is regulated by PKA-mediated phosphorylation. Our results demonstrate that, indeed, protein phosphorylation is involved in the regulation of that enzyme. Two evidences support this notion: first, the activity of ACTH-activated 115 kDa PTP was

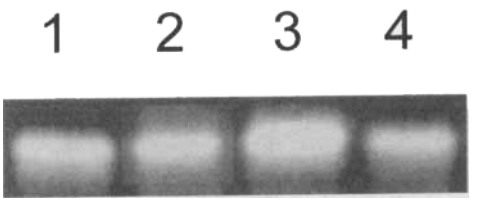

FIGURE 2
Effect of the dephosphorylation catalyzed by PAP on the activity of 115 kDa PTP from rat ZF. Adult male rats were treated with ACTH for 15 min. Soluble ZF proteins (20.μg) were incubated with PAP (1 U) for 15 min at 30°C or 0°C and subjected to in-gel PTP assay. The autoradiogram shows the activity displayed by the 115 kDa PTP. Lanes 1 and 2, proteins from unstimulated ZF incubated at 30°C and 0°C respectively. Lanes 3 and 4, proteins from ACTH-stimulated ZF incubated at 0°C and 30°C respectively. This is a representative experiment, independently performed twice.

reduced by incubation with phosphatase; second, the effect of phosphatase treatment was reversed by subsequent incubation with PKA.

PAP treatment reduced PTP activity although not completely and it is noteworthy that the enzyme is active even in control ZF. Those results could be due to an incomplete action of PAP or could also indicate that the enzyme displays negligible activity in a dephosphorylated state. Then, PKA-mediated phosphorylation might not be essential for enzyme activity. Rather, that event could modulate PTP activity, perhaps by enhancing the affinity of the enzyme for its substrate(s).

Given the fact that PKA reverses the action of PAP upon the activity displayed by the 115 kDa PTP, we suggest that the activation is due to phosphorylation of the enzyme. However, still we can not discern whether this is a direct effect of PKA on the 115 kDa PTP or an indirect phosphorylation event mediated by a second kinase present in the sample.

At this point, the identity of the 115 kDa PTP is unknown. Although several serine/threonine phosphorylation-regulated PTPs have been described (7,8), there are few potential candidates among the known PTPs that match the PTP described here. One such candidate could be PTP H1, a human PTP of 120 kDa member of an expanding group of PTPs characterized by N-terminal segments with homology to the

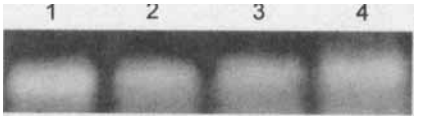

FIGURE 3
Effect of the incubation with PKA on the activity of the dephosphorylated 115 kDa PTP. Soluble ZF proteins from ACTH-treated animals were analyzed by in-gel PTP assay after incubation with PAP and subsequent incubation PKA as described in Methods. The autoradiogram shows the activity displayed by the 115 kDa PTP present in samples incubated with PAP for 10 min at 0°C (lane 1) or 30°C (lane 2) and in samples incubated with PKA for 10 min at 0°C or 30°C after incubation with PAP for 10 min at 30°C (lanes 3 and 4 respectively).

band 4.1 protein (9). PTP H1 is phosphorylated *in vitro* to different levels by several protein serine/threonine kinases, including PKA and the phosphorylation increases the affinity of PTP H1 for its substrates (10).

In summary, we have demonstrated that serine/threonine phosphorylation can regulate the activity of an ACTH-activated PTP of 115 kDa. Thus, the regulation of total PTP activity by ACTH might be attributed, at least in part, to the phosphorylation of this PTP by PKA. Further characterization of the 115 kDa PTP may help to determine the identity of the protein, and most importantly, its role in ACTH signaling pathway.

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