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Regulation of AMP-activated protein kinase by cAMP in adipocytes: Roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis

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ABSTRACT

AMP-activated protein kinase (AMPK) is an important regulator of cellular energy status. In adipocytes, stimuli that increase intracellular cyclic AMP (cAMP) have also been shown to increase the activity of AMPK. The precise molecular mechanisms responsible for cAMP-induced AMPK activation are not clear. Phosphodiesterase 3B (PDE3B) is a critical regulator of cAMP signaling in adipocytes. Here we investigated the roles of PDE3B, PDE4, protein kinase B (PKB) and the exchange protein activated by cAMP 1 (Epac1), as well as lipolysis, in the regulation of AMPK in primary rat adipocytes. We demonstrate that the increase in phosphorylation of AMPK at T172 induced by the adrenergic agonist isoproterenol can be diminished by co-incubation with insulin. The diminishing effect of insulin on AMPK activation was reversed upon treatment with the PDE3B specific inhibitor OPC3911 but not with the PDE4 inhibitor Rolipram. Adenovirus-mediated overexpression of PDE3B and constitutively active PKB both resulted in greatly reduced isoproterenol-induced phosphorylation of AMPK at T172. Co-incubation of adipocytes with isoproterenol and the PKA inhibitor H89 resulted in a total ablation of lipolysis and a reduction in AMPK phosphorylation/activation. Stimulation of adipocytes with the Epac1 agonist 8-pCPT-2′O-Me-cAMP led to increased phosphorylation of AMPK at T172. The general lipase inhibitor Orlistat decreased isoproterenol-induced phosphorylation of AMPK at T172. This decrease corresponded to a reduction of lipolysis from adipocytes. Taken together, these data suggest that PDE3B and PDE4 regulate cAMP pools that affect the activation/phosphorylation state of AMPK and that the effects of cyclic AMP on AMPK involve Epac1, PKA and lipolysis.

1. Introduction

AMP-activated protein kinase (AMPK) is a critical regulator of energy homeostasis. It functions as a cellular energy sensor, responding to increases in the AMP:ATP ratio by inactivating energy consuming processes such as lipogenesis and cholesterol biosynthesis, while activating energy producing processes such as fatty acid oxidation [1]. AMPK is a heterotrimeric enzyme with alpha, beta and gamma subunits, the alpha subunit being the catalytic subunit [2]. Activation of AMPK is known to be mediated by phosphorylation of the catalytic subunit at threonine 172 (T172) by the upstream kinases LKB1 and Ca^{2+}-calmodulin kinase kinase β [3–5]. It is also thought that regulation of AMPK activity is increased directly by the binding of AMP to the regulatory subunit which also makes the holoenzyme resistant to dephosphorylation by phosphatases [6]. It has been shown that ischemia, oxidative stress and exercise increase the cellular AMP:ATP ratio and activate AMPK [7–9]. It has also been demonstrated in cultured adipocytes that agents which increase intracellular cAMP also increase the activity of AMPK [10,11]. In contrast, in cultured pancreatic β-cells agents that stimulate cAMP production resulted in decreased AMPK activity [12]. Although many of the downstream effects of cAMP are mediated by cAMP dependant protein kinase (PKA), exchange proteins directly activated by cAMP (Epac1 and Epac2) have more recently been shown to mediate signals downstream of cAMP independent of PKA [13,14]. Also, in some cell types, cAMP and cGMP mediate effects by binding to cyclic nucleotide gated ion channels [15]. The intracellular level of cAMP is regulated by the balance between production of cAMP by adenylate cyclase and the breakdown of cAMP by the actions of the 11 member superfamily of cyclic nucleotide phosphodiesterases (PDE1-11). Phosphodiesterase enzymes differ in their primary structure, affinities for cAMP and cGMP, responses to specific effectors and inhibitors, and mechanisms through which they are regulated [16].

PDE3B and PDE4 enzymes are the most abundant PDEs in adipocytes. Whereas little is known regarding the role of PDE4s in adipocyte metabolic functions, the role and regulation of PDE3B have been extensively studied [17,18]. Results from knockout and transgenic mouse models, as well as results from isolated primary

Abbreviations: PDE, phosphodiesterase; cAMP, cyclic AMP; Epac, exchange proteins directly activated by cAMP; AMPK, AMP-activated protein kinase; PKA, protein kinase A; PKB, protein kinase B; ACC, acetyl CoA carboxylase; ISO, isoproterenol; INS, insulin.

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adipocytes and β-cells demonstrate an important role of PDE3B in the regulation of glucose and lipid metabolism, in insulin secretion and in adipocyte functions [19–21]. For example, in adipocytes, insulin-induced phosphorylation and activation of PDE3B, mediated via protein kinase B (PKB) [22,23], is the major mechanism whereby insulin inhibits catecholamine-induced lipolysis. Furthermore, PDE3B plays an important role in the regulation of insulin-induced glucose uptake and lipogenesis in adipocytes [21].

It has been recently shown that lipolysis is at least partially required for the cAMP-induced AMPK activation in 3T3-L1 adipocytes [24]. The re-esterification of fatty acids that occurs during lipolysis leads to a depletion of ATP and an increase in the AMP:ATP ratio, thus activating AMPK [24]. Further studies have demonstrated that fatty acids themselves can lead to an increased activation of AMPK in HepG2 cells [25]. The mechanisms involved in the regulation of AMPK in response to changes in the intracellular level of cAMP in primary adipocytes are at this point unclear. It is currently believed that cAMP exists in cells as discrete pools, each with local expression of proteins that regulate, and are regulated by, cAMP. In this study we investigate the role of PDE3B, PDE4, PKA, PKB, Epac and lipolysis in the phosphorylation and activation of AMPK in primary adipocytes.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (from B&K Universal Stockholm, Sweden) between 36 and 42 weeks of age were used for the isolation of primary adipocytes. All experiments have been approved by the Animal Ethics Committee, Lund, Sweden.

2.2. Chemicals and reagents

Isoproterenol (SIGMA), Insulin (Novo Nordisk) and the Epac agonist 8-pCPT-2′-*O*-Me-cAMP (Biolog life-science institute) were used for the treatment of adipocytes. The PDE3 inhibitor OPC3911 (Otsuka pharmaceuticals), general lipase inhibitor Orlistat (kind gift of Dr. Maria Sörhede Winzell), PKA inhibitor H89 and PDE4 inhibitor Rolipram (both from Biomol international) were used as inhibitors. All antibodies used for immunoblotting, with the exception of anti-PDE3B (raised in-house in rabbits against the peptide CGYYGSGKMFRRPSLP from PDE3B) and total PKB (a kind gift from Dario Alessi, University of Dundee, raised against a peptide corresponding to residues 466–480 of rat PKBα), were from Cell Signaling Technologies. AMPKα1 antibody used for the AMPK activity assays was a kind gift of Professor Grahame Hardie, University of Dundee (U.K.). The peptide AMARAASAAALRRR (AMARA) used for the AMPK activity assays was synthesized by Dr. Graham Bloomberg, University of Bristol (U.K.).

2.3. Isolation of primary adipocytes

Primary rat adipocytes were isolated from epididymal fat pads as previously described [26]. Adipocytes were diluted to a concentration of 10% (v/v) in Krebs Ringer (KRH) buffer containing 25 mM Hepes, 200 nM adenosine, 2 mM glucose and 1% bovine serum albumin (BSA). One milliliter of cell suspension was incubated for 37 °C as indicated in Results. Incubations were stopped by washing the adipocytes in BSA free KRH buffer and homogenization in a buffer containing 50 mM TES, 2 mM EGTA, 1 mM EDTA, 250 mM sucrose, 40 mM phenylphosphate, 5 mM NaF, 1 mM dithioerythriol, 0.5 mM sodium orthovanadate, 10 μg/mL antipain, 10 μg/mL leupeptin, 1 μg/mL pepstatin A, pH 7.4. Homogenates were centrifuged at 5000 ×*g* for 5 min at 4 °C, the fat cake removed and the supernatants solublized in 1% (v/v) Nonidet P40 on ice for 15 min. Solublized lysates were centrifuged at 10,000 ×*g* for 10 min at 4 °C and protein concentrations were determined by the method of Bradford [27].

2.4. Adenovirus mediated overexpression of PDE3B and constitutively active PKB

Primary rat adipocytes (2.5 mL of 12.5% (v/v)) were infected with high titer virus stocks (1×10^{10} PFU/mL) encoding β-Galactosidase (Ad-β-GAL),

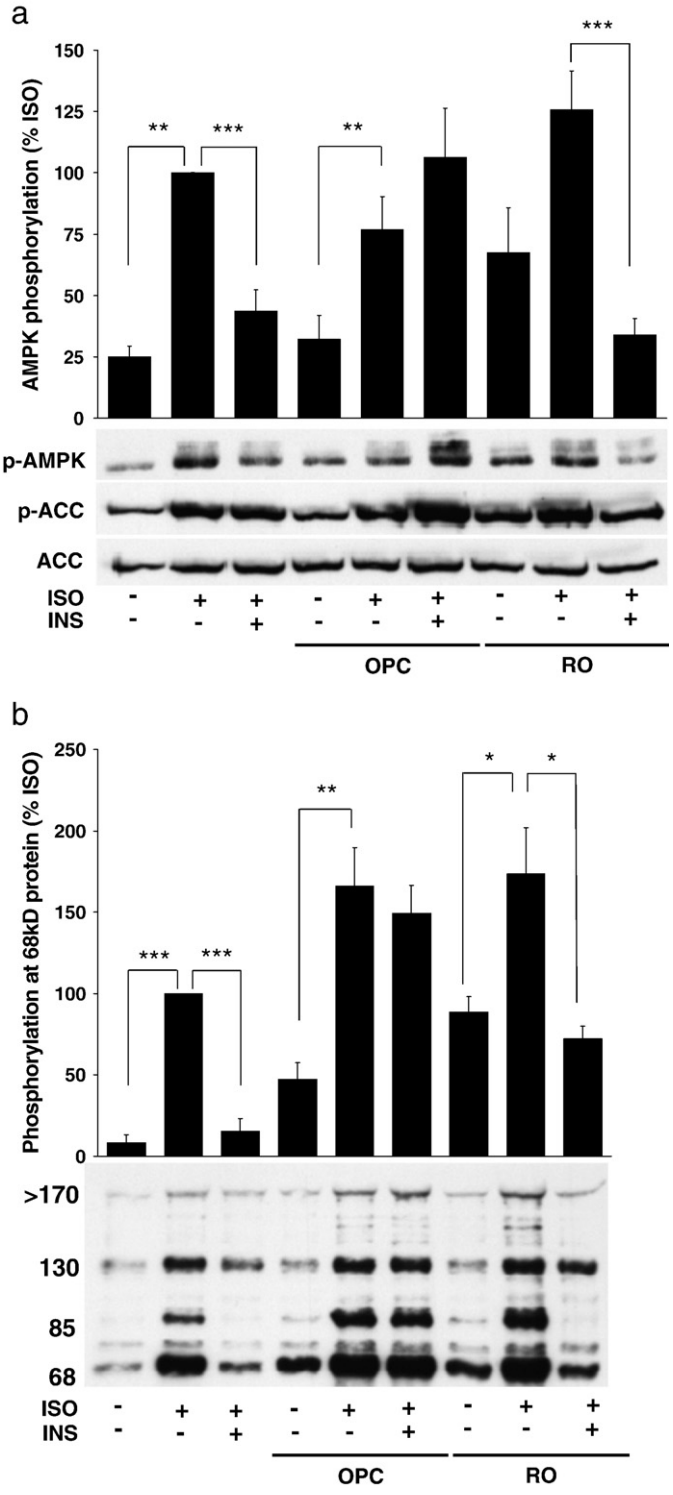

Fig. 1. PDE3B and PDE4 are involved in the regulation of AMPK. Primary adipocytes were stimulated with 30 nM isoproterenol (ISO) alone or in combination with 1 nM insulin (INS) for 10 min. Adipocytes were pre-treated for 10 min with OPC3911 (OPC) or Rolipram (RO) prior to stimulation with hormones as indicated. Representative immunoblots for (a) phospho-AMPK T172, phospho-ACC S79 and total ACC and (b) for phospho-PKA substrates are shown. Data is presented as the mean ± SEM of the quantified phosphorylation relative to the isoproterenol stimulated sample. $n = 3–7$. $^{**}p < 0.01$, $^{***}p < 0.001$.