Role of AMP-Activated Protein Kinase in Cyclic-AMP-dependent Lipolysis In 3T3-L1 Adipocytes

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Running title: AMPK and lipolysis
ABBREVIATIONS

AICAR: 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AC: adenylate cyclase; AMPK: AMP-activated protein kinase; 8-Bromo-cAMP: 8-Bromoadenosine-3’, 5’-cyclic monophosphate Na; cAMP: cyclic AMP; HSL: hormone-sensitive lipase; PI 3’-kinase: phosphatidylinositol-3’-kinase; PKA: protein kinase A; Sp-cAMPs: Sp-adenosine-3’, 5’-cyclic monophosphorothioate triethylamine salt.
ABSTRACT

AMP-activated protein kinase (AMPK) is a phylogenetically conserved intracellular energy sensor that has been implicated as a major regulator of glucose and lipid metabolism in mammals. However, its possible role in mediating or influencing the adrenergic control of lipolysis in adipocytes remains uncertain. In this study, we utilized the murine cultured preadipocyte line 3T3-L1 to examine this question. Treatment of adipocytes with isoproterenol or forskolin promoted the phosphorylation of AMPK at a critical activating Thr172 residue in a dose- and time-dependent manner. This correlated well with a stimulation of the activity of AMPK, as measured in the immune complex. Analogs of cAMP mimicked the effect of isoproterenol and forskolin on AMPK phosphorylation. Treatment of adipocytes with insulin reduced both basal and forskolin-induced AMPK phosphorylation via a pathway dependent on PI 3’-kinase. Overexpression of a dominant-inhibitory mutant of AMPK blocked isoproterenol-induced lipolysis by approximately 50%. These data indicate that there exists a novel pathway by which cAMP can lead to the activation of AMPK, and in adipocytes this is required for maximal activation of lipolysis.

Key words: AMP-activated protein kinase, β-adrenergic receptor, cAMP, insulin, and lipolysis
INTRODUCTION

One of the challenges that all eukaryotic cells face is adapting to periods of nutritional or oxygen deprivation. The strategies utilized to combat such stresses have been remarkably well conserved over long evolutionary periods, and one of the most critical adaptive pathways centers around the AMP-activated protein kinase (AMPK). AMPK is widely expressed and consists of a catalytic $\alpha$ subunit and regulatory $\beta$ and $\gamma$ subunits. It can be activated by phosphorylation at Thr172 of the catalytic subunit by AMPK kinase, a protein that has so far eluded molecular identification. In addition, the allosteric binding of AMP to AMPK enhances both its activity as well the stability of phosphorylated state (1). AMPK was initially described as an activity that phosphorylates and inactivates HMG-CoA reductase and acetyl-CoA carboxylase, thereby decreasing the rates of cholesterol and fatty acid biosynthesis, respectively (1). Subsequent studies have led to the idea that AMPK acts as an intracellular energy sensor, stimulated by the increased intracellular AMP/ATP ratio when cells are stressed by conditions such as hypoxia/ischemia in the heart and excessive contraction in skeletal muscle. Activated AMPK accelerates ATP-producing pathways, such as fatty acid and glucose oxidation, while reducing ATP consumption, ultimately leading to the preservation or restoration of adequate high energy phosphates (2).

In recent years, the study of AMPK in mammalian organisms has been directed in part towards the identification of physiological processes in which the enzyme is important, and the search for extracellular factors that regulate its activity. In regard to the former, much attention has centered on the role of AMPK in both the short- and long-term response to exercise (3-5). Perhaps more surprising has been the identification of circulating factors implicated as
modulators of AMPK activity. Two adipocyte-derived hormones, leptin and adiponectin, which regulate energy homeostasis and glucose and lipid metabolism, induce the activation of AMPK. Leptin stimulates phosphorylation and activation of the $\alpha_2$ subunit of AMPK in skeletal muscle, and this has been invoked as a principal mediator of the hormone’s action to stimulate fatty-acid oxidation (6). Adiponectin promotes phosphorylation and activation of AMPK in both liver and skeletal muscle, and again this has been suggested to account for the stimulatory effect of adiponectin on glucose utilization and fatty-acid oxidation (7,8). In addition, the anti-diabetic drugs metformin and the thiazolidinediones appear to activate AMPK in liver and skeletal muscle (9-12).

Whereas the importance of AMPK to control lipid metabolism in liver and muscle is well established, its role in regulating lipolysis in adipose tissue has remained controversial. The $\beta$-adrenergic signaling pathway represents a prime regulator of triglyceride breakdown, acting via the accumulation of cAMP and subsequent PKA-dependent phosphorylation of hormone-sensitive lipase (HSL). The activity of HSL, the rate-limiting enzyme that catalyzes the hydrolysis of triglyceride to diglyceride and monoglyceride, is regulated by both phosphorylation and translocation to the lipid droplet (13,14). PKA phosphorylates HSL at Ser563, Ser659 and Ser660, though the first has long been thought responsible for HSL activation. In 1989, Gardon et al. reported AMPK phosphorylates HSL at Ser 565 in vitro without any direct effect on HSL activity, but this abolished the further phosphorylation of HSL by PKA at Ser563 (15). 5-aminoimidazale-4-carboxamide ribonucleoside (AICAR), a cell permeable adenosine analog that can be phosphorylated to form 5-aminoimidazale-4-carboxamide-1-$\beta$-D-ribofuranosyl-5’-monophosphate (ZMP), stimulates AMPK activity and glucose uptake in both muscle and
adipose tissues (16-19). Pre-incubation of isolated rat adipocytes with AICAR reduced the response of these cells to the lipolytic agent isoproterenol (20,21). These observations led to the hypothesis that AMPK antagonizes lipolysis in adipocytes, presumably to prevent futile cycling and depletion of ATP. However, in 1998, Anthonsen et al demonstrated that, whereas mutating Ser659 and Ser660 abolished the enzymatic activity of HSL, replacing Ser563 by alanine did not reduce activity of the enzyme, questioning the importance of inhibiting modification of this residue (22). In addition, Moule and Denton (23) reported that isoproterenol stimulated AMPK phosphorylation and kinase activity in isolated rat epididymal fat cells, an observation seemingly at odds with an anti-lipolytic role for AMPK. In order to clarify the role of AMPK in regulating lipolysis in adipose tissue, we utilized 3T3-L1 adipocytes as a model system, measuring AMPK phosphorylation and activity after the treatment with different activators and/or inhibitors of the components along the β-adrenergic signaling pathway. Our data support the idea that β-adrenergic agents activate AMPK via an intermediary rise in cAMP, and that this serves to enhance the lipolytic rate.
EXPERIMENTAL PROCEDURES

Materials. Sp-cAMPs and glycerokinase were from BIOMOL (Plymouth Meeting, PA) and Roche (Basel, Switzerland), respectively. Isoproterenol and all other reagents were purchased from Sigma (St. Louis, MO). Anti-phospho-AMPK antisera has been described (5), and phospho-AKT and phospho-PKA substrate antibodies were obtained from Cell Signaling (Beverly, MA). SAMS peptide was synthesized by Sigma-Genosys (Stanford, CA).

Cell culture. 3T3-L1 fibroblasts were cultured and differentiated as described (24). 3T3-L1 adipocytes were used for experiments 7-12 days after differentiation. In all experiments except for the glycerol release assays, 3T3-L1 adipocytes were preincubated in DMEM + 1% fatty acid free BSA for 2 hrs before the all the treatments.

Adenovirus infection. pcDNA3-Myc-tagged wild-type and dominant inhibitory mouse AMPKα2 (AMPKα2WT and AMPKα2KD), which have been described previously (5), were subcloned into adeno-X viral DNA from Clontech (Palo Alto, CA). Recombinant adenoviral DNA was obtained by transforming E. Coli. The positive recombinant adenoviral DNA was linearized by Pac I and transfected into HEK 293 cells to produce recombinant adenovirus. The adenoviral infection was optimized using poly-o-lysine enhancement method as described by Orlicky and Schaack (25). The degree of expression for different titers of adenovirus was measured by Western blot analysis and, and the multiplicity of infection (M. O. I.) for each adenovirus adjusted to give equivalent levels for each of the different proteins. Lipolysis was measured 4 days after adenovirus infection when protein expression reaches maximum (26).
**Western immunoblot analysis.** Tagged proteins were detected by Western immunoblots as described previously (5). 3T3-L1 adipocytes were solubilized in adipocyte lysis buffer containing 50mM Tris (pH7.4), 150mM NaCl, 50mM NaF, 5mM sodium pyrophosphate, 0.5% triton X-100, 1mM EDTA, 1mM EGTA, supplemented with the protease inhibitors or in SDS buffer containing PBS + 0.05% SDS. All the SDS-PAGE gels used were 10% polyacrylamide.

**Immunocomplex kinase assay.** *In vitro* immunocomplex assays were performed as described previously (5). Briefly, the pre-clarified cell lysates were incubated with anti-AMPK antibody for an hour and protein A agarose beads for another hour. The beads were washed three times with the 3T3-L1 adipocytes lysis buffer and once with the kinase reaction buffer. SAMS peptide was resolved by Coomassie blue staining after electrophoresis on a 4-12% NuPage precast gels (Invitrogen, Carlsbad, CA). The autoradiograms were quantitated using NIH Image 1.61 software.

**Glycerol release assay.** The stimulation of adipocytes and measurement of glycerol release was performed as described previously (27). Prior to assaying glycerol release, adipocytes were incubated in KRP (136mM NaCl, 4.7mM KCl, 10mM NaPO₄ pH7.4, 0.9mM MgSO₄ and 0.9mM CaCl₂) + 5% fatty acid free BSA for 3 hrs. The samples were diluted two-fold for the amount of glycerol to be in the linear range of the standard curve. Released glycerol was expressed relative to the cellular protein content.
Statistical analysis. The data are presented as the mean ± SD. Significance was assessed by Student’s two sample t-tests. P<0.05 was considered significant.
RESULTS

Isoproterenol and forskolin stimulate AMPK phosphorylation and activation in 3T3-L1 adipocytes

To reevaluate the role of AMPK in the regulation of lipolysis, we first considered the phosphorylation state and activity of the enzyme after exposure of 3T3-L1 adipocytes to agents that raise intracellular cAMP. The β-adrenergic agent isoproterenol increased phosphorylation of AMPK at the critical activating threonine residue, T172, as determined by its immunoreactivity with and antibody specific for the phosphorylated state (Fig. 1). This was blocked by preincubation with the β-adrenergic agent antagonist, propranolol. Treatment of adipocytes with forskolin, which activated adenylyl cyclase independent of the receptor, also increased AMPK phosphorylation (Fig. 1). As a positive control, we exposed cells to AICAR, a cell permeable AMP analog and AMPK activator, which also induced phosphorylation of AMPK to a comparable degree (Fig. 1). The effect of isoproterenol on AMPK phosphorylation was dose-dependent, characterized by a detectable increase at 1mM and reaching the maximum level at 10mM, where phosphorylation was 4-fold above basal (Fig. 2a,b). Similarly, forskolin produced phosphorylation of AMPK at 1μM and a maximal response of 7-fold at 10μM (Fig. 2c, d). As a positive control, all extracts were immunoblotted using an antisera that recognizes the consensus phosphorylation site for protein kinase A. Multiple reactive bands were noted after treatment with isoproterenol or forskolin, and the dose-dependency roughly paralleled that for AMPK T172 phosphorylation (Fig. 2)
The phosphorylation of AMPK was detectable 1 min after addition of isoproterenol, and reached maximum by 15 min (Fig. 3a, b). The effect of forskolin was slower, not achieving a maximal T172 phosphorylation till 30 min after addition of drug (Fig. 3c, d).

Phosphorylation of T172 on the alpha subunit of AMPK generally correlates well with AMPK activity, and appears to the major site of phosphorylation by the activating upstream protein kinase AMPKK (28). To assess directly the effect isoproterenol or forskolin on the activity of AMPK, an immune complex kinase assay was performed on immunoprecipitates of AMPK prepared from adipocytes treated with drugs. Isoproterenol or forskolin increased AMPK activity of 1.8 and 2.5 fold, respectively, correlating with the increased T172 phosphorylation (Fig. 4).

**Stimulation of AMPK phosphorylation by membrane permeant analogues of cAMP.** Since isoproterenol and forskolin increase the activity of both adenylyl cyclase and AMPK, the most probable mechanism is that the elevated level of cAMP is indirectly responsible for the augmented phosphorylation of AMPK. To test the possibility, 3T3-L1 adipocytes were treated with cAMP. As shown in Fig. 5, both Sp-cAMPS and 8-Bromo-cAMP increased AMPK phosphorylation in a dose-dependent manner that correlated well with general protein phosphorylation as detected by an antisera that recognizes a consensus protein kinase A phosphorylation site. Whereas these data do not establish whether cAMP is acting via protein kinase A to induce phosphorylation of AMPK, they do provide strong support for the notion that the cyclic nucleotide represents an important mediator for β adrenergic agent dependent AMPK activation.
Insulin reduces AMPK phosphorylation through a PI 3'-kinase-dependent pathway. A major regulator of anabolic metabolism, insulin antagonizes adipocyte lipolysis, in part by reducing intracellular cAMP. This is believed to occur by a PI 3'-kinase and Akt/PKB-dependent phosphorylation and activation of cAMP phosphodiesterase 3B (13,29,30). If isoproterenol and forskolin stimulate AMPK by increasing the level of intracellular cAMP, one might expect that insulin would antagonize the activation of AMPK. To test this idea, we first treated 3T3-L1 adipocytes with forskolin, followed after 20 min by the exposure to insulin, and then measured AMPK phosphorylation after an additional 10 minutes. As predicted, insulin substantially reduced the effect of forskolin on AMPK phosphorylation (Fig. 6a, b). To test whether this effect of insulin is mediated by PI 3'-kinase, cells were also treated with the PI 3'-kinase inhibitor wortmannin. Pre-incubation of wortmannin completely abolished the effect of insulin to antagonize forskolin-induced AMPK phosphorylation, whereas pre-incubation with wortmannin did not change either basal or forskolin-induced phosphorylation of AMPK (Fig. 6a, b). The effect of insulin on AMPK phosphorylation is likely to represent a physiological meaningful event mediated by interaction with the insulin receptor, as the decreased phosphorylation of AMPK was evident after treatment with 1nM insulin, and achieved a maximum at 10nM insulin (Fig. 6c, d). This dose-dependency correlated well with phosphorylation of Akt/PKB, a serine/threonine kinase now recognized as an important mediator of the metabolic actions of insulin (Fig. 6c, d).

AMPK is required for optimal isoproterenol-induced lipolysis. The major physiological target of increased cAMP in the fat cell is the augmentation of lipolysis, i.e. the breakdown of triglyceride to free fatty acid and glycerol. Though the experiments described above show clearly
that cAMP positively regulates AMPK, they leave open the question of the contribution of AMPK to the control of lipolysis. In order to resolve this issue, we infected 3T3-L1 adipocytes with recombinant adenoviruses overexpressing either wild type (AMPK α2WT) or dominant inhibitory mutant (AMPK α2KD) of AMPK α2 subunit. To determine that infection by each of the adenoviruses led to expression of protein at roughly equivalent levels, Western blots were performed using anti-AMPKα antibody (Fig. 7a). Note that the endogenous proteins were not detected at this exposure, indicating a significant degree of overexpression. In addition, the overexpressed proteins were visualized by immunoreactivity with the Myc tag. These data demonstrate that, under the conditions used, both control and mutant AMPK subunits were expressed at equal levels (Fig. 7a).

Under basal conditions, the relatively modest rate of glycerol release, a direct measure of lipolysis, was unaffected by infection by any of the viruses. Treatment of adipocytes with isoproterenol for 40 min increased lipolytic rate approximately 10-fold; this was completely blocked by co-incubation with propranolol. Infection of adipocytes with adenovirus expressing α2WT did not affect either basal or isoproterenol-induced lipolysis. Overexpression of α2KD reduced isoproterenol-stimulated glycerol release by about 50% when measured either 20 min or 40 min after the addition of drug (Fig. 7b).
DISCUSSION

In this study, we investigated the role of AMPK in isoproterenol-induced lipolysis in 3T3-L1 adipocytes. Some contradictory data have existed pertaining to this issue, with most reports indicating that AMPK antagonizes triglyceride breakdown in adipocytes. In contrast, our results show clearly that AMPK activation is required for the maximal increase in lipolysis induced by isoproterenol. Moreover, and perhaps more surprisingly, our study also revealed a novel mechanism by which a rise in cAMP levels can lead to an increase in AMPK activity.

Since AMPK has been suggested to be a “metabolic switch” driving the cellular response to nutritional stress, a fair amount of attention has been focused on understanding its control of lipogenesis and fatty acid oxidation. However, the contribution of AMPK to the regulation of lipolysis has remained controversial, largely due the lack of reagents to specifically reduce AMPK activity, as well as the potential non-specificity of AICAR, the drug generally employed to activate AMPK. We have overcome these problems by relying on adenoviral transduction of cultured 3T3-L1 adipocytes with AMPK mutants specifically recognized to either block or activate the enzyme. AMPK exists as a stable heterotrimer; overexpression of a mutant alpha subunit leads to displacement and degradation of the endogenous wildtype subunit, and the production of a catalytically inactive enzyme complex (5). Using this reagent we show here that the maximal stimulation of lipolysis by agents that raise cAMP depends on AMPK activity. The 50% reduction of glycerol release in shown Fig. 7 is most likely an underestimate of AMPK’s contribution to lipolysis, as it is unlikely that more than 75% of the adipocytes were productively infected with recombinant adenovirus under these conditions. Generation of a constitutively active AMPK variant depended on the identification of a series of spontaneous mutations in the
non-catalytic gamma subunit, initially in a pig with hypertrophy skeletal muscles and then humans with cardiac conduction disturbances (31-33). The activating nature of the mutation has been confirmed in tissue culture lines, though precise mechanism remains unclear (34,35). Active AMPK alone had a minimal effect on glycerol release, which probably only emphasizes the critical role of the canonical cAMP pathway through HSL (Yin and Birnbaum, unpublished observations) (13,36).

The traditional view that AMP, creatine phosphate and possibly other metabolites are the sole regulators of AMPK has been challenged by the recent reports that leptin and adiponectin can stimulate the activity of the enzyme (6,8). Thus far, there has been little understanding of how these hormones transmit the signal from their plasma membrane receptors to the intracellular AMPK. One of the surprising results in the present studies is that agents that increase cAMP or the nucleotide itself activate AMPK in intact cells. This is consistent with the report of Moule and Denton (23) that isoproterenol increases AMPK phosphorylation and kinase activity. In addition to isoproterenol, other β-adrenergic agents such as forskolin also increased AMPK phosphorylation and kinase activity via an intermediary increase in cAMP, as might be expected if AMPK were required for optimal lipolysis. Enthusiasm about the identification of a novel intracellular messenger capable of leading to the activation AMPK is tempered by uncertainty about the mechanism. T172, the phosphorylation site on AMPK responsible for most of the increase in AMPK activity, is not a consensus PKA phosphorylation site, which suggests that even if PKA regulates AMPK activity through an indirect mechanism, such as phosphorylating AMPKK or another unknown upstream kinase(s), or that the effect of cAMP is independent of PKA. There are now several such downstream signaling pathways recognized, including the
Epac-Rap1 pathway, which has been implicated in regulating many critical cellular events, ranging from cell proliferation and cell differentiation, to cell adhesion and morphogenesis (37). However, to our knowledge, this pathway has not been reported to be active in adipocytes.

The other critical question is how AMPK regulates lipolysis. HSL is the rate-limiting enzyme in this process of lipolysis, and both its activity and subcellular location are regulated by phosphorylation. There are three PKA phosphorylation sites present on the regulatory module of HSL, Ser 563, Ser 659, and Ser 660, the latter two being most important for regulation of activity \textit{in vitro} (22). However phosphorylation by PKA alone cannot account for the maximal activity of HSL, as \textit{in vitro} PKA phosphorylation only causes a 1.5-2 fold increase of HSL lipase activity, while isoproterenol stimulates more than 50 fold increase of fatty acid release \textit{in vivo} (27,38). AMPK has been shown to phosphorylate Ser 565, another serine residue within the regulatory domain of HSL \textit{in vitro}. One possibility is that phosphorylation of S565 is critical to the translocation of HSL to the lipid droplet. In fact, Su et al. (39) have found that mutation of this residue abolishes the ability of HSL to translocation to the lipid droplet. Alternatively, there are number of other proteins involved in lipolysis, including perilipin and lipotransin, and any of these might be direct or indirect targets of AMPK (40,41).

In conclusion, our study demonstrates that the activity of AMPK is required for the maximal lipolysis induced by isoproterenol, and provides a novel mechanism of how AMPK is activated in 3T3-L1 adipocytes. Our data suggest that upon stimulation by β-adrenergic agents, AMPK is phosphorylated and activated, and this activation contributes to the maximal response of lipolysis, possibly by phosphorylating HSL at Ser 565 and promoting the HSL translocation.
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References:


FIGURE LEGENDS

Fig. 1 Isoproterenol and forskolin stimulate the phosphorylation of AMPK in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with isoproterenol (10μM, 20min) without or with the addition of propranolol (200mM), propranolol alone (200mM, 20min), AICAR (500μM, 30min), or forskolin (50μM, 45min). Cell extracts were prepared and equal amounts of proteins (30 μg) analyzed by SDS-PAGE and immunoblotting with anti-phosphoAMPK and anti-AMPK antibodies, as described in experimental procedures.

Fig. 2 Isoproterenol and forskolin stimulate AMPK phosphorylation in 3T3-L1 adipocytes in a dose-dependent manner. 3T3-L1 adipocytes were treated with increasing doses of either isoproterenol (a,b) for 15 min or forskolin (c,d) for 30 min. Extracts were prepared and analyzed by Western blots using anti-phosphoAMPK, anti-AMPK and anti-phosphoPKA substrate antibodies (α-PKAS). All Western blots shown are the representative of four independent experiments, which were quantitated displayed in graphical format in panels b and d. Data are expressed relative to basal phosphorylation.

Fig. 3 Isoproterenol and forskolin stimulate the phosphorylation of AMPK in 3T3-L1 adipocytes in a time-dependent manner. 3T3-L1 adipocytes were treated with 10μM isoproterenol (a) or 10μM forskolin (c) for different periods of time. Extracts were prepared and the Western Blot results were analyzed as in Fig. 2. The Data (mean ± STD, n=4) are expressed relative to basal phosphorylation.
**Fig. 4** Isoproterenol and forskolin stimulate the activity of AMPK in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with 10μM isoproterenol for 15 min or 10μM forskolin for 30 min. Cell extracts were prepared and AMPK was immunoprecipitated with anti-AMPK antibody. The kinase activity of AMPK was measured using SAMS peptide as a substrate as described in experimental procedures. The autoradiogram on the upper panel showed the data representative of four independent experiments. Band intensity was quantitated by densitometer and the normalized to the basal kinase activity. The values indicate the fold increase as the mean ± STD.

**Fig. 5** The cAMP analogs Sp-cAMPS and 8-Bromo-cAMP increase AMPK phosphorylation in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with two different cAMP analogs: Sp-cAMPS (a) or 8-Bromo-cAMP (b) as indicated. Cell extracts were prepared, fractionated by SDS-PAGE and immunoblotted with anti-AMPK, anti-phosphoAMPK and α-PKAS.

**Fig. 6** Insulin reduces basal and forskolin-stimulated phosphorylation of AMPK in 3T3-L1. (a) 3T3-L1 adipocytes were treated with 10μM forskolin for 30 min with or without 10nM insulin, in the presence or the absence of 100 nM wortamamin. Cell extracts were prepared and analyzed by Western Blots using anti-phosphoAMPK and anti-phosphoAKT antibodies. Shown in (a) are representative Western blots from four independent experiments, which are quantitated and displayed graphically in panel b. (c) 3T3-L1 adipocytes were treated with increasing concentrations of insulin for 10 min. Cell extracts were prepared and the level of phosphorylation of AMPK or Akt assessed by Western blots. The normalized data from four independent experiments are shown in (d).
Fig. 7 The role of AMPK in isoproterenol-stimulated lipolysis. (a) 3T3-L1 adipocytes were infected by AMPK-α2WT or AMPK-α2KD adenoviruses. Cell extracts from uninfected cells and virus infected cells were prepared and Western blots performed to determine total AMPK using anti-AMPK antibody, and overexpressed protein using anti-Myc antibody. (b) 3T3-L1 adipocytes were preincubated in KRP+5% FFA-free BSA for 3 hrs before the glycerol release assays were conducted. Aliquots of the culturing media were collected at 0, 20, 40 min, and the amount of glycerol released in the presence or absence of isoproterenol was measured as described in the experimental procedures. The release of glycerol to the medium at 20 and 40 min was normalized to basal release at 0 min and expressed as mean ± STD of triplicate determinations. The result shown is the representative of six independent experiments. *: P< 0.05.
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- PhosphoAMPK

- AMPK

Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

(a) Sp-cAMPs

Forskolin

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(b) 8-Bromo-cAMPs

Forskolin

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Figure 6
Figure 7

(a) Adenovirus - 2WT 2KD
   -AMPK 2
   -Myc

(b) Mol Glycerol/mg protein

- Isoproterenol (10 M)
- Time after isoproterenol
- 20 min
- + 20 min
- - 40 min
- + 40 min

- No virus
- 2WT-AMPK
- 2KD-AMPK

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