Biochemical and Biophysical Research Communications 443 (2014) 435–440

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ATP sensitive bi-quinoline activator of the AMP-activated protein kinase

John W. Scott [a,*], Jonathan S. Oakhill [a], Naomi X.Y. Ling [a], Christopher G. Langendorf [a], Richard C. Foitzik [b], Bruce E. Kemp [a], Olaf-Georg Issinger [c]

[a] St. Vincent's Institute and Department of Medicine, University of Melbourne, 41 Victoria Parade, Fitzroy 3065, Victoria, Australia
[b] Cancer Therapeutics CRC Pty. Ltd., Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3052, Victoria, Australia
[c] Biomedical Research Group, BMB, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark

ARTICLE INFO

Article history:
Received 11 November 2013
Available online 8 December 2013

Keywords:
AMPK
Allosteric
Drug
Activator
Phosphorylation
Isoform-specific

ABSTRACT

The AMP-activated protein kinase (AMPK) regulates cellular and whole-body energy balance in response to changes in adenylate charge and hormonal signals. Activation of AMPK in tissues such as skeletal muscle and liver reverses many of the metabolic defects associated with obesity and Type 2 diabetes. Here we report a bi-quinoline (JJO-1) that allosterically activates all AMPK αβγ isoforms *in vitro* except complexes containing the γ3 subunit. JJO-1 does not directly activate the autoinhibited α subunit kinase domain and differs among other known direct activators of AMPK in that allosteric activation occurs only at low ATP concentrations, and is not influenced by either mutation of the γ subunit adenylate-nucleotide binding sites or deletion of the β subunit carbohydrate-binding module. Our findings indicate that AMPK has multiple modes of allosteric activation that may be exploited to design isoform-specific activators as potential therapeutics for metabolic diseases.

1. Introduction

The AMP-activated protein kinase (AMPK) is an important regulator of cellular and whole-body energy homeostasis that coordinates metabolic pathways to balance energy supply with demand. At the cellular level, AMPK functions primarily as a fuel gauge monitoring the relative levels of adenylate nucleotides (ATP/ADP/AMP), which can be regarded as a molecular read-out of cellular energy charge [1,2]. AMPK protects cells from stresses (fuel deprivation, hypoxia, exercise) that lower cellular energy charge by orchestrating a switch in metabolism in favour of ATP-producing catabolic pathways while inhibiting ATP-consuming anabolic processes. It achieves this largely by direct phosphorylation of enzymes that catalyse the rate-limiting steps of major metabolic pathways such as fatty acid, sterol, and carbohydrate metabolism. AMPK also plays an adaptive role to chronic energy challenges by reprogramming the expression of metabolic genes via phosphorylation of transcription factors and co-regulators [3,4]. In addition to regulating energy balance at the cellular level, AMPK is also a central regulator of whole-body energy metabolism, integrating a variety of hormonal (leptin, ghrelin and adiponectin) and nutritional signals in the central nervous system and periphery to control feeding behaviour and body weight [5]. Consequently, there is now considerable interest in developing direct activators of AMPK for the treatment of metabolic diseases including Type 2 diabetes and obesity.

AMPK exists as a heterotrimeric complex composed of an α subunit that contains the kinase and autoinhibitory domains (AID), as well as regulatory β and γ subunits that contain a carbohydrate-binding module (CBM) and the allosteric adenylate nucleotide-binding sites, respectively. Multiple isoforms exist for each subunit in mammals (α1, α2, β1, β2, γ1, γ2 and γ3), therefore at least twelve heterotrimeric combinations are possible giving rise to differences in tissue distribution, regulation and function. There are four potential adenylate-binding sites on the γ subunit, however site 2 does not appear to be functional [6–8]. Sites 1 and 3 are exchangeable sites that can bind either ATP, ADP or AMP, whereas binding of ATP to site 4 occludes nucleotide binding at site 3 [9]. The key step in the activation of AMPK is phosphorylation of Thr172 in the activation loop of the α subunit by either LKB1 or Ca^{2+}/calmodulin-dependent protein kinase kinase-2 (CaMKK2) [1]. Binding of ADP or AMP to the γ subunit promotes phosphorylation of Thr172 by the upstream kinases provided that the β subunit is myristoylated [6,10]. In addition, both ADP and AMP maintain the active state of AMPK by inhibiting dephosphorylation of Thr172 by protein phosphatases [8]. AMP, but not ADP, causes a further allosteric activation of phosphorylated AMPK, however this effect is relatively modest compared with Thr172 phosphorylation but sufficient to stimulate downstream signalling in cells [11].

The first small-molecule direct activator of AMPK reported was the thienopyridone drug A769662 [12]. Whereas AMP can activate all twelve possible αβγ isoform combinations of AMPK, A769662

* Corresponding author. Fax: +61 39416 2676.
E-mail address: jscott@svi.edu.au (J.W. Scott).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.130

selectively activates complexes containing the β1 subunit [13]. Activation of AMPK-β1 containing complexes by A769662 is dependent on the CBM and, in particular, the Ser108 autophosphorylation site that resides within this domain [13,14]. Several direct small-molecule AMPK activators have since been reported including the furanothiazolidine derivative PT1 and the naturally-occurring molecule sanguinarine [15–17]. PT1 is thought to activate AMPK by relieving inhibition of kinase activity by the AID in the α subunit. Sanguinarine selectively activates AMPK complexes containing the α1 and γ1 subunits, but is not selective for the β1 isoform like A769662. These findings provide a potential pathway for the development of isoform-selective activators of AMPK and may ultimately be exploited to target the activation of AMPK in specific tissues.

In the present study, we report the discovery of a small-molecule (JJO-1) that directly activates AMPK in cell free assays but only at low ATP concentrations. We show that JJO-1 activates all AMPK αβγ isoforms except complexes containing the γ3 subunit, and that activation occurs independently of the β subunit CBM. JJO-1 now joins a growing list of isoform-selective activators of AMPK.

2. Materials and methods

2.1. Molecular biology

Plasmid constructs for AMPK α1, α2, β1, β2 and γ1, γ2 and γ3 subunits for expression in COS7 cells were generated as described previously [13]. Point mutations were generated using Quikchange site-directed mutagenesis (Stratagene) and deletion mutants as described previously [6,13]. All plasmid constructs were verified by sequencing the entire open reading frame. DNA for transfection was prepared using HiSpeed MaxiPrep kit (QIAGEN) and quantified by absorbance at 260 nm.

2.2. Expression of recombinant AMPK and mutant variants

COS7 cells were grown in Dulbecco's Modified Eagle Medium (Sigma) with 10% foetal calf serum at 37 °C with 5% CO_2. Cells were transfected at 60% confluency with 1 μg of the various plasmids expressing human AMPK subunits (pcDNA3 GSTα1, GSTα2, myc-tagged β1, myc-tagged β2 and pMT2 HA-tagged γ1, γ2 and γ3) and deletion/point mutants using FuGene 6 (Roche) according to the manufacturers instructions. Transfected cells were harvested after 36 h by washing with ice-cold phosphate-buffered saline (PBS) followed by rapid lysis *in situ* using 1 ml of lysis buffer (50 mM Tris.HCl [pH 7.4], 150 mM NaCl, 50 mM NaF, 1 mM NaPPi, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1% [v/v] Triton X-100) containing protease inhibitors (Roche). For drug treatments, transfected COS7 cells (36 h post-transfection) were incubated with fresh media for 1 h after which either JJO-1, phenformin (Sigma) or a DMSO control was added for a further 1 h, then harvested as described above. Cellular debris was removed by centrifugation and total protein was determined using the Bradford protein assay (Bio-Rad).

2.3. AMPK purification and activity assays

AMPK complexes were purified by glutathione-Sepharose pull down from 50 μg of lysate using 10 μl of a 50% slurry of glutathione Sepharose (GE Healthcare) pre-equilibrated with lysis buffer (see above), followed by successive washes in lysis buffer containing 1 M NaCl and finally into assay buffer (50 mM HEPES [pH 7.4], 1 mM DTT, 0.02% [v/v] Brij-35). AMPK activity was determined in the pulldowns by phosphorylation of the SAMS peptide using 100 μM SAMS, 200 μM [γ-^{32}P]-ATP (Perkin Elmer), 5 mM $MgCl_2$ in the presence and absence of either JJO-1, A769662 (Creagen), PT1 (Tocris Bioscience) or AMP (Roche) in a standard 25 μl volume assay at 30 °C. Reactions were terminated after 10 min by spotting 15 μl onto P81 phosphocellulose paper (Whatman) and washing in 1% phosphoric acid (Merck). Radioactivity was quantified by scintillation counting. Kinase activity was corrected for variations in protein expression by immunoblotting for the α1 subunit and densitometry. AMPK phosphorylation and dephosphorylation assays were performed as described previously [6]. For the chemical library screen, AMPK activity was determined using a luminescence ATP detection assay system (Perkin Elmer) following the manufacturers instructions.

2.4. Immunoblotting

Glutathione-Sepharose purified or immunoprecipitated AMPK was denatured in SDS–PAGE sample buffer, separated by SDS–PAGE and transferred to nitrocellulose membranes. The membranes were blocked for 1 h in PBS-T supplemented with 2% non-fat milk, and then incubated with conjugated primary anti-α1 (IR800 dye) and PT172 (IR680 dye) antibodies. Binding was detected using the LiCor Odyssey Infra-red dual detection system.

2.5. Statistical analysis

Results are expressed as the mean ± standard error of mean (SEM). Statistical analysis, where indicated, was performed using one-way analysis of variance (ANOVA), with the alpha level set at 0.05 for each test. *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Identification of a novel direct activator of AMPK

Using recombinant AMPK-α1β1γ1 and an ATP depletion luminescence kinase assay, we screened the Diverse Compound Library Set 1 from the National Cancer Institute and identified a bi-quinoline compound (JJO-1) as a direct activator of AMPK (Fig. 1A). To confirm the screening hit, we resynthesised the compound and measured activation of AMPK using the standard [γ-^{32}P]-ATP based assay. Although JJO-1 activated AMPK using the luminescence assay, we were unable to demonstrate activation using the standard assay. A key difference between the assays is the final concentration of ATP that is used (1 μM for the luminescent assay compared with 200 μM for the standard assay), therefore we investigated whether this could explain the discrepancy. Indeed, ATP concentrations of 50 μM or lower in the standard assay restored the activation of AMPK by JJO-1 (Fig. 1B). At 20 μM ATP, JJO-1 activated AMPK approximately 2.7-fold with a half-maximal concentration of 1.8 ± 0.2 μM (Fig. 1C).

The subunit isoform composition of an AMPK complex is an important factor in determining sensitivity to AMP and A769662 [13,18]. To test whether activation of AMPK by JJO-1 is influenced by the presence of a particular subunit isoform, we expressed all twelve possible AMPK αβγ and measured kinase activity in the presence and absence of either JJO-1, A769662 or AMP at 20 μM ATP. Unlike A769662, which selectively activates AMPK-β1 containing complexes, JJO-1 activated all AMPK complexes except those containing the γ3 subunit (Fig. 1D). In contrast, A769662 did not activate β2 containing complexes but did activate β1γ3 containing complexes, whereas AMP activated all the subunit isoform combinations.

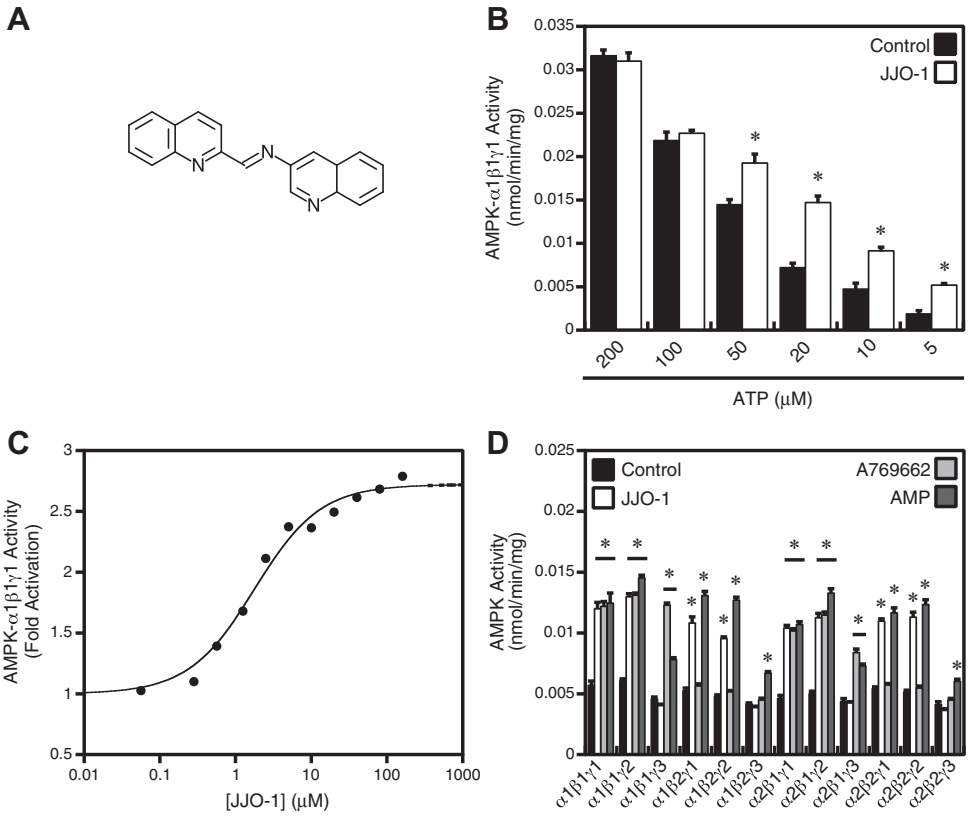

Fig. 1. JJO-1 directly activates all AMPK-αβγ isoforms at low ATP concentrations except complexes containing the γ3 subunit. (A) Chemical structure of JJO-1. (B) Activation of AMPK-α1β1γ1 by JJO-1 (50 μM) measured over a range of ATP concentrations (5–200 μM). Results are shown as the mean ± SEM for four independent experiments. (C) Activation of AMPK-α1β1γ1 measured over a range of JJO-1 concentrations (0–160 μM) at 20 μM ATP. The results were fitted to the equation: Activity = Basal + (((Fold Stimulation × Basal) − Basal) × ([JJO − 1]/($A_{0.5}$ + [JJO − 1])). (D) Activity of all twelve αβγ combinations of AMPK complex measured at 20 μM ATP in the presence and absence of either JJO-1 (50 μM), A769662 (20 μM), or AMP (100 μM). Results are shown as the mean ± SEM for four independent experiments. * denotes significantly different from control ($P < 0.02$).

3.2. JJO-1 does not increase Thr172 phosphorylation in cell free assays or intact cells

ADP and AMP promote phosphorylation of Thr172 by the upstream kinases, in addition to protecting against dephosphorylation by protein phosphatases, therefore it was of interest to determine whether JJO-1 can regulate AMPK phosphorylation in a similar manner. As shown in (Fig. 2A), phosphorylation of Thr172 by CaMKK2 was stimulated approximately 1.9-fold by AMP *in vitro*, however JJO-1 had no effect. Similarly, dephosphorylation of Thr172 by protein phosphatase-2C (PP2C) was unaffected by JJO-1, whereas AMP protected against dephosphorylation by approximately 41% (Fig. 2B). These results indicate JJO-1 behaves solely as a direct allosteric activator of Thr172 phosphorylated AMPK.

3.3. JJO-1 activates AMPK γ1 adenylate-binding site mutants

Given that allosteric activation of AMPK by JJO-1 is sensitive to the concentration of ATP, and that complexes containing the γ3 subunit are insensitive to JJO-1, we considered the possibility that the drug binds in proximity to the regulatory adenylate nucleotide-binding sites. We reported previously that mutation of conserved aspartate residues in the adenylate-binding sites 1, 3 and 4 (D90A, D245A and D317A) of the γ1 subunit altered the allosteric activation of AMPK by AMP [6]; therefore, we tested whether these mutations affect activation by JJO-1. The γ1 mutants were co-expressed with GSTα1 and β1 subunits in COS7 cells, and then purified and assayed at either 20 μM ATP or 200 μM ATP, in the presence and absence of either JJO-1, A769662 or AMP. The D317A mutant has substantially increased basal Thr172 phosphorylation compared with the wild-type enzyme (Fig. 3A), therefore all the complexes were first dephosphorylated with λ phosphatase and then phosphorylated with CaMKK2 in order to obtain comparable levels of Thr172 phosphorylation and kinase activities for the different mutants in the absence of the activators. (Fig. 3B) shows that JJO-1 allosterically activated all the AMPK γ1 subunit mutant complexes to a similar extent at 20 μM ATP, but had no effect at 200 μM ATP. In contrast, A769662 activated all the γ1 mutants irrespective of the ATP concentration in the assay. Although the D317A site 4 mutant was insensitive to allosteric activation by AMP at 200 μM ATP, it regained sensitivity when assayed at 20 μM ATP. JJO-1 had no effect on the allosteric activation of AMPK-α1β1γ1 by AMP (Fig. 3C).

3.4. Activation of AMPK by JJO-1 occurs independently of domains in the α and β subunits

We next explored whether JJO-1 can activate the autoinhibited α subunit kinase domain, or is dependent on the β subunit CBM. To investigate whether JJO-1 might relieve inhibition by the AID, we expressed and purified isolated α1 kinase domain with (α1 (1–394)) or without (α1 (1–315)) this region. The α1 kinase domain constructs were activated with CaMKK2 prior to measuring allosteric activation with JJO-1. (Fig. 4A) shows that JJO-1 does not activate the α1 kinase domain at 20 μM ATP regardless of whether the AID is present or not. Consistent with previous reports, PT1 activated the α1-(1–392) but not the α1-(1–315) construct. AMP

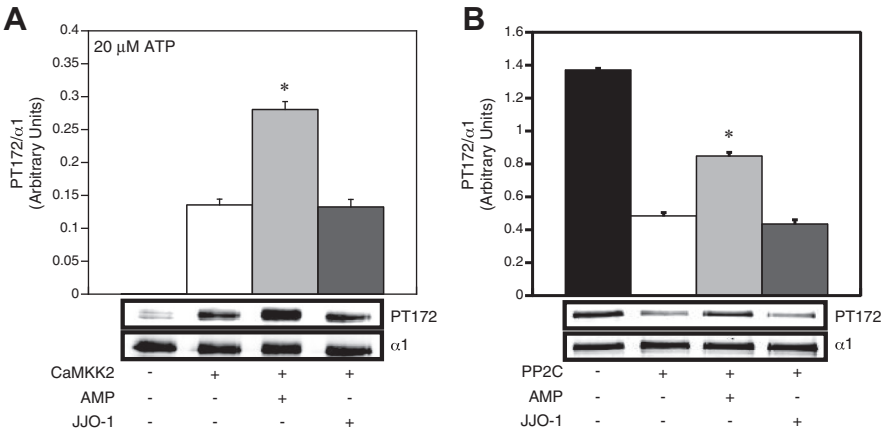

Fig. 2. JJO-1 does not promote phosphorylation of Thr172. (A) AMPK-α1β1γ1 was phosphorylated by CaMKK2 in the presence and absence of either JJO-1 (100 μM) or AMP (200 μM). (B) AMPK-α1β1γ1 was dephosphorylated by PP2C in the presence and absence of either JJO-1 (100 μM) or AMP (200 μM). Results are displayed as Thr172 phosphorylation relative to control as determined by immunoblotting using the infra-red dual detection system, and are the means ± SEM for three independent experiments. A representative blot is shown below the graph. ∗ denotes significantly different from control ($P < 0.002$).

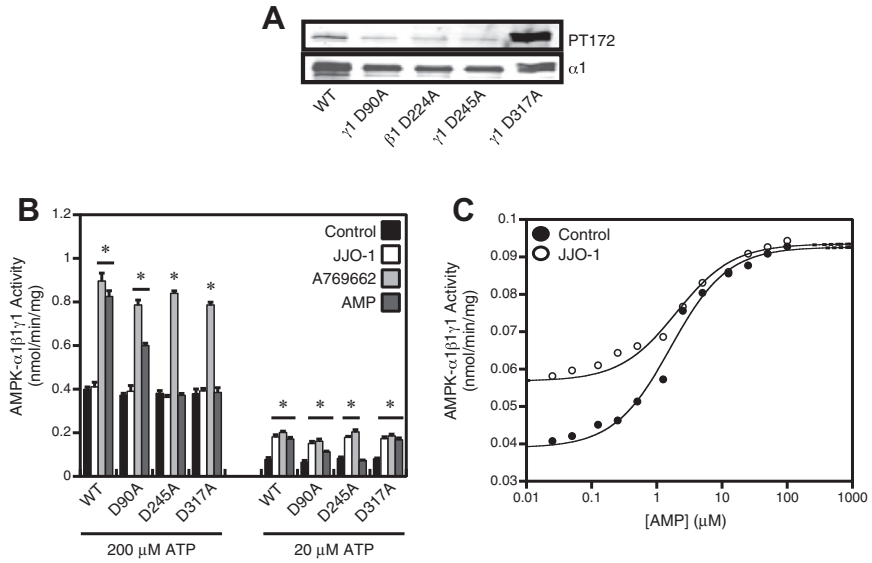

Fig. 3. JJO-1 activates AMPK independent of the allosteric adenylate-binding sites. (A) Activity of AMPK-α1β1γ1 adenylate-binding site mutants measured at either 200 μM or 20 μM ATP, in the presence and absence of either JJO-1 (50 μM), A769662 (20 μM) or AMP (100 μM). Results are shown as the mean ± SEM for four independent experiments. (B) Activation of AMPK-α1β1γ1 measured over a range of AMP concentrations (0–100 μM) at 20 μM ATP, in the presence and absence of JJO-1 (10 μM). The results were fitted to the equation: Activity = Basal + (((Fold Stimulation × Basal) − Basal) × ([AMP]/($A_{0.5}$ + [AMP])). ∗ denotes significantly different from control ($P < 0.003$).

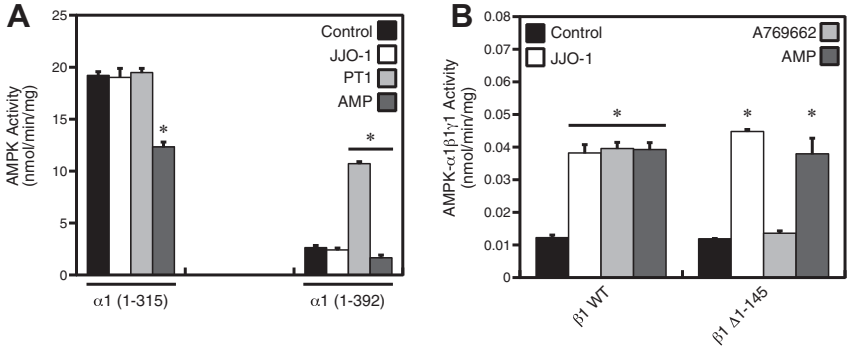

Fig. 4. The α subunit AID and β subunit CBM are not required for activation of AMPK by JJO-1. (A) Activity of AMPK-α1β1γ1 mutant that lacks the N-terminus and CBM (Δ1–145), measured at 20 μM ATP in the presence and absence of either JJO-1 (50 μM), A769662 (20 μM) or AMP (100 μM). Results are shown as the mean ± SEM for four independent experiments. (B) Activity of CaMKK2 activated GST-α1 (1–392) and GST-α1 (1–315) measured at 20 μM ATP, in the presence and absence of either JJO-1 (50 μM), PT1 (50 μM) or AMP (100 μM). Results are shown as the mean ± SEM for four independent experiments. ∗ denotes significantly different from control ($P < 0.005$).

(100 μM) caused a considerable inhibition of both kinase constructs, however this is most likely due to competition with ATP at the active site.

To assess whether JJO-1 activation of AMPK is dependent on the CBM, we assayed an AMPK mutant (β1 (Δ1–145)) that lacks the N-terminal sequence and entire CBM of the β1 subunit [13] at 20 μM ATP. As expected, A769662-mediated activation of the β1 Δ(1–145) deletion mutant was abolished; however, activation by JJO-1 and AMP was maintained (Fig. 4B). These results show that activation by JJO-1 is independent of the CBM.

4. Discussion

In this study we have identified a novel compound (JJO-1) that directly activates AMPK but only at ATP concentrations of 50 μM or lower. Under low ATP conditions, JJO-1 stimulated AMPK with a half-maximal concentration of 1.8 μM, which is similar to AMP and the furanothiazolidine PT1, but less potent than A769662 [16,19]. JJO-1 activated all AMPK αβγ heterotrimer combinations except those that contain the γ3 subunit, therefore it joins the ranks of A769662 and sanguinarine as an isoform-selective activator.

Although able to allosterically activate AMPK at low ATP concentrations, JJO-1 does not stimulate phosphorylation of Thr172 by CaMKK2, or protect against dephosphorylation by PP2C. The dependency of allosteric activation by JJO-1 on the concentration of ATP may be an indication that the drug-binding site is close to, or overlaps with the regulatory adenylate-binding sites on the γ subunit. However, JJO-1 was still able to activate a number of adenylate-binding site mutants (D90A, D245A and D317A) [8]. This indicates that JJO-1 activates AMPK via a mechanism that is distinct from AMP. An important finding to arise from our studies was that mutation of site-4 (D317A) abolished allosteric activation by AMP when assayed at 200 μM ATP, but activation was restored when assayed at 20 μM ATP. Recent crystal structures with ATP bound to site-4 provide a potential explanation for the restoration of AMP activation at low ATP as well as the increased basal phosphorylation of Thr172 of the D317A mutant in unstressed cells. These structures demonstrate a 'switch mechanism' in which the orientation of the side-chain of His297 in the γ1 subunit couples nucleotide occupancy states of sites-3 and -4, the two nucleotide sites shown to be most important for mediating allosteric regulation of AMPK [6,9]. The 5′-phosphate of AMP at site-4 forms an electrostatic interaction with His297, orientating it away from site-3 (open conformation) and allowing ATP to bind at this site. Conversely, exchange for ATP at site-4 re-orientates His297 toward site-3 (closed conformation), preventing its occupation by ATP but presumably not AMP. This switch mechanism is incapacitated in the D317A mutant through disruption of nucleotide binding at site-4, therefore His297 would be free to stabilize ATP binding at site-3 in preference to AMP and completely suppress AMP activation at 200 μM ATP, but not at 20 μM ATP. Mutation of site-4 (D317A) in the γ1 subunit does not impede stimulation of Thr172 phosphorylation in response to ADP [10], which is consistent with our observation that the D317A mutant has increased basal Thr172 phosphorylation in unstressed cells. These findings illustrate the complex interactions that occur between the adenylate nucleotides when bound to the γ subunit, and is consistent with previous direct binding studies that showed that nucleotide binding is highly-co-operative [20].

JJO-1 does not alter the α1 kinase domain activity irrespective of whether the AID is present or not. Moreover, JJO-1 also activates AMPK in a CBM-independent manner, therefore our results indicate that JJO-1 acts in a manner that is distinct from the mechanisms utilised by PT1 and A769662. Multiple allosteric drug-binding sites have been identified on other enzymes including fructose-1,6-bisphosphatase (F1,6 BPase) and glycogen phosphorylase (GP) [21,22]. Both F1,6 BPase and GP share some interesting parallels with AMPK as they are also multimeric in structure, and are allosterically regulated by AMP. The novel anilinoquinazoline drug allosteric site discovered in F1,6 BPase is distinct from the regulatory AMP and fructose-2,6-bisphosphate binding sites, as well as the fructose-6-phosphate substrate-binding site, and is located at the interface between its monomeric subunits. In a similar fashion, the allosteric site in GP also extends across the interface between its subunits [21]. Since JJO-1 does not appear to utilise known effector domains (AID, CBM, adenylate-binding sites) to allosterically activate AMPK, there is the possibility that, like F1,6 BPase and GP, the drug-binding site spans more than one subunit. This may also be the case for A769662, which is dependent on the β1 subunit CBM for activation of AMPK, but does not bind to the isolated domain as shown by NMR [13].

Given its limitations, JJO-1 has no practical use as a routine AMPK activator. Nonetheless, it does have isoform-selectivity and potentially acts via an alternate allosteric site. Due to the differential expression of AMPK isoforms across various tissues, our study highlights the feasibility of developing isoform-selective activators, which may allow for targeted activation of AMPK.

Acknowledgments

This study was supported by grants from the Australian Research Council and National Health and Medical Research Council, the Australian Government Co-Operative Research Centre's (CRC) Initiative, and the Victorian Government Operational Infrastructure Support Scheme. BEK is an NHMRC Research Fellow and JSO an ARC Future Fellow. OGI received a travel grant from the Danish Cancer Society (No. DR08055).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.130.

References

[1] D.G. Hardie, D. Carling, S.J. Gamblin, AMP-activated protein kinase: also regulated by ADP?, Trends Biochem Sci. 36 (2011) 470–477.
[2] G.R. Steinberg, B.E. Kemp, AMPK in health and disease, Physiol. Rev. 89 (2009) 1025–1078.
[3] S.L. McGee, M. Hargreaves, AMPK and transcriptional regulation, Front. Biosci. 13 (2008) 3022–3033.
[4] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, Nat. Cell Biol. 13 (2011) 1016–1023.
[5] B.B. Kahn, T. Alquier, D. Carling, D.G. Hardie, AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism, Cell Metab. 1 (2005) 15–25.
[6] J.S. Oakhill, Z.P. Chen, J.W. Scott, R. Steel, L.A. Castelli, N. Ling, S.L. Macaulay, B.E. Kemp, {beta}-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK), Proc. Natl. Acad. Sci. USA 107 (2010) 19237–19241.
[7] B. Xiao, R. Heath, P. Saiu, F.C. Leiper, P. Leone, C. Jing, P.A. Walker, L. Haire, J.F. Eccleston, C.T. Davis, S.R. Martin, D. Carling, S.J. Gamblin, Structural basis for AMP binding to mammalian AMP-activated protein kinase, Nature 449 (2007) 496–500.
[8] B. Xiao, M.J. Sanders, E. Underwood, R. Heath, F.V. Mayer, D. Carmena, C. Jing, P.A. Walker, J.F. Eccleston, L.F. Haire, P. Saiu, S.A. Howell, R. Aasland, S.R. Martin, D. Carling, S.J. Gamblin, Structure of mammalian AMPK and its regulation by ADP, Nature 472 (2011) 230–233.
[9] L. Chen, J. Wang, Y.Y. Zhang, S.F. Yan, D. Neumann, U. Schlattner, Z.X. Wang, J.W. Wu, AMP-activated protein kinase undergoes nucleotide-dependent conformational changes, Nat. Struct. Mol. Biol. 19 (2012) 716–718.
[10] J.S. Oakhill, R. Steel, Z.P. Chen, J.W. Scott, N. Ling, S. Tam, B.E. Kemp, AMPK is a direct adenylate charge-regulated protein kinase, Science 332 (2011) 1433–1435.
[11] G.J. Gowans, S.A. Hawley, F.A. Ross, D.G. Hardie, AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation, Cell Metab. 18 (2013) 556–566.

[12] B. Cool, B. Zinker, W. Chiou, L. Kifle, N. Cao, M. Perham, R. Dickinson, A. Adler, G. Gagne, R. Iyengar, G. Zhao, K. Marsh, P. Kym, P. Jung, H.S. Camp, E. Frevert, Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome, Cell Metab. 3 (2006) 403–416.

[13] J.W. Scott, B.J. van Denderen, S.B. Jorgensen, J.E. Honeyman, G.R. Steinberg, J.S. Oakhill, T.J. Iseli, A. Koay, P.R. Gooley, D. Stapleton, B.E. Kemp, Thienopyridone drugs are selective activators of AMP-activated protein kinase beta1-containing complexes, Chem. Biol. 15 (2008) 1220–1230.

[14] M.J. Sanders, Z.S. Ali, B.D. Hegarty, R. Heath, M.A. Snowden, D. Carling, Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family, J. Biol. Chem. 282 (2007) 32539–32548.

[15] J. Choi, N. He, M.K. Sung, Y. Yang, S. Yoon, Sanguinarine is an allosteric activator of AMP-activated protein kinase, Biochem. Biophys. Res. Commun. 413 (2011) 259–263.

[16] T. Pang, Z.S. Zhang, M. Gu, B.Y. Qiu, L.F. Yu, P.R. Cao, W. Shao, M.B. Su, J.Y. Li, F.J. Nan, J. Li, Small molecule antagonizes autoinhibition and activates AMP-activated protein kinase in cells, J. Biol. Chem. 283 (2008) 16051–16060.

[17] H. Yun, J. Ha, AMP-activated protein kinase modulators: a patent review (2006–2010), Expert Opin. Ther. Pat. 21 (2011) 983–1005.

[18] P.C. Cheung, I.P. Salt, S.P. Davies, D.G. Hardie, D. Carling, Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding, Biochem. J. 346 (Pt 3) (2000) 659–669.

[19] O. Goransson, A. McBride, S.A. Hawley, F.A. Ross, N. Shpiro, M. Foretz, B. Viollet, D.G. Hardie, K. Sakamoto, Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase, J. Biol. Chem. 282 (2007) 32549–32560.

[20] J.W. Scott, S.A. Hawley, K.A. Green, M. Anis, G. Stewart, G.A. Scullion, D.G. Norman, D.G. Hardie, CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations, J. Clin. Invest. 113 (2004) 274–284.

[21] V.L. Rath, M. Ammirati, D.E. Danley, J.L. Ekstrom, E.M. Gibbs, T.R. Hynes, A.M. Mathiowetz, R.K. McPherson, T.V. Olson, J.L. Treadway, D.J. Hoover, Human liver glycogen phosphorylase inhibitors bind at a new allosteric site, Chem. Biol. 7 (2000) 677–682.

[22] S.W. Wright, A.A. Carlo, M.D. Carty, D.E. Danley, D.L. Hageman, G.A. Karam, C.B. Levy, M.N. Mansour, A.M. Mathiowetz, L.D. McClure, N.B. Nestor, R.K. McPherson, J. Pandit, L.R. Pustilnik, G.K. Schulte, W.C. Soeller, J.L. Treadway, I.K. Wang, P.H. Bauer, Anilinoquinazoline inhibitors of fructose 1,6-bisphosphatase bind at a novel allosteric site: synthesis, in vitro characterization, and X-ray crystallography, J. Med. Chem. 45 (2002) 3865–3877.