REVIEW ARTICLE

Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis

Guy A. RUTTER[1], Gabriela DA SILVA XAVIER and Isabelle LECLERC

Henry Wellcome Laboratories of Integrated Cell Signalling and Department of Biochemistry, University Walk, University of Bristol, Bristol BS8 1TD, U.K.

AMPK (5′-AMP-activated protein kinase) is emerging as a metabolic master switch, by which cells in both mammals and lower organisms sense and decode changes in energy status. Changes in AMPK activity have been shown to regulate glucose transport in muscle and glucose production by the liver. Moreover, AMPK appears to be a key regulator of at least one transcription factor linked to a monogenic form of diabetes mellitus. As a result, considerable efforts are now under way to explore the usefulness of AMPK as a therapeutic target for other forms of this disease. Here we review this topic, and discuss new findings which suggest that AMPK may play roles in regulating insulin release and the survival of pancreatic islet β-cells, and nutrient sensing by the brain.

Key words: 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), 5′-AMP-activated protein kinase (AMPK), diabetes mellitus, glucose homoeostasis, insulin secretion, pancreatic islet β-cell.

INTRODUCTION

Diabetes mellitus is characterized by elevated levels of blood glucose (hyperglycaemia), fatty acids and, in severe cases, ketone bodies [1]. The disease is associated with absent [Type I (insulin-dependent) diabetes] [2], low, normal or raised [Type II (non-insulin-dependent) diabetes] [3] levels of blood insulin [1]. Diabetes now affects 3–5 % of the population in Westernized countries [4], and the treatment of diabetic complications (heart disease, kidney failure, peripheral neuropathy, retinopathy, etc.) accounts for almost 10 % of health care costs in both Europe and North America. The incidence of Type II diabetes, already at near-epidemic proportions, is projected to double by 2025 [5], in large part as a result of a dramatic increase in the prevalence of obesity [6]. New treatments of diabetes are thus urgently required.

AMPK (5′-AMP-activated protein kinase) is emerging as a potentially interesting drug target for the treatment of diabetes. Work by several groups during the past 15 years, especially those of Hardie [7], Kemp [8], Witters [9] and Carling [10], has revealed that AMPK probably serves as a key metabolic sensor in both insulin-sensitive and other tissues [7] that is capable of responding to metabolic stresses (and in particular depletion of intracellular ATP) by shutting down the synthesis of fatty acids and cholesterol, two major energy-consuming pathways. The possibility that AMPK may play such a role in mammals is particularly appealing, given that homologues of AMPK both in the budding yeast *Saccharomyces cerevisiae* [11] and in plants [12] are activated in response to glucose depletion, and control the expression of genes that permit growth on alternative carbon sources.

An important goal of this review is to provide an update on recent findings that implicate AMPK in the control of blood glucose homoeostasis in humans and other mammals, and described in a surge of recent publications during the past 1–2 years (Figure 1). Readers are referred to an excellent article on a similar theme for further background and a review of work up to 1999 [13]. We will first survey molecular aspects of AMPK function before discussing the potential role of AMPK in insulin-sensitive tissues, as well as in the insulin-secreting pancreatic β-cell and in other tissues.

HISTORICAL BACKGROUND

The first reports of an enzyme that would later be named AMPK appeared in 1973. Gibson and co-workers [14] described an activity capable of inhibiting hydroxymethylglutaryl-CoA reductase, while Carlson and Kim [15] described the regulation of ACC (acetyl-CoA carboxylase) by reversible phosphorylation. The protein kinase involved, whose ability to phosphorylate ACC was later found to be highly dependent on the presence of 5′-AMP, was first characterized in 1980 [16]. The molecular identification [17] and cloning [18–20] of AMPK came some years later. Strikingly, this achievement revealed the existence of homologues of mammalian AMPK both in *S. cerevisiae* (SNF-1, for sucrose non-fermenting-1) [11] (Table 1) and in plants (SnRK, for SNF-1 related kinase) [12,21]. In each of the latter cases, the AMPK homologue acted as a switch to reset gene expression appropriately for survival on alternative sugars [7,9], via the phosphorylation and inactivation of transcriptional repressors, such as yeast Mig1 (maltose-inducible gene-1) [22–24].

Until quite recently, conclusive evidence that AMPK might play an equally important role in the normal physiology of animal cells was lacking. Instead, activation of AMPK was thought to act principally as part of a late response to pathological conditions such as severe metabolic shock (e.g. ischaemia or metabolic poisoning) or other stresses (e.g. heat), in order to disable anabolic

Abbreviations used: ACC, acetyl-CoA carboxylase; ACRP30, adipocyte complement-related protein of 30 kDa; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, 5′-AMP-activated protein kinase; AMPKK, AMPK kinase; $[Ca^{2+}]_c$, cytosolic free Ca^{2+} concentration; CBS, cystathionine β-synthase; CHO, Chinese hamster ovary; ChREBP, carbohydrate response element binding protein; eIF, eukaryotic initiation factor; FAS, fatty acid synthase; HNF4α, hepatocyte nuclear factor 4α; HSL, hormone sensitive lipase; IRS-1, insulin receptor substrate-1; K_{ATP} channel, ATP-sensitive potassium channel; L-PK, liver-type pyruvate kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositode 3′-kinase; PKA, protein kinase A; PKC, protein kinase C; PPARγ, peroxisome proliferator activated receptor γ; SNF-1, sucrose non-fermenting; SREBP, sterol regulatory element binding protein.

[1] To whom correspondence should be addressed (e-mail g.a.rutter@bris.ac.uk).

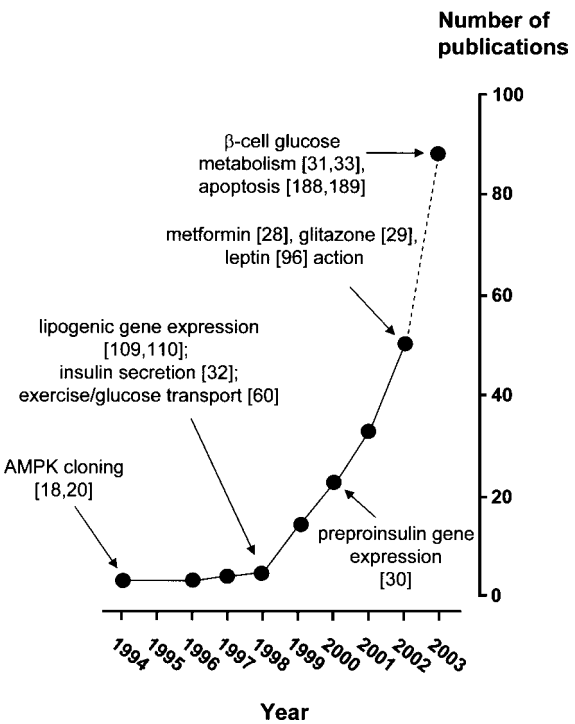

Figure 1 Publications on AMPK and diabetes or glucose metabolism, 1994–2003

Published articles were identified using Pubmed (www.ncbi.nlm.nih.gov/entrez/query.fcgi) using the search parameter 'AMPK plus glucose'. Only references on mammalian systems are included in the yearly totals. The value for 2003 was calculated by extrapolation of the number of papers published up to 20 May 2003.

Table 1 AMPK orthologues in mammals and in *Saccharomyces cerevisiae*

Chromosomal locations of the human subunits were determined using the PubMed (www.ncbi.nlm.nih.gov/entrez/) and Ensemble (www.ensembl.org) databases.

AMPK	Gene map locus in *Homo sapiens*	*SNF1*	Function
$\alpha 1$ $\alpha 2$	5p13 1p32	Snf1p	Catalytic
$\beta 1$ $\beta 2$	12q24.1 1q21.2 Gal83	Sip1 Sip2	Adapter/cellular localization
$\gamma 1$ $\gamma 2$ $\gamma 3$	12q13.1 7q36.1 2q36.1	Snf4	Regulator

pathways involved in lipid and cholesterol synthesis, while stimulating fatty acid oxidation. In this way, ATP might be spared for other, more urgent, uses, including the execution of an apoptotic programme if necessary [25,26]. However, more recent findings demonstrated that AMPK activity is regulated in some mammalian tissues by physiological stimuli, including hormones and nutrients, as well as by 'routine' changes in ATP consumption, e.g. during the contraction of skeletal muscle. As a result, changes in AMPK activity now seem very likely to be involved in fuel selection in animal tissues [13,27] as well as in yeast and plants. Recent data suggest that several drugs currently in wide usage in the treatment of obesity and diabetes, including metformin [28] and rosiglitazone [29], may act, at least in part, by regulating AMPK activity. These observations re-inforce the view that modulation of AMPK activity may be an important means of regulating fuel (specifically glucose and fatty acid) usage in such tissues. However, and as discussed below, data from this [30,31] and several other [32–34] laboratories suggest that changes in AMPK in the pancreatic islet β-cell may also be important for the regulation of insulin synthesis and secretion. In addition, AMPK seems also to be involved in regulating satiety centres in the hypothalamus [35], as discussed below.

SUBUNIT STRUCTURE OF MAMMALIAN AMPK

Mammalian AMPK is a trimeric enzyme comprising a catalytic α subunit (63 kDa) and non-catalytic β and γ subunits [36,37] (Table 1). Multiple isoforms of each mammalian enzyme exist ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$–$\gamma 3$), each encoded by a different gene. The β subunits have a calculated molecular mass of 30 kDa, but migrate on SDS/PAGE with apparent masses of 38 kDa ($\beta 1$) and 34 kDa ($\beta 2$), whereas the three γ isoforms have molecular masses of 37 kDa ($\gamma 1$), 63 kDa ($\gamma 2$) and 55 kDa ($\gamma 3$) [38]. While AMPK complexes containing $\alpha 2$ (the first catalytic isoform to be cloned) [39] predominate in skeletal and cardiac muscle [8], approximately equal levels of $\alpha 1$ and $\alpha 2$ complexes are present in the liver [40]. In contrast, pancreatic islet β-cells largely express $\alpha 1$ complex activity [30,32]. Less is known about the tissue distribution of the non-catalytic subunits, although $\beta 2$ subunits predominate in muscle [41]. $\gamma 1$ and $\gamma 2$ subunits are expressed ubiquitously [42], whereas $\gamma 3$ expression is confined to skeletal muscle [38].

At present, only limited crystallographic data are available to provide details of the three-dimensional structure of the individual AMPK subunits or the holoenzyme [37]. However, three domains are clearly evident in the primary structure of the α (catalytic) subunit: an N-terminal catalytic domain, a central auto-inhibitory region, and a C-terminal regulatory domain to which the β and γ subunits seem likely to bind [9] (Figure 2).

Members of the β-subunit family (*S. cerevisiae* homologues Gal 83p and Sip2p) are myristoylated at the N-terminus, and may also be phosphorylated at two or three sites [43,44]. Myristoylation may contribute to the association with membranes seen in some cases, e.g. with $\alpha 2$-subunit-containing complexes in pancreatic MIN6 β-cells [30]. The β subunit also possesses a glycogen binding domain with high similarity to starch binding domains, whose potential physiological relevance will be discussed below [45,46]. Finally, the γ subunits (*S. cerevisiae* homologue Snf4p) seem likely to be the site of nucleotide binding [38], perhaps via paired CBS (cystathionine β-synthase) domains [47]. Correspondingly, several mutations in the CBS domains of the $\gamma 2$ subunit [48] are associated with hypertrophic cardiomyopathy and Wolff–Parkinson–White syndrome [49], and mutations in the CBS domains of AMPK $\gamma 3$ (R200Q) are associated with abnormally high muscle glycogen content in the Hampshire pig [50]. At present, however, no linkage has been reported between mutations in any of the human AMPK subunits and Type II diabetes [51]. Potential changes in the interactions between the individual subunits, which may contribute to the regulation of enzyme activity by 5′-AMP, are shown in Figure 2.

REGULATION OF AMPK IN MAMMALIAN CELLS: GENERAL PRINCIPLES

AMPK lies at the foot of an activating signal transduction cascade, in which the α subunit is phosphorylated on Thr-172 by an upstream kinase (AMPKK) [52] (Figure 2). Although not yet defined in molecular terms, an AMPKK activity has been purified

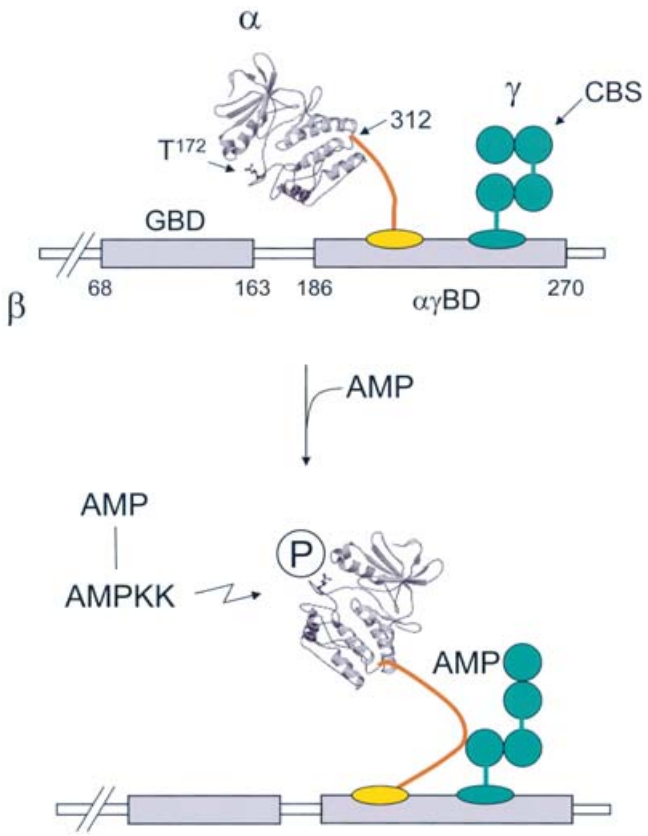

Figure 2 Possible subunit interactions involved in the activation of AMPK by phosphorylation and by allosteric regulation by 5′-AMP

The β subunit may act as a scaffold, binding the α and γ subunits via a C-terminal binding domain ($\alpha\gamma$BD). Phosphorylation of the α subunit at Thr-172 by AMPKK may lead to a conformational change in the α subunit autoinhibitory domain (brown), alterting its interaction with CBS domains of the γ subunit (green), relieving inhibition of catalytic activity in the α subunit. GBD, glycogen binding domain. The three-dimensional structure of the α subunit is adapted from Curr. Biol., **13**, Polekhina, G., Gupta, A., Michell, B. J., van Denderen, B., Murthy, S., Feil, S. C., Jennings, I. G., Campbell, D. J., Witters, L. A., Parker, M. W. et al., AMPK beta subunit targets metabolic stress sensing to glycogen, pp. 867–871, Copyright (2003), with permission from Elsevier [46]. Overall architecture is adapted from [38].

from liver extracts [53], and is co-immunoprecipitated by antibodies against the β subunit of AMPK, suggesting that the two kinases (AMPKK and AMPK) may normally be associated in a complex. In addition to covalent modification, AMPK activity is regulated acutely by AMP both (a) through a direct, allosteric mechanism, and (b) by making the enzyme a poorer substrate for dephosphorylation. This complex regulatory system probably provides exquisite sensitivity to even very small changes in intracellular AMP levels [52], which, thanks to the equilibrium reaction catalysed by adenylate kinase [54], are also highly sensitive to small changes in the intracellular [ATP]/[ADP] ratio.

REGULATION OF AMPK IN SKELETAL MUSCLE AND HEART

Uptake of glucose into skeletal muscle accounts for $>70\,\%$ of glucose disposal in humans [3,55], so this process is of paramount importance for normal glucose homoeostasis. Moreover, glucose uptake into skeletal muscle is markedly inhibited in subjects with Type II diabetes *in vivo* [56] and in skeletal muscle isolated from diabetic patients [57]. Glucose uptake is stimulated both by insulin, largely through the recruitment to the plasma membrane of the glucose transporter Glut4 [58–60], and during muscle contraction [61] by an insulin-independent mechanism. Recent evidence [13,62] suggests that AMPK may play an important role in Glut4 translocation to the cell surface, at least in the response to exercise. Furthermore, the action of a 'hepatoportal vein glucose sensor', which appears to mediate an insulin-independent uptake of glucose into muscle following increases in the portal glucose concentration after a meal [63], requires the presence of AMPK in muscle [64].

Enhanced ATP consumption during exercise [65] suggests that increases in AMPK activity might be expected, and such changes have been demonstrated in skeletal muscle by direct enzymic assay [66–68]. Activation of AMPK is probably then responsible for inactivating ACC II (the muscle isoform of ACC) by phosphorylation on Ser-221, causing a fall in intracellular malonyl-CoA levels, de-inhibition of carnitine palmitoyltransferase I and stimulation of the β-oxidation of long-chain acyl-CoAs by mitochondria [27,68,69]. At the same time, a decrease in the mitochondrial oxidation of glucose ensues, favouring ATP synthesis from glycolysis in this tissue (the 'Randle' or glucose/fatty acid cycle) [70]. Interestingly, changes in heart work rate were associated with parallel increases in [AMP] in the range 0.5–30 μM, with no significant change in [ATP] and with a near 10-fold increase in AMPK activity, suggesting that AMPK has an effective K_m for AMP in the low micromolar range *in vivo* [71]. Moreover, these observations suggest that increases in [AMP], even in the absence of changes in [ATP], which are usually tightly buffered in most tissues, are alone sufficient to bring about changes in AMPK activity.

Many important recent findings with regard to glucose homoeostasis and the role of AMPK have resulted from the use of the cell-permeant adenosine analogue, AICAR (5-aminoimidazole-4-carboxamide ribonucleoside), which is rapidly taken up into cells and phosphorylated to form the AMP mimetic, ZMP, usually without changing the intracellular levels of AMP or ATP [7]. Treatment with AICAR causes enhanced uptake of glucose into isolated or perfused muscle *in vitro* [72,73], and into the skeletal muscle of conscious rats [74]. These effects are thus likely to contribute to the stimulation of glucose clearance during exercise [61,75]. Importantly from a mechanistic perspective, the effects of AICAR and exercise are not additive [76], suggesting that activation of AMPK is the common downstream mediator of both forms of stimulation. The effects of AICAR have been shown to be due, in large part, to the recruitment to the plasma membrane of Glut4 in both heart cells [76] and skeletal muscle [77] (although direct activation of Glut1 has also been reported recently in epithelial cells [78]). In contrast, the effects of insulin on the recruitment to the plasma membrane of Glut4 are independent of AMPK activation. Thus the ability of insulin to provoke Glut4 redistribution is completely inhibited by wortmannin, an inhibitor of PI3K (phosphoinositide 3-kinase), whereas the effects of AICAR are unaffected by this drug [72].

Although an important tool, AICAR's utility is limited by: (1) accumulation of the triply phosphorylated form, which may act as an ATP analogue (see below with reference to insulin secretion); (2) the fact that ZMP very probably mimics the effects of AMP by mechanisms outside of its actions on AMPK, including the stimulation of other AMP-sensitive enzymes such as glycogen phosphorylase [79]; and (3) the fact that AICAR may also act via adenosine receptors [80], adenosine transporters [81] or both. Molecular approaches have therefore been sought in order to activate or inactivate AMPK selectively. In a recent elegant study [62], blockade of AMPK selectively in the skeletal muscle of mice was achieved by targeted expression of a dominant-negative, 'kinase dead' form of AMPK α2, mutated in the ATP binding site (K45R),

and under the control of the muscle-specific creatine kinase promoter. Expression of dominant-negative AMPK reduced the effects of exercise partially, and those of AICAR and hypoxia completely, on glucose uptake by muscle. The latter result is of particular interest, since it indicates that the stimulation of glucose uptake in response to exercise involves both AMPK-dependent and -independent signalling events [62]. The nature of these pathways is unclear, although the involvement of PKC (protein kinase C) or changes in $[Ca^{2+}]_c$ (cytosolic free Ca^{2+} concentration) are likely possibilities (and indeed atypical PKCs as well as the protein tyrosine kinase Pyk2 [82] are also implicated as downstream mediators of the effects of AMPK on glucose transport) [83].

Direct measurements of exercise-induced changes in the activity of each isoform in skeletal muscle suggest that changes in $\alpha 2$ activity are much greater than those in $\alpha 1$ [84]. Moreover, mice overexpressing dominant-negative AMPK $\alpha 2$ show markedly greater fatigue, demonstrating the importance of AMPK in the normal responses to the stresses imposed on muscle by increased workload. Nevertheless, it has been proposed that changes in AMPK $\alpha 1$, rather than $\alpha 2$, activity may play some role in the defective contraction-induced glucose metabolism in diabetes [82]. Thus only changes in $\alpha 1$ activity were affected in skeletal muscle from a model of Type II diabetes, the Zucker diabetic fatty rat [85].

Another recently identified target of AMPK is the bifunctional regulatory enzyme phosphofructokinase/fructose-2,6-bisphosphatase, the stimulation of which enhances glycolytic flux in the heart when [AMP] is elevated. Phosphorylation of this enzyme at Ser-466 during ischaemia of the heart leads to an enhancement of the V_{max} of the kinase relative to the phosphatase activity, and consequent accumulation of fructose 2,6-bisphosphate. By activating phosphofructokinase-1, fructose 2,6-bisphosphate contributes to the increase in glycolytic flux during ischaemia (the Pasteur effect) [86]. The effects of ischaemia on fructose 2,6-bisphosphate levels, which could be mimicked by AICAR, were blocked by a dominant-negative form of AMPK, thus demonstrating the essential involvement of this enzyme. Interestingly, insulin decreased the activation of AMPK during ischaemia, while having no effect on the change in intracellular [AMP], suggesting that insulin may regulate the enzyme by a distinct but as yet undefined signalling pathway [86]. That the regulation of glycolytic flux by changes in AMPK activity may not be a phenomenon confined to heart cells is suggested by the finding that inducible phosphofructokinase-2, the isoform expressed in monocytes, also appears to be regulated by AMPK during hypoxia [87]. In contrast with the effects of ischaemia, the well known effect of increased workload to stimulate glycolysis in the heart [88] was shown recently not to affect total ATP, AMP, phosphocreatine or creatine ratios [89], consistent with some earlier studies [90], but in conflict with others [64] (see above; [91]). Correspondingly, AMPK activity was unchanged by work rate in the recent studies [89], indicating that distinct mechanisms may regulate glucose transport and glycolysis in cardiac and skeletal muscle, at least when glucose (and insulin) are present [91].

Very recently, Viollet and colleagues [92] have developed mice in which both alleles of the $\alpha 2$ isoform of AMPK are inactivated through homologous recombination. These animals display abnormal glucose tolerance and are insulin resistant. Perhaps surprisingly, glucose transport was normal in isolated AMPK $\alpha 2^{-/-}$ muscles, and islets from these animals displayed normal glucose- or arginine-stimulated insulin secretion [92]. On the other hand, catecholamine levels were increased significantly in this model, and blockers of α- (and to a lesser extent β-) adrenoreceptors rescued the abnormalities in glucose metabolism. These studies are, however, complicated by a marked compensatory increase in AMPK $\alpha 1$ expression, at least in muscle (the levels of AMPK $\alpha 1$ were not measured in pancreatic islets). Nevertheless, they do suggest that AMPK $\alpha 2$ activity is essential for the function of a central glucose sensor, most probably located in the hypothalamus, which governs sympathetic tone and thus adrenal catecholamine release (see below). It should be mentioned that double knockout of both AMPKα isoforms (i.e. $\alpha 1$ and $\alpha 2$) caused early embryonic lethality [92], and it seems likely that further investigation of the importance of the separate isoforms will require conditional, tissue-specific inactivation of the two genes through Cre–*LoxP* strategies [93].

In addition to workload and ischaemia, it now appears that leptin (a satiety factor secreted by adipocytes [94]), insulin-sensitizing drugs of the glitazone family, such as rosiglitazone [29], and metformin [95] may all regulate AMPK activity in skeletal muscle. Thus Minokoshi and colleagues [96] demonstrated that intrahypothalamic or intravenous injection of recombinant leptin into mice led to activation of AMPK $\alpha 2$ in soleus muscle within 15 min. The effect of intravenous leptin was apparently direct, being (a) unaffected when the sciatic nerve was severed, (b) reproducible in isolated soleus muscle, and (c) absent from the muscle of *db*/*db* mice, which lack functional leptin receptors. These direct effects of leptin also appeared to be mediated, at least in part, by increases in intracellular [AMP]. In contrast, the neurally mediated effects of leptin appeared to involve α-adrenoreceptors, acting by a mechanism that did not involve any increase in intracellular AMP level. Importantly, these studies provide an explanation for earlier observations that leptin both decreases triacylglycerol stores [97] and increases insulin sensitivity [98] in skeletal muscle, once again making the regulation of AMPK a central factor in the control of glucose homoeostasis. Furthermore, 2-week infusion of leptin into rats, achieved with a subcutaneous pump, increased the expression of AMPK $\alpha 2$ and $\beta 2$ in soleus and gastronemius muscle, without causing any detectable changes in the total concentrations of ATP, ADP or AMP in soleus muscle, although a small (but not significant) increase in calculated free [AMP] was reported in soleus muscle [99]. In marked contrast with the above findings, recent data suggest that leptin partitions fatty acids towards oxidation rather than triacylglycerol storage in the heart by a mechanism that does not involve changes in AMPK phosphorylation and activity. Thus other, AMPK-independent, mechanisms are also utilized by leptin to regulate fatty acid metabolism, at least in cardiac muscle [100].

A further level of control of AMPK in muscle (and possibly other tissues) has emerged recently. Thus Wojtaszewski and colleagues [101] demonstrated that activation of AMPK $\alpha 2$ by AICAR in skeletal muscle is suppressed as glycogen levels are increased, whereas Yeaman and colleagues [102] observed an increase in AMPK $\alpha 1$ and $\alpha 2$ activity upon incubation of muscle cells at low glucose concentrations, and an associated decrease in the proportion of glycogen synthase in the active form. These observations are compatible with a direct interaction between glycogen and a recently identified glycogen binding domain within the AMPK β subunit, the presence of which is essential for an association between AMPK and glycogen particles [45,46] (Figure 2). Although the physiological significance of this interaction is not yet fully understood, it may be important for bringing the kinase into close proximity with glycogen-bound phosphatases. Interestingly, the relationship between AMPK activation and glycogen synthase activity may be a reciprocal one. Thus treatment of a muscle cell line with AICAR leads to a decrease in the proportion of glycogen synthase in the active, dephosphorylated form [102]. During metabolic stress, this effect, combined with enhanced glucose uptake, is expected to enhance the

flow of glucose carbon through glycolysis and away from glycogen storage.

ROLE OF AMPK IN THE CONTROL OF GLUCOSE AND FATTY ACID METABOLISM IN THE LIVER AND ADIPOSE TISSUE

Liver is central to the regulation of glucose homoeostasis in mammals, and inactivation of insulin receptors selectively in this tissue causes diabetes [103], despite the fact that the tissue is responsible for only ~30% of glucose disposal [104].

Recent studies by Zhou and colleagues [95] have revealed that AICAR is a powerful suppressor of glucose output from hepatocytes. Moreover, these studies made the important observation that the effects of AICAR, at least in hepatocyes, are likely to be due largely to the regulation of AMPK, since they were fully reversed by a selective inhibitor of the enzyme, 'Compound C'. Strikingly, metformin, a biguanide in widespread use for the treatment of Type II diabetes and obesity [105], and shown previously to suppress glucose production by the liver [106], had the same effect as AICAR on glucose output, and this effect of metformin was reversed by Compound C. Whether the effects of metformin represent a genuinely important mechanism by which this drug acts *in vivo* is, nonetheless, open to question, since very high concentrations, well in excess of levels usually achieved in the blood (micromolar), were required to observe clear effects on glucose output. However, studies by both Halestrap and colleagues [107] and Zhou et al. [95] provided evidence that much lower concentrations of metformin can exert similar effects if the period of exposure to the drug is longer. This probably reflects the relatively slow permeation of the drug across cell membranes and its subsequent accumulation within the mitochondrial matrix, where it exerts a dose-dependent inhibitory effect on complex I of the respiratory chain. The effects of metformin reported by Zhou and colleagues [95] were not associated with changes in total intracellular AMP or ATP concentrations, although these workers did not measure free adenine nucleotide concentrations. Moreover a recent, longer term (10-week) study of the effects of metformin treatment in diabetic human subjects reported both an increase in AMPK $\alpha 2$ activity in muscle biopsies and a decrease in muscle ATP content [108].

Recently, both Hardie and colleagues [28] and Carling and colleagues [29] have presented data arguing for a distinct, non-metabolic effect of metformin. In those studies, metformin failed to decrease total ATP or increase AMP levels, while stimulating AMPK to a greater extent than was achieved using well known metabolic poisons. However, since metformin may act on mitochondria by mechanisms (including uncoupling) distinct from those of either oligomycin or rotenone, it remains conceivable that the biguanide may have caused changes in the free concentrations of adeninine nucelotides selectively in the cell cytosol that were masked in measurements using total cell extracts [107,108]. On the other hand, the observation by Hawley et al. [28] that AMPK activity was increased by metformin in CHO (Chinese hamster ovary) cells, which are largely glycolytic and resistant to mitochondrial poisoning with oligomycin or rotenone, also points to an effect of the drug that is independent of changes in mitochondrial ATP synthesis [28]. Moreover, metformin increased [ADP]/[ATP] values in H-2K muscle cells by 1.6-fold [29], although total AMP levels were too low to be quantified accurately in these studies. The latter report also provided independent evidence that AMPK activity, and phosphorylation at Thr-172, may be controlled by an upstream pathway that does not involve an increase in intracellular AMP. Thus osmotic stress imposed by 600 mM sorbitol activated both AMPK $\alpha 1$- and $\alpha 2$-containing complexes ~3-fold without inducing any measurable changes in total ATP, ADP or AMP. The same fold induction of AMPK activity by the mitochondrial uncoupler dinitrophenol was associated with a 2-fold increase in the total [AMP]/[ATP] ratio, whereas the 5–7-fold induction of AMPK activity caused by metformin was only mimicked by a concentration of dinitrophenol sufficient to increase [AMP]/[ATP] by 6-fold. So, in spite of the above caveats, a mechanism of AMPK regulation by metformin that is independent of [AMP] remains a strong possibility. While the nature of the putative AMP-independent pathway remains elusive, its existence raises the intriguing possibility that multiple kinases may phosphorylate AMPK directly. Although the identity of these kinases remains obscure, pharmacological investigations would appear to eliminate a role for MAPK (mitogen-activated protein kinase), the stress-activated MAPK family member p38, PKC and PI3K [29].

Effects of AMPK on gene expression in the liver

Given the role played by AMPK in the control of genes involved in fuel selection in yeast [11], an analogous role for the enzyme in the control of gene expression in mammals might be predicted. Correspondingly, the activation of AMPK either by AICAR [95,109,110] or by overexpression of constitutively active AMPK $\alpha 1$ [40] had marked effects on the expression of a number of glycolytic and lipogenic enzymes in the liver [109,110] that are normally under the control of nutrients and hormones, including insulin. These enzymes included L-PK (the liver isoform of pyruvate kinase), fatty acid synthase [95] and ACC, as well as 'Spot14'. Moreover, phosphoenolpyruvate kinase gene expression was reported to be activated by AICAR in some studies [111], but inhibited [112] or unaffected [109] in others.

An interesting finding has been the demonstration that AICAR causes marked decreases in the levels of both SREBP1c (sterol regulatory element binding protein 1c) [95], a key regulator of lipogenic genes [113,114], and HNF4α (hepatocyte nuclear factor 4α) [115]. The latter factor is a member of the steroid/thyroid hormone receptor superfamily [116] and regulates the expression of a number of key genes in both liver and pancreatic β-cells [117], including those encoding Glut2, L-PK and possibly the preproinsulin gene in islet β-cells (see below) [118]. Mutations in HNF4α (F75fsdelT, F99fsdelAA, R154X, Q268X, G115S, E276Q, V393I) are responsible for an autosomal-dominant, early-onset form of non-insulin-dependent diabetes mellitus, MODY-1 (maturity-onset diabetes of the young-1) [117,119–123]. HNF4α is phosphorylated by AMPK *in vitro* at multiple sites, including a consensus AMPK phosphorylation site located at Ser-304, and mutation of this residue to aspartate (S304D) decreases the ability of HNF4α to dimerize and to bind DNA, and also decreases the stability of the expressed protein in CHO cells [115,124]. Importantly, phosphorylation and destabilization of HNF4α may also have a role in the β-cell (the other major site of expression of this transcription factor), since both treatment of mouse islets with AICAR (I. Leclerc, unpublished work) or expression of active AMPK $\alpha 1$ in INS-1 cells [33] decreases HNF4α protein levels by ~50%. Interestingly, the levels of expression of HNF1α and HNF1β were unaffected by expression of active AMPK $\alpha 1$ in the latter studies [33], indicating that the effects of AMPK on HNF4α were rather selective. The important implication of the above findings is that diminished expression of HNF4α is expected to lead to lower levels of both insulin and glucose-sensing proteins (Glut2 and L-PK), and may therefore contribute to the inhibition of β-cell glucose metabolism observed in response to overexpression of a constitutively active form of AMPK $\alpha 1$ (see below) [31,32,34].

The forkhead transcription factor FKHR (now referred to as Foxo1a) [125] has also been shown recently to be down-regulated upon incubation of H4 hepatoma cells with AICAR [126]. Inhibited expression of this factor may in turn repress the glucose-6-phosphatase gene, at least in clonal liver cells (H4IIE) [112]. Moreover, activation of AMPK by AICAR decreases the expression of SREBP1c both in hepatocytes [95] and in pancreatic islets [127], an effect that probably involves both decreased transcription of the SREBP1 gene and a post-transcriptional action on the expressed protein. Thus AICAR treatment (G. Zhou, personal communication) or overexpression of constitutively active AMPK α1 (F. Foufelle, personal communication) decreases the stability of mature SREBP1c in hepatocytes by enhancing proteasome-dependent degradation, although this effect is not observed in pancreatic islets [127]. Finally, the newly identified glucose-sensitive transcription factor, ChREBP (carbohydrate response element binding protein) [128], which is implicated in the regulation of L-PK transcription, appears also to be a substrate for AMPK in the liver [129]. Moreover, fatty acids, presumably through activation and the generation of AMP, reduced the activation by glucose of the L-PK promoter in liver cells and led to the phosphorylation of ChREBP. In common with HNF4α, an aspartate mutant of the phosphorylated serine (Ser-568) in ChREBP has only weak DNA binding and *trans*-activation capacity towards the L-PK promoter.

PPARγ (peroxisome proliferator-activated receptor γ), a key nuclear factor of adipocyte differentiation [130] and an important receptor for the glitazone class of insulin-sensitizing drugs [131], may also be a target for regulation by AMPK. Stressing the importance of PPARγ in normal glucose homoeostasis, mutations that destabilize the transactivation domain (P467L) lead to the formation of a dominant-negative form of the protein and to severe insulin resistance, diabetes mellitus and hypertension [132]. Recent studies have demonstrated that PPARγ interacts directly with AMPK γ subunits in yeast two-hybrid assays [133] and that activation of AMPK in 3T3-L1 adipocytes suppresses PPARγ gene expression [134], possibly through phosphorylation of the co-activator subunit, p300, at Ser-89 [133]. Thus AMPK plays a critical role in the regulation of a number of glucose- and/or insulin-regulated transcription factors, at least one of which (HNF4α) is known to be linked to monogenic forms of diabetes [122].

A perplexing aspect of the role of AMPK in the normal regulation of gene expression in the liver is that changes in the activity of the enzyme have been difficult to measure in primary isolated hepatocytes, under conditions in which alterations in lipogenic and/or gluconeogenic gene expression are clearly evident [40,135]. Moreover, while expression of a constitutively active form of AMPK α1 completely abrogated the effects of glucose to increase the levels of mRNAs encoding lipogenic enzymes, inhibition of AMPK activity with dominant-negative AMPK α1 failed to alter the suppression of these genes by low glucose concentrations [40]. Thus active AMPK would appear to serve in the liver as an efficient silencer of lipogenic gene expression, and this role may be important in mediating the effects of fatty acids during starvation [129]. On the other hand, it seems likely that additional mechanisms are involved in the normal stimulatory actions of high glucose (and insulin) levels on the hepatic expression of lipogenic genes.

Regulation of AMPK in adipose tissue

One study has examined the effects of AICAR in 3T3-L1 adipocytes [136]. In marked contrast with the muscle system, AICAR was found to have little effect on glucose transport in the absence of insulin, and to reverse the stimulatory effect of the hormone. Another interesting aspect of the effect of active AMPK on lipid metabolism is the phosphorylation of HSL (hormone-sensitive lipase). Thus stimulation of AMPK activity occurs in primary rat adipocytes in response to β-adrenoreceptor stimulation [137], an effect previously assumed, on the basis of studies using AICAR [138], to lead to the inhibition of lipolysis. This effect was proposed to result from phosphorylation by AMPK of HSL at Ser-565, blocking the subsequent phosphorylation at a distinct activatory site by PKA (protein kinase A) [139]. In this way, activation of AMPK was proposed simultaneously to inhibit both lipogenesis and lipolysis, thus preventing the re-esterification of fatty acids generated by lipolysis ('futile cycling') [7]. However, recent evidence from M. Birnbaum (personal communication) has shown that expression of a dominant-negative form of AMPK reduces, but does not completely abolish, the lipolytic response to the β-adrenoreceptor agonist isoprenaline, implying that activation of AMPK may in fact play a positive role in the stimulation of lipolysis. Whether cAMP is able to stimulate AMPK activity via PKA-dependent or -independent [140–143] mechanisms remains to be explored.

Another mechanism by which changes in AMPK activity seem likely to control ATP (and thus glucose) consumption in all cell types is via alterations in the rate of protein synthesis. Activation of AMPK leads to inhibition of the elongation phase of protein synthesis in isolated hepatocytes as a result of the phosphorylation of eEF2 (eukaryotic elongation factor 2α) [144]. Moreover, injection of AICAR led to the apparent inaction of signalling via protein kinase B and mTOR (mammalian target of rapamycin), and to a decrease in the amount of eIF4E (eukaryotic initiation factor 4E) in the active form (associated with eIF4G) [145], suggesting that distinct mechanisms may be involved in the regulation of protein synthesis by AMPK in muscle and liver.

A further role for AMPK in the control of gene expression has been reported recently, and involves the regulation of mRNA stability. Thus AMPK-mediated phosphorylation leads to the shuttling from the cytosol into the nucleus of HuR, a ubiquitously expressed RNA-binding protein which binds mRNAs via AU-rich regions, consequently increasing the half-lives of the bound messenger [146].

ROLE OF AMPK IN THE PANCREATIC ISLET β-CELL: REGULATION OF INSULIN SECRETION AND INSULIN GENE EXPRESSION

Role of AMPK in the acute regulation of insulin secretion

In contrast with the liver, where changes in intracellular adenine nucleotide levels in response to nutrients and hormones are usually small or non-existent [147], glucose has marked effects on oxidative metabolism and total intracellular ATP [148,149] and AMP [32] levels in pancreatic islet β-cells. Importantly, these changes are reflected in clear increases in the free concentration of ATP [150]. Such changes are implicated in the regulation of insulin secretion, since [ATP] changes in response to an elevation of glucose close K_{ATP} channels (ATP-sensitive K^+ channels) [151] and trigger membrane depolarization and the influx of Ca^{2+} [152], which in turn leads to exocytosis of insulin [153]. Thus, on theoretical grounds alone, AMPK might seem to be an attractive candidate to participate in regulating the secretory activity of these fuel-sensing cells.

At resting glucose concentrations, β-cells are effectively 'nutrient deprived', since both glucose uptake (by the high-K_m glucose transporter Glut2) [154,155] and glucose phosphorylation, which is mediated by glucokinase (hexokinase IV; $K_m \sim 10$ mM) [156,157], are minimally active. On the other hand, as glucose

concentrations in the blood increase, β-cell oxidative metabolism is enhanced [158,159], leading to observable increases in oxygen consumption [160–162], mitochondrial nicotinamide nucleotide fluorescence [159,163–165] and both total [149] and free ATP concentrations in the cytosol, mitochondria and subplasma membrane region [150], and corresponding decreases in total ADP and AMP concentrations [32,149]. The existence of such demonstrable changes in both [ATP] and [AMP] therefore make alterations in AMPK activity as the glucose concentration rises seem very likely. Correspondingly, elevations in glucose concentration over the physiological range markedly reduced AMPK activity in both clonal β-cell lines [30–32] and primary islets [166] (Figure 3). These changes were associated with a progressive and near complete dephosphorylation of AMPK at Thr-172 as glucose concentrations were increased from 3 to 10 mM [31] (Figure 3A).

While there has been great interest in the use of AICAR or other activators of AMPK as potentially a highly selective tool to achieve normoglycaemia (by stimulating glucose uptake by muscle and inhibiting hepatic glucose production; see above), the effects of this drug on insulin synthesis and release from pancreatic islet β-cells are disputed. This is clearly an important issue, since were the activation of AMPK by AICAR (or by any other means) to exert deleterious or inhibitory effects on β-cell function, then such agents would evidently be contra-indicated for the treatment of diabetes. In two early studies [167,168] AICAR was found to stimulate insulin release from isolated islets at both low and high glucose concentrations. However, one potential problem in the interpretation of these studies is that relatively high concentrations (> 1 mM) of AICAR, or long incubation times (> 1 h), may act in part to increase intracellular levels of ZTP (an ATP analogue), thus leading to the closure of K_{ATP} channels, Ca^{2+} influx [152] and the triggering of insulin secretion [153]. Interestingly, Salt and colleagues [32] found that whereas AICAR weakly stimulated insulin release from primary pancreatic islets at low (5.5 mM) glucose concentrations, the drug markedly inhibited secretion at a stimulatory glucose concentration (16.7 mM). Similarly, incubation with relatively low concentrations of AICAR causes a profound inhibition of glucose-stimulated insulin secretion in insulinoma-derived INS-1 [34] or MIN6 [31] cells and in primary rat islets [31]. This effect may be due in part to the suppression of malonyl-CoA synthesis, and thus to enhanced β-oxidation and a decline in the intracellular acyl-CoA concentration [27]. Consistent with this view, AICAR caused a substantial increase in ACC phosphorylation at Ser-79 in INS-1 [34,169] and in MIN6 [31] β-cells. Whether AMPK also plays a role in regulating insulin release from more glucose-responsive β-cell lines, such as INS-832/13 cells [170], remains to be seen.

In an attempt to circumvent the problems associated with the use of AICAR alone, da Silva Xavier et al. [31] explored the effects of overexpressing constitutively active (amino acids 1–312; T172D) or dominant-negative (D157A) (Figure 3B) forms of AMPK α1 on glucose-stimulated insulin secretion. Adenovirus-mediated expression of the former construct caused a near-total suppression of glucose-induced insulin secretion in clonal β-cells (MIN6; Figure 3C) and suppressed glucose-induced increases in both glycolysis and mitochondrial oxidative metabolism [31]. Moreover, constitutively active AMPK α1 blocked increases in $[Ca^{2+}]_c$ induced by 30 mM glucose, and strongly inhibited $[Ca^{2+}]_c$ increases provoked by cell depolarization with the sulphonylurea tolbutamide [31]. Essentially similar findings have recently been described by Eto and colleagues [33], using INS-1 cells and adenoviral expression of constitutively active AMPK α1. Although insulin secretion is stimulated more weakly by elevated

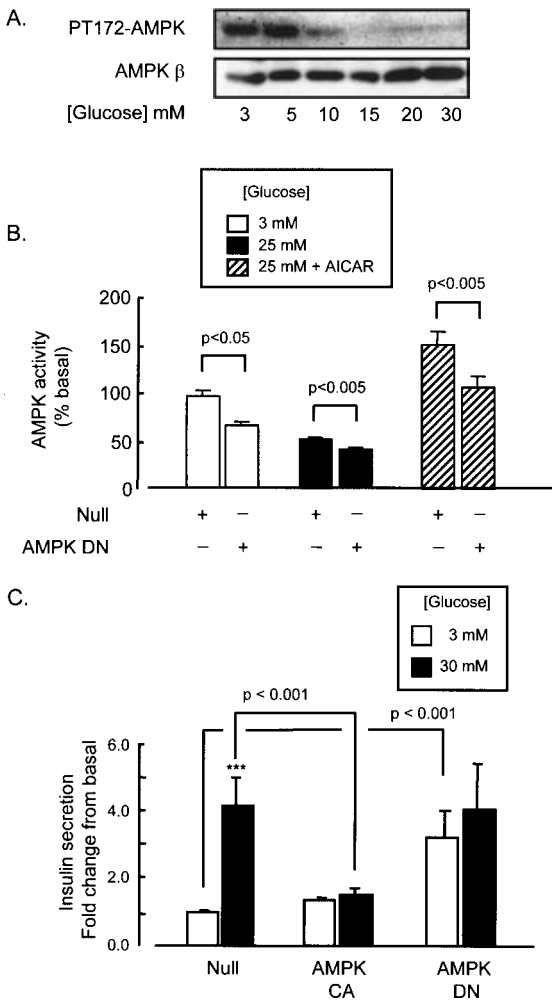

Figure 3 Regulation by glucose and role of AMPK activity in pancreatic MIN6 β-cells

(**A**) Elevated glucose concentrations decrease the phosphorylation of AMPK on Thr-172 (PT172-AMPK) in clonal MIN6 β-cells. Cells were cultured for 1 h at the indicated glucose concentrations prior to homogenization and analysis by SDS/PAGE using a polyclonal anti-phosphospecific (AMPK α-Thr-172) antibody. (**B**) Corresponding changes in AMPK activity, measured by phosphotransfer assay, in response to glucose, adenoviral expression of dominant-negative (DN) AMPK α1 or AICAR. (**C**) Adenoviral transduction with constitutively active (AMPK CA) or dominant-negative (AMPK DN) forms of AMPK α1. Insulin released during a 30 min incubation at the indicated glucose concentrations was quantified by RIA and normalized to total cellular insulin content. Data are modified from [31].

glucose concentrations in native INS-1 cells (~2-fold) than in MIN6 cells or primary islets [31], expression of active AMPK α1 caused a significant decrease in glucose-, tolbutamide- and KCl-stimulated insulin secretion, and also decreased insulin content, as expected [30]. Similarly, the latter study demonstrated a clear (~40%) decrease in the rate of [6-^{14}C]glucose oxidation [33], presumably reflecting an inhibition of mitochondrial metabolism [31].

In MIN6 cells, the inhibitory effects of overexpressing active AMPK were largely mimicked by acute exposure to AICAR, suggesting that the effects of active AMPK were mediated for the most part through the phosphorylation of pre-existing proteins [31]. Moreover, the extent of AMPK activation, as assessed by the level of its phosphorylation at Thr-172, or by phosphorylation of the downstream target ACC1 at Ser-79, was similar in cells

incubated with 3 mM glucose or with 30 mM glucose in the presence of AICAR [31,169], implying that increases in AMPK activity as postprandial glucose concentrations fall over the normal physiological range are capable of exerting a strong inhibitory effect on insulin secretion. While the identity of the molecular target(s) for AMPK whose phosphorylation may mediate the inhibitory effects of changes in the activity of the kinase remains unclear, one might speculate that inhibition and inactivation of ACC, and the consequent activation of fatty acid oxidation, may lead to decreases in pyruvate dehydrogenase activity, and thus to the entry of glucose carbon atoms into the citrate cycle [70], an alteration likely to inhibit glycoytic flux in β-cells [31].

As a counterpoint to the above findings, the expression of a dominant-negative acting form of AMPK α1, expected to inhibit both α1- and α2-containing complexes, had no apparent effects on glucose metabolism or glucose-induced increases in $[Ca^{2+}]_c$, but did cause an increase in the release of insulin. Intriguingly, this activation of insulin release was insensitive to decreases in $[Ca^{2+}]_c$ imposed by the hyperpolarizing agent diazoxide, implying the activation of a Ca^{2+}-independent pathway of secretion [31]. An important conclusion from these results is that the inhibition of AMPK by high glucose concentrations (mimicked here with the dominant-negative AMPK α1) is not in itself sufficient to close K_{ATP} channels or to provoke cell depolarization and Ca^{2+}-stimulated insulin release. On the other hand, increases in intracellular malonyl-CoA or acyl-CoA content, which might enhance insulin secretion by increasing the sensitivity of the secretory machinery to ambient $[Ca^{2+}]_c$ [171–173], seem unlikely to be involved, since these were not detected in cells overexpressing dominant-negative AMPK [31].

At present, the targets of AMPK that may mediate the above effects of the activated enzyme are not fully elucidated. However, bioinformatics analysis (G. A. Rutter, unpublished work) reveals that the list of these targets is likely to go substantially beyond known metabolic enzyme substrates. Intriguingly, up to one-half of all proteins encoded by the human genome contain one or more consensus phosphorylation sites for AMPK [^{-5}h(*b*/X)XX(S/T)XXX^{+4}h, where *h* is a hydrophobic and *b* is a positively charged amino acid] [7,174]. While it is likely that only a small proportion of these sites become phosphorylated in living cells when AMPK is activated, it is of interest that a number of proteins that may play a key controlling role in the acute regulation of insulin secretion are candidates for phosphorylation. Thus proteins bearing consensus phosphorylation sites include the motor proteins kinesin [175] and myosin [176], as well as the cortical actin network regulator gelsolin [177]. Supporting a possible involvement of the latter two proteins, expression of dominant-negative AMPK α1 in β-cells causes a complete collapse of the cortical actin network (T. Tsuboi and G. A. Rutter, unpublished work). Two key β-cell ion channels are also potential substrates for phosphorylation by AMPK, bearing conserved consensus phosphorylation sites. The channel-forming subunit of the K_{ATP} channel, Kir6.2 [178], possesses a consensus AMPK phosphorylation site at Thr-71. This site, which is absent from related members of the inwardly rectified K^+ channel family, is predicted [179] to lie in a cytosolic loop immediately N-terminal to the first membrane-spanning helix (M1) of the channel. Interestingly, Thr-71 is believed to interact with the second membrane-spanning domain (M2) of Kir6.2, and mutation of this residue to lysine or glutamate has marked effects on channel opening and pH sensitivity [180]. The sulphonylurea receptor subunit of this channel (SUR1), a member of the 'ABC cassette' family of nucleotide binding proteins [181], also bears an excellent potential AMPK phosphorylation site at the beginning of the cytosolic loop that is implicated in ATP binding (nucleotide binding fold-1) [182]. Correspondingly, treatment with AICAR significantly reduced insulin secretion (or the release of a co-transfected insulin surrogate) and increases in $[Ca^{2+}]_c$ provoked by the sulphonylurea tolbutamide in MIN6 cells [31]. Finally, a consensus AMPK sequence also exists in the calmodulin binding domain of the voltage-sensitive (L-type) Ca^{2+} channel α1D subunit (the isoform expressed in β-cells) [183], a region associated with Ca^{2+}-dependent channel inactivation [184,185]; this is conserved in *Caenorhabditis elegans*, where the phosphorylated serine is replaced by histidine, a target for regulatory phosphorylation in this organism [186]. Further studies are required to determine whether phosphorylation of these targets might thus contribute to the regulation of insulin release by AMPK (Figure 4).

A potential complicating effect of the therapeutic use of AICAR is the possibility that this agent may prompt β-cell apoptosis, as reported in Fto2B hepatoma and primary liver cells [187], through the activation of JNK (c-Jun N-terminal kinase). Moreover, AICAR has recently been reported to increase apoptotic rates in clonal MIN6 β-cells via a similar mechanism [188,189]. Such an effect, were it observed *in vivo*, would seem likely in the long run to have deleterious consequences for insulin synthesis and release. On the other hand, at least at time points less than 24 h, expression of neither constitutively active nor dominant-negative AMPK α1 affected apoptosis in MIN6 cells [31], raising the question of the involvement of other effects of AICAR in the apoptotic response. Interestingly, the apparent effect of AMPK activation to enhance apoptosis in β-cells [189] would appear to set this cell type apart from others, since activation of AMPK diminishes apoptotic rates in a number of other systems, including neurons [190] and astrocytes [191], and is required for the resistance of pancreatic tumour cells to nutrient depletion [192]. This important difference might be related to the apparently unique effect of AMPK activation in the β-cell to inhibit glucose metabolism and ATP production (see above), in stark contrast with the situation in most other cell types, where AMPK activation serves to stimulate glycolytic flux and to spare ATP consumption [193], or possibly to the inhibition of insulin release (see above) [31].

An important consequence of the findings discussed above is that agents that activate AMPK, including AICAR, metformin, the glitazones and leptin (see above), may also be expected to lead to an acute inhibition of insulin secretion *in vivo*. Indeed, AICAR [194] and metformin [195] both decrease circulating plasma insulin levels in humans, although this effect is normally assumed to result from the enhancement of insulin sensitivity, and in particular by suppressing glucose output from the liver [196]. Moreover, metformin activates AMPK in both MIN6 cells and human islets (I. Leclerc, unpublished work), and leptin acutely inhibits insulin secretion from β-cell lines and islets [197–199] and opens K_{ATP} channels in the pancreatic β-cell line CRI-G1 [200]. On the other hand, metformin potentiates the stimulation of insulin secretion at high (16.7 mM) glucose, from both the perfused rat pancreas [201] and isolated human islets [202], suggesting that other, positive effects of the drug (interaction with K_{ATP} channels?) [203] may outweigh the inhibitory effects of AMPK activation. Tests of β-cell function *in vivo* [204] will be required to determine whether these agents do, in fact, inhibit β-cell function or mass in living animals.

Effects of AMPK on gene expression in β-cells

Given the effects of AICAR on the expression of glucose-regulated genes in the liver (see above), it seemed possible that

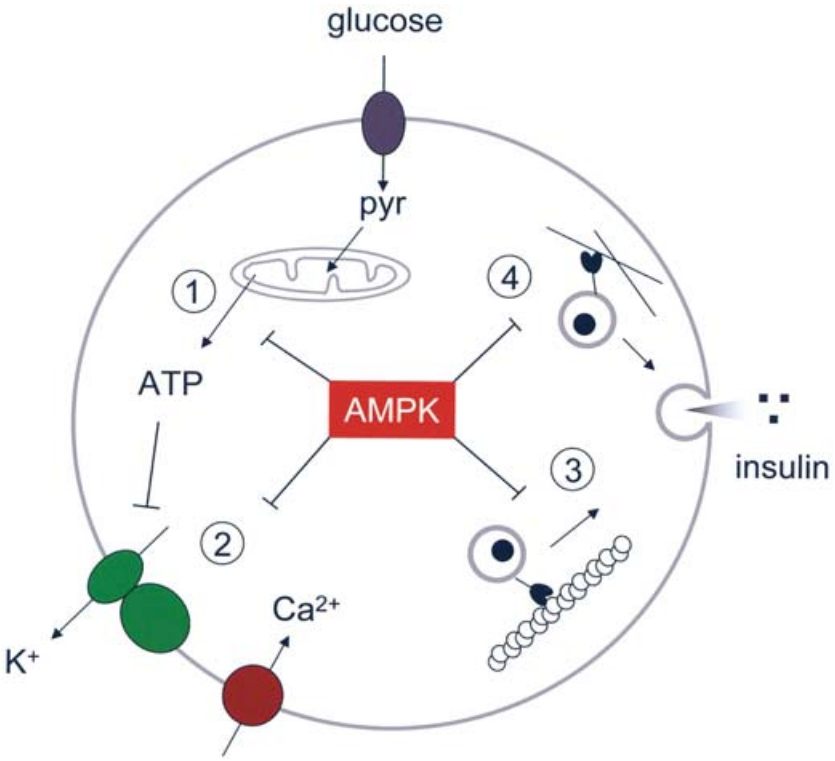

Figure 4 Potential mechanisms involved in the regulation of insulin secretion by AMPK

(1) Increases in glucose concentration lead to enhanced mitochondrial metabolism and ATP synthesis. By poorly defined mechanisms which may involve activation of fatty acid oxidation and the operation of a β-cell Randle cycle, AMPK stimulation inhibits glucose oxidation and ATP synthesis in β-cells [31,33]. pyr, pyruvate. (2) AMPK may lead to the activation of K_{ATP} channels (green; see the text), causing decreases in glucose-stimulated Ca^{2+} influx (brown) [31]. Phosphorylation of kinesin (3), myosin (4) or syntaxin may then affect vesicle trafficking to the cell surface and fusion.

AMPK may play a similar role in β-cells. Microinjection of reporter constructs and imaging revealed that introduction of anti-AMPK α2, but not anti-AMPK α1, antibodies fully mimicked the effects of glucose on both the preproinsulin and L-PK promoters in MIN6 β-cells [30]. This result was unexpected, since (a) the substrate specificities of these two AMPK isoforms had been believed previously to be essentially identical [174], and (b) the α1 isoform was the more abundant isoform in β-cells. One possible explanation for these results is that inhibition of AMPK α2 function in the nucleus (AMPK α1 is excluded from the nucleus in MIN6 cells) [30], and its sequestration in the cytosol, are essential to allow a tonic blockade of transcription. However, an alternative possibility is that, since the anti-AMPK α2 antibodies used in this study were more effective at inhibiting AMPK phosphorylation by AMPKK, inhibition of the α2 isoform alone was sufficient to decrease total cellular AMPK activity to below a threshold level for transcriptional inhibition.

Role of AMPK in the protection of β-cells against glucolipotoxicity

Accumulation of triacylglycerol by pancreatic islets may represent a mechanism through which chronic hyperglycaemia leads to diminished insulin secretion in Type II diabetes 'glucolipotoxicity' [205–207]. Correspondingly, transduction of clonal MIN6 β-cells [208] or primary rat islets [127] with an adenovirus expressing a truncated, constitutively active form of the transcription factor SREBP1c [114] leads to the activation of fatty acid synthase gene expression, accumulation of triacylglycerol, a fall in total islet ATP content and a profound inhibition of glucose-stimulated insulin release. Similar findings have also been reported using INS-1 cells [209]. Interestingly, the effects of SREBP1c overexpression on triacylglycerol accumulation can be reversed in part by treatment with AICAR [127], suggesting that AICAR may act under conditions of β-cell lipid loading to favour preservation of β-cell function.

ROLE OF AMPK IN GLUCOSE SENSING BY THE HYPOTHALAMUS

Glucose, and possibly insulin, signalling in the hypothalamus is important for regulating feeding behaviour in mammals and thus, in the longer term, in the regulation of body mass [210,211]. Thus both the arcuate nucleus and paraventricular nucleus contain neurons that are either excited or inhibited by glucose, through mechanisms which are still poorly understood [211,212]. These neurons appear to express glucokinase [213–215] and require functional K_{ATP} channels for normal glucose sensing [216], and may thus be anticipated to respond to glucose in a manner perfectly analogous to islet β-cells [217]. However, changes in lactate output from neighbouring astroglial cells may play an important role in setting neuronal ATP (and, presumably, AMP) levels in these cells [218,219]. The involvement of changes in AMPK activity in the response to glucose, as well as to insulin and leptin, and elucidation of the key molecular targets of the enzyme in these cells is likely to be an important area of future research. In particular, the elevated levels of catecholamines in the AMPK $\alpha 2^{-/-}$ mouse (see above) [92] are consistent with an effect

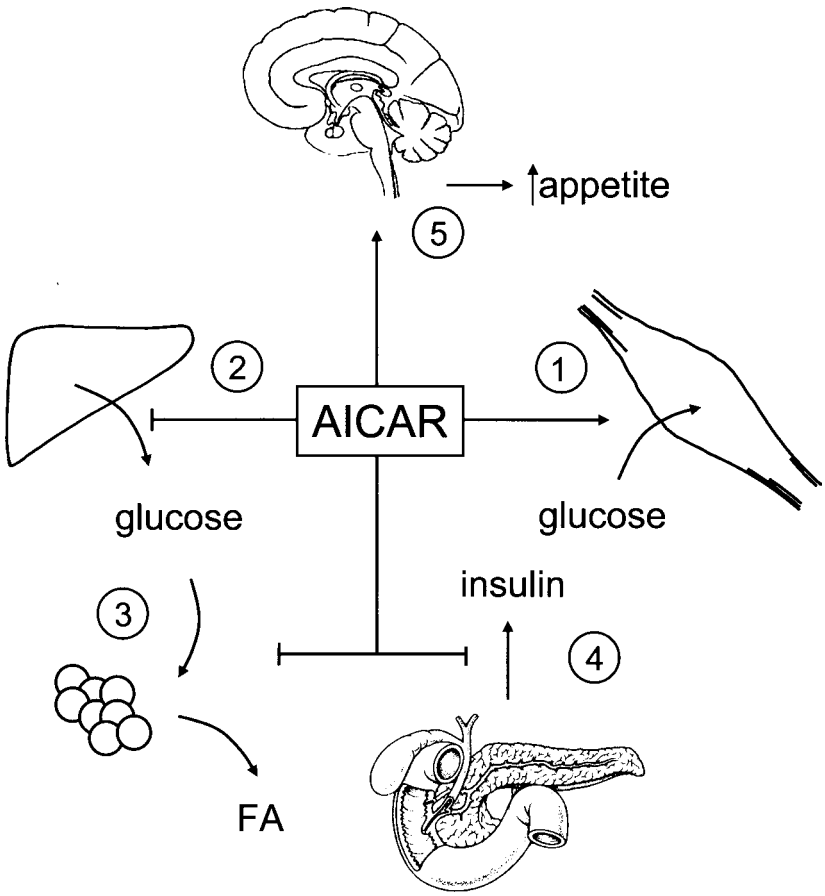

Figure 5 Likely effects of AICAR (and other AMPK activators) on glucose homoeostasis *in vivo*

AICAR (1) stimulates glucose uptake into muscle through the membrane recruitment of Glut4, (2) inhibits hepatic glucose output and triacylglycerol synthesis, (3) inhibits both glucose uptake and lipolysis by adipose tissue, (4) acutely suppresses insulin release from pancreatic islets, and (5) activates glucose-responsive neurons in the paraventricular and arcuate nuclei of the hypothalamus, potentially stimulating appetite. Effects (1)–(4) probably explain the glucose-lowering effects of AICAR (and contribute to the effects of metformin and glitazones) and are likely to be beneficial in Type II diabetes. Effects (4) and (5) may be contra-indicated. FA, fatty acids.

on the hypothalamic regulation of adrenal function. Moreover, exciting recent unpublished studies from Y. Minokoshi and B. Kahn (personal communication) now show that AMPK $\alpha 2$ is expressed abundantly in the neurons of the paraventricular nucleus of the hypothalamus, and may be selectively activated by insulin-induced hypoglycaemia. Moreover, leptin seems to decrease the phosphorylation of AMPK $\alpha 2$, exactly the opposite effect to that observed in skeletal muscle. The mechanisms involved in glucose-induced changes in AMPK phosphorylation and activity in hypothalamic neurons are not yet clear, however.

OTHER ROLES FOR AMPK IN THE CONTROL OF GLUCOSE HOMOEOSTASIS

Role of AMPK in signalling by the insulin receptor

In contrast with the likely beneficial effects of AMPK activation to potentiate the effects of insulin on glucose transport in muscle cells (see above), AICAR treatment has also been reported to cause the phosphohorylation on serine residues of IRS-1 (insulin receptor substrate-1). Thus serine phosphorylation of IRS-1, for example in response to cytokines [220,221], is generally believed to impede the interaction of IRS-1 with PI3K, and thus to inhibit insulin signalling [222]. Similarly, insulin signalling to the preproinsulin promoter [223,224] is partially impeded by the expression of constitutively active AMPK $\alpha 1$ in clonal β-cells [31]. However, in C2C12 myocytes, marked increases in IRS-1 phosphorylation at Ser-789 after AICAR treatment were not associated with decreased binding, but rather by an enhanced interaction between IRS-1 and PI3K [225]. Further work will be necessary to elaborate the importance of the latter phosphorylation event in insulin signalling. Whether insulin-induced increases in the phosphorylation of downstream effectors of PI3K, such as PDK-1 (phosphoinositide-dependent kinase-1) [226] or p70S6 kinase [227], are also affected by AMPK remains to be explored.

AMPK signalling downstream of the adiponectin receptor

Adiponectin, the globular subunit of ACRP30 (adipocyte complement-related protein of 30 kDa) [228], is a hormone secreted by adipocytes which appears to regulate energy homoeostasis in mammals, and whose levels are depressed in obesity and Type II diabetes [229]. ACRP30 was shown recently to activate AMPK in skeletal muscle [230,231] and liver [228], and to lower blood glucose concentrations *in vivo* [230]. *In vitro*, ACRP30 increases total

intracellular AMP levels and activates both isoforms of AMPK transiently, stimulating the phosphorylation of ACC and the β-oxidation of fatty acids [229]. Thus AMPK appears to play the key role in signal transduction downstream of the adiponectin receptor, an action which underlies the effects of this factor on whole-animal glucose homoeostasis.

CONCLUSIONS

While stimulation of AMPK has both positive and negative effects on metabolism by insulin-sensitive tissues *in vitro*, the principal effect of AICAR, and other agents (e.g. metformin and rosiglitazone) which activate AMPK *in vivo*, is usually to decrease blood glucose concentrations. Moreover, long-term AICAR administration has been reported to exert a range of other favourable effects, e.g. on plasma triacylglycerol and non-esterified fatty acid levels, high-density lipoprotein cholesterol and intra-abdominal fat content [232], at least in *fa/fa* fatty diabetic rats. Similarly, the long-term use of metformin is reported to have a beneficial effect on the evolution of Type II diabetes in obese patients, lowering both blood glucose concentrations and levels of glycosylated haemoglobin, albeit less effectively than other tested monotherapies or insulin [233]. As discussed above, the overall metabolic effects of activating AMPK in multiple tissues will undoubtedly be complex, and may well involve side effects such as the acute suppression of insulin secretion, and even changes in feeding behaviour (Figure 5). More *in vivo* studies will be required to explore these potential complications, which are of particular concern, since they may exacerbate the tendency towards obesity [6]. However, given that different combinations of AMPK isoforms appear to exist in different tissues and cell types, it is conceivable that future therapies for diabetes may be designed around isoform-selective activators (or inhibitors) of this key regulatory enzyme.

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