Endocrine (2012) 42:292–298
DOI 10.1007/s12020-012-9732-x

MINI REVIEW

Points of integration between the intracellular energy sensor AMP-activated protein kinase (AMPK) activity and the somatotroph axis function

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Received: 19 March 2012 / Accepted: 14 June 2012 / Published online: 27 June 2012

Abstract AMP-activated protein kinase (AMPK), an enzyme functioning as a cellular sensor of low energy, stores and promotes adaptive changes in growth, differentiation, and metabolism. While AMPK is primarily thought of as a regulator of systemic metabolism, it has been clearly established that it also has a role in the regulation of cell growth and may be a therapeutic target for proliferative disorders. Growth hormone (GH) secretion from the anterior pituitary and GH-induced synthesis and release of insulin-like-growth-factor-1 (IGF-1) from the liver determine linear growth before puberty. Actually, GH and IGF-1 are potent growth factors affecting cell growth and differentiation in different tissues, and still have anabolic functions and serve as essential regulators of fuel metabolism in adulthood, as well. A variety of peripheral hormonal and metabolic signals regulate GH secretion either by acting directly on the anterior pituitary and/or modulating GH-releasing hormone or somatostatin release from the hypothalamus. Actually, intracellular transduction of endocrine and metabolic signals regulating somatotroph function is still debated. Based on the previously summarized contents, the aim of the present work has been to review currently available data suggesting a role of AMPK in the interplay between GH axis activity and metabolic functions.

Keywords AMP-activated protein kinase · Growth hormone · Pituitary · Pituitary adenomas

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Introduction

AMP-activated protein kinase (AMPK)

AMPK, an enzyme functioning as a cellular sensor of low energy, stores and promotes adaptive changes in growth, differentiation, and metabolism. AMPK (PRKA) is expressed in all eukaryotic cells as a heterotrimeric protein [1]. AMPK is activated under conditions that increase cellular AMP levels such as glucose deprivation and hypoxia. Upon binding to AMPK gamma subunit, AMP allosterically activates the enzyme to fivefold. AMP also facilitates the phosphorylation of threonine-172 in the activation loop within the alpha subunit by the upstream serine/threonine kinase-11 (STK11 or LKB1). Phosphorylated AMPK is 100-fold more active than the native protein [2–5]. It has been shown that various growth factors and hormones can promote AMPK activation through threonine-172 phosphorylation in LKB1-dependent or independent manner [3, 6–12]. Reportedly, calcium/calmodulin-dependent protein kinase (CAMKK) [8], transforming growth factor-beta-activated kinase (TAK1) [13], and ataxia telangiectasia mutated protein [14] may function as AMPK kinases.

Activated AMPK phosphorylates enzymes and regulatory proteins involved in metabolic pathways, leading to adaptive changes in liver, adipose tissue, skeletal muscle, and central nervous system (CNS) in response to caloric

restriction and negative energy balance [4, 15–20]. As to CNS, AMPK is expressed at high levels in hypothalamic nuclei modulating energy intake, such as the arcuate, dorsomedial, paraventricular, and ventromedial nuclei suggesting a role in modulating feeding [7, 19]. AMPK was originally identified as the upstream kinase for acetyl-CoA carboxylase (ACC1 and ACC2) and HMG-CoA reductase. In general, AMPK switches off anabolic pathways like fatty acid, cholesterol, glycogen and protein synthesis, and switches on catabolic pathways producing ATP (i.e., glucose uptake and glycolysis) [4, 15–18, 20].

While the AMPK system may be primarily thought of as a regulator of systemic metabolism, it has been clearly established that AMPK also has a role in the regulation of cell growth [21–31]. AMPK activation may cause opposite effects in different cells. Although it has been shown to cause cell cycle arrest and to overcome the growth-stimulatory signaling via inhibition of mammalian target of rapamycin (mTOR) pathway in normal and tumor cells, AMPK may also act as a metabolic survival factor in specific tumor cells [27].

As to the therapeutic implications of AMPK activation, there is evidence that it can cooperate with glycemic control in type-2 diabetes. Metformin, a well-known insulin-sensitizing agent, decreases cellular ATP production by inhibiting the mitochondrial electron-transport chain. The resulting activation of AMPK in skeletal muscle and liver is thought to mediate the metformin-induced decrease in hepatic glucose output, the increase in peripheral glucose consumption, and the decrease in fat accumulation [4, 20, 32]. Furthermore, a number of recent preclinical studies suggest that AMPK may also be a therapeutic target for proliferative disorders, and chronic activation of AMPK has been suggested as a strategy for slowing aging [20].

Growth hormone (GH) secretion

GH secretion from the anterior pituitary and GH-induced synthesis and release of insulin-like-growth-factor-1 (IGF-1) from the liver determine linear growth before puberty. Actually, GH and IGF-1 are potent growth factors affecting cell growth and differentiation in different tissues and still have anabolic functions and serve as essential regulators of fuel metabolism in adulthood, as well [33–36]. A reduction in GH secretion with healthy aging is observed. Multiple neurotransmitter pathways, as well as a variety of peripheral hormonal and metabolic signals, regulate GH secretion either by acting directly on the anterior pituitary and/or modulating GH-releasing hormone (GHRH) or somatostatin (SS) release from the hypothalamus [33, 36, 37]. As to the role of metabolic substrates in the regulation of GH axis, it is well known that oral glucose administration has a rapid and transient inhibitory effect on GH release, most likely acting at the hypothalamic level. Indeed, glucose does not influence GH secretion from in vitro cultured pituitary cells. In turn, insulin-induced hypoglycemia causes a marked GH-secretory response in humans. Free fatty acids (FFA) also participate in the regulation of pituitary GH secretion since increased FFA levels block GH secretion provoked by virtually all stimuli. A direct pituitary inhibitory effect of FFA has been suggested [33, 36]. Intracellular transduction of endocrine and metabolic signals regulating somatotroph function at the hypothalamic and pituitary levels is still debated.

AMPK and GH–IGF-1 axis

Functional interactions

Based on the previously summarized contents, the aim of the present work is to briefly review currently available data suggesting a role of AMPK in the interplay between GH axis activity and metabolic functions. Indeed, hormones which are important regulators of GH secretion (namely, ghrelin, glucocorticoids, insulin, and leptin) are known to affect AMPK activity in different tissues [4, 6, 38]. For example, hypothalamic AMPK activation is believed to mediate the orexigenic effects of ghrelin [39, 40]. Furthermore, a direct crosstalk between AMPK and cellular anabolic pathways, like the insulin/IGF-1 signaling pathway, has been shown [4, 41, 42]. Although insulin and AMPK activation cooperate to regulate glucose metabolism in skeletal muscle and liver, in other cases their pathways oppose each other. Indeed, as an adaptive change to starvation, AMPK activation antagonizes the stimulatory effects of insulin and IGF-1 on protein synthesis and cell growth at the post-receptor level, by inhibiting mTOR response to the growth factors [4, 21, 23] (Fig. 1). We may conclude that factors regulating AMPK activity have the potential to interfere with the IGF-1-mediated anabolic effects of GH axis. In addition, since it has been shown that the rapid activation of protein synthesis in hepatoma cells by GH requires signaling through mTOR, AMPK activators may be hypothesized to affect GH-induced IGF-1 synthesis in the liver and the direct anabolic effects of GH in different tissues [43]. In turn, the insulin/IGF-1 signaling pathways can interfere with the AMPK response to cellular ATP depletion in some tissues. In cardiac muscle, there is evidence that insulin antagonizes AMPK activation by Akt (PKB)-dependent phosphorylation of the AMPK alpha subunit [44, 45] (Fig. 1). Conversely, various growth factors, including IGF-1, have been shown to induce phosphorylation of threonine-172 within the activation loop of AMPK in ATM-dependent and LKB1-independent manner

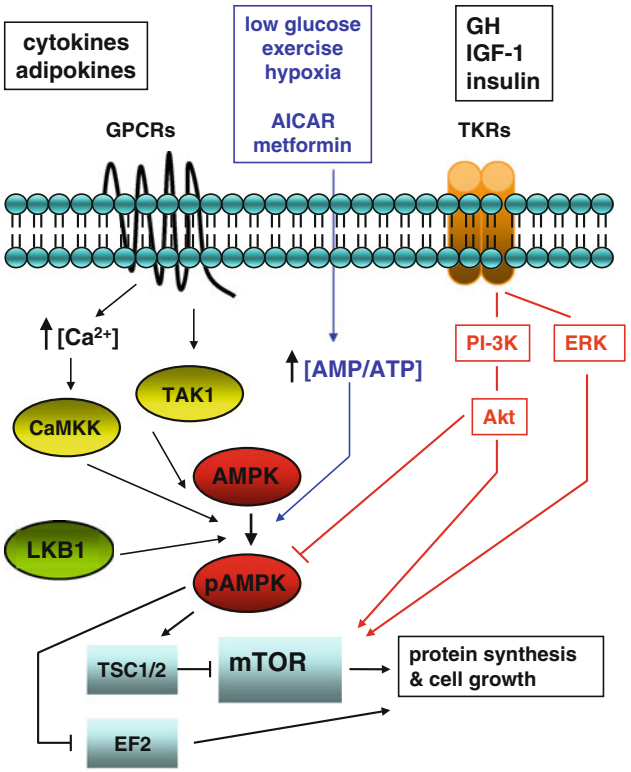

Fig. 1 AMPK is activated by phosphorylation of threonine-172 in the alpha subunit catalyzed by LKB1, which is constitutively active, or by extracellular signal-regulated kinases like TAK1 and CaMKK. Phosphorylation is enhanced and dephosphorylation is inhibited by conformational changes triggered by binding of AMP to the gamma subunit of AMPK. The functional interactions between AMPK and growth factors including GH and IGF-1, or insulin at the level of intracellular signaling pathways regulating protein synthesis and cell growth are shown. The ser/thr mTOR is a key regulator of protein translation/synthesis. The mTOR pathways are activated by amino acids and by growth factors through PI-3k-Akt and ERK pathways. Through phosphorylation of TSC2 (tuberin), AMPK increases the activity of TSC1–TSC2 complex to inhibit mTOR. Moreover, AMPK directly phosphorylates and inhibits mTOR. Additionally, AMPK limits protein synthesis through the inhibition of translation elongation factor 2 (EF2). Akt/PKB can phosphorylate AMPK catalytic subunit in sites other than threonine-172 and antagonize its activation triggered by threonine-172 phosphorylation. (⊣ inhibition; → activation)

in human fibroblasts and HeLa cells [4, 14]. The latter results may suggest a feedback regulatory loop via AMPK which down-regulates the cellular response to the stimulatory effects of growth factors on protein synthesis and cell cycle progression.

Finally, there is evidence that plasma GH can affect AMPK expression and AMPK phosphorylation levels in different tissues in vivo [7]. Total and phosphorylated AMPK levels were higher in the hypothalamus of spontaneous dwarf rats, a model of GH deficiency, versus controls. Similar results were obtained in GH receptor knockout mice as far as the liver is concerned [7, 46, 47]. Accordingly, total and phosphorylated AMPK levels were reduced in skeletal muscle and liver of mice overexpressing GH compared to wild-type animals [7, 48, 49] (Fig. 2). Remarkably, GH deficiency did not impair the decrease in the hepatic levels of phosphorylated AMPK after food deprivation, as compared with wild-type Lewis rats. In fact, both active and total AMPK levels dropped in the liver of the spontaneous dwarf rats after food deprivation [50]. Actually, controversial data have been reported about the link between food restriction and hepatic AMPK activity and it has been hypothesized that species, age, and duration of the fasting may profoundly affect the results [50].

AMPK and somatotroph cell function

To our knowledge, no data have been shown about a role, if any, of AMPK in the regulation of GH axis at the level of hypothalamic nuclei. Conversely, the effects of AMPK activation or inhibition on somatotroph function in the anterior pituitary have been recently investigated by our research group [51]. We showed that the AMP mimetic compound 5-aminoimidazole-carboxamide ribonucleoside (AICAR) markedly stimulated the phosphorylation of the AMPK activation loop in dispersed normal rat pituitary cells in a time-dependent manner. AICAR-induced

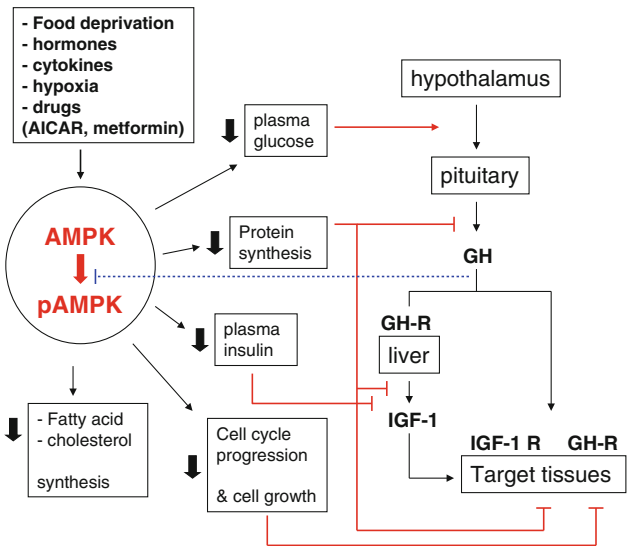

Fig. 2 AMPK is expressed in all eukaryotic cells and is activated through theonine-172 phosphorylation (pAMPK) by upstream kinases under conditions that increase cellular AMP levels (starvation and hypoxia) and in response to hormones, cytokines, or drugs. AMPK activation can negatively affect the signaling pathways downstream of GH and IGF-1 receptors (GH-R and IGF-1 R) in their target tissues (like mTOR signaling pathway, as shown in Fig. 1), and GH and IGF-1 synthesis and release in the pituitary and the liver, respectively. AMPK may also affect the GH–IGF-1 axis in an indirect manner by driving changes in plasma levels of metabolic substrates (glucose and FFAs) and hormones (insulin). See related paragraphs for details. (⊣ inhibition; → activation)

activation of AMPK was followed by a decrease of GH release and intracellular GH storage (Fig. 2). Conversely, the treatment of pituitary cells with compound C, a specific AMPK inhibitor, slightly potentiated GH secretion in response to GHRH. AICAR and SS-14 exerted an additive inhibitory effect on GHRH-stimulated GH release. In summary, our data suggest that AMPK activity can affect somatotroph function at the pituitary level, acting through the control of GH synthesis, and support the hypothesis that AMPK can be an intracellular transducer mediating direct effects of circulating hormones or metabolic factors on GH-secreting cells. At this purpose, it is worth to comment briefly on further data we have obtained in vivo and in vitro. Chronic dexamethasone (DEX) administration to young male rats induced a significant decrease of phosphorylated AMPK in pituitary homogenates. These results were not unexpected because AMPK has been previously shown to mediate various glucocorticoid-induced metabolic changes [38]. Glucocorticoids are known to exert important and complex effects on GH axis activity both in humans and in experimental animals [36]. Prolonged DEX administration reduces prepubertal growth by suppressing GH secretion through enhanced SS-14 hypothalamic tone [34, 52]. However, glucocorticoids are also enhancers of GH gene transcription in somatotrophs [34, 36], and reduced AMPK activity might facilitate an increase in GH synthesis in response to glucocorticoids. The effects of DEX on AMPK activity in pituitary cells may be indirect. In fact, we did not observe any significant change in AMPK phosphorylation and activity in rat GH-secreting adenomatous cells (GH3) and in normal rat pituitary cells in vitro, after treatment with DEX. As to any endocrine and metabolic alterations related to DEX treatment which might mediate the effects on pituitary AMPK, DEX-treated animals were euglycemic versus controls and had normal insulin concentrations but, as expected, they showed reduced plasma IGF-1 levels and reduced auto-phosphorylation of insulin/IGF-1 receptors in the pituitaries. These data, along with the negative effects of a specific IGF-1 receptor inhibitor on AMPK activity in GH3 cells, suggest that IGF-1 can enhance AMPK activity in pituitary cells and suggest to further investigate a possible role of AMPK in mediating the negative feedback regulatory activity of IGF-1 on somatotrophs. Interestingly, our in vitro studies also evidenced divergent effects of insulin versus IGF-1 in the regulation of AMPK in pituitary cells (GH3 cells). Indeed, in agreement with its previously discussed activity in cardiac muscle, insulin antagonized AMPK activation in GH3 cells.

Taking into account the effects of glucose load and insulin-induced hypoglycemia (see previous paragraphs) on GH axis activity, the rise in circulating insulin after meal might facilitate intracellular GH storage in somatotrophs through AMPK inhibition, thus predisposing cells to an increase in GH release after glucose lowering, in response to an anabolic state. Actually, based on the previously mentioned studies, any conclusion about the role of pituitary AMPK in the physiological regulation of GH secretion is not yet possible and further in vivo investigations are required.

AMPK and hepatic IGF-1 production

As to the in vivo effects of AMPK activators on plasma IGF-1 concentrations, clinical data of normal-weight subjects and patients with PCOS treated with metformin have been previously reviewed [20]. In brief, IGF-1 was reported to decrease in normal-weight subjects but no change or a modest increase was observed in patients with PCOS. Actually, a decrease of the ratio between IGF-1 and IGF-1-binding protein-1 (IGFBP-1) levels was associated with metformin therapy in PCOS. Since IGFBP-1 is a functional antagonist of IGF-1, it has been suggested that the decrease of free IGF-1 activity may account for up-regulation of GH release and normal or elevated levels of total IGF-1, due to reduced negative feedback on the hypothalamus–pituitary axis. The effects of metformin on systemic IGF-1 are most likely insulin mediated. Insulin activity is an important regulator of IGF-1 production since insulin promotes IGF-1 and suppresses IGFBP-1 synthesis in liver. Hence, by down-regulating fasting and postprandial plasma insulin levels, metformin is expected to decrease plasma-free IGF-1 (Fig. 2). Actually, data obtained in rat hepatoma cells in vitro suggest that AMPK activation in hepatocytes can also stimulate IGFBP-1 production via a direct intracellular pathway [53].

GH-secreting tumors

Apart from its role in cellular physiology, there is a growing interest in AMPK activity as a therapeutic target in different kind of tumors since AMPK is known to negatively regulate intracellular signaling downstream of growth factor receptors. Sporadic human GH-secreting pituitary adenomas are mostly benign tumors which are characterized by unrestrained hormone secretion. SS analogs are currently used to inhibit GH hypersecretion, but there are also data suggesting that they can also affect tumor growth. Actually, it has long been known that there is a subset of tumors manifesting partial or full resistance to the available analogs. Furthermore, more effective treatment options are required for recurring invasive macroadenomas [54–56]. To this end, the efficacy of mTOR inhibitors (rapamycin and everolimus) and octreotide as single agents or in combination are being actively studied in neuroendocrine tumors, including pituitary GH-secreting

tumors [57]. We have recently suggested that AMPK activators may represent an alternative way to inhibit mTOR in these kind of tumors [51, 58]. Furthermore, the effects of AMPK activation on GH-secreting adenomatous cells is relevant to the management of acromegaly since metformin, an indirect AMPK activator, may be administered to acromegalic patients as an antidiabetic drug [59]. Our in vitro studies in GH3 cells and in normal rat pituitary cells suggest that AMPK activators and SS-14 may cooperate in the control of cell growth and hormone secretion by independent signaling pathways which most likely converge in inhibition of p70S6K activity [58].

Conclusions and final remarks

Currently available data support the hypothesis of a role of AMPK-mediated intracellular signaling pathway in the control of GH–IGF-1 axis activity and in the regulation of tissue response to GH–IGF-1. Actually, further studies are necessary to come to a final conclusion about its relevance within the complex network of signals regulating somatotrophic function. It is worth to remark the importance to further investigate the interplay between cellular AMPK activity and the GH–IGF-1 axis activity, due to the growing interest in the favorable impact of caloric restriction on longevity and the role played by down-regulation of both GH and IGF-1 levels in mediating the beneficial effects of reduced energy intake on life span, as observed in rodents [60, 61]. More detailed studies in mutant mice with GH deficiency or resistance suggest that the effects of the GH–IGF-1 axis on longevity in aging mammals may be negative. Remarkably, the impact on longevity of reduced IGF-1 levels or activity revealed to be smaller than the effects of decreased GH synthesis and actions. Mechanisms linking decreased GH and IGF-1 signaling with longevity have been recently reviewed and discussed [61]. We limit to observe that the effects of GH and IGF-1 on cellular metabolic activities like protein synthesis, mitochondrial function, and fat oxidation have been included and AMPK is known to play an important role within the intracellular signaling pathways regulating these functions. In detail, AMPK activation is expected to antagonize the anabolic effects of GH and IGF-1, especially the mTOR-mediated effects on protein synthesis (Fig. 2).

Conflict of interest The authors declare that they have no conflict of interest.

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