Targeting cAMP/PKA pathway for glycemic control and type 2 diabetes therapy

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Abstract

In mammals, cyclic adenosine monophosphate (cAMP) is an intracellular second messenger that is usually elicited by binding of hormones and neurotransmitters to G protein-coupled receptors (GPCRs). cAMP exerts many of its physiological effects by activating cAMP-dependent protein kinase (PKA), which in turn phosphorylates and regulates the functions of downstream protein targets including ion channels, enzymes, and transcription factors. cAMP/PKA signaling pathway regulates glucose homeostasis at multiple levels including insulin and glucagon secretion, glucose uptake, glycogen synthesis and breakdown, gluconeogenesis, and neural control of glucose homeostasis. This review summarizes recent genetic and pharmacological studies concerning the regulation of glucose homeostasis by cAMP/PKA in pancreas, liver, skeletal muscle, adipose tissues, and brain. We also discuss the strategies for targeting cAMP/PKA pathway for research and potential therapeutic treatment of type 2 diabetes mellitus (T2D).

Key Words

- ▶ PKA
- ▶ diabetes
- ▶ glucose
- ▶ insulin
- ▶ glucagon

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cAMP/PKA pathway

cAMP was initially discovered as an intracellular mediator of the glycogenolytic effect of glucagon and epinephrine in the liver (Berthet *et al.* 1957). It was later revealed in skeletal muscle that the glycogenolytic effect of cAMP depends on a cascade of protein phosphorylation: cAMP/PKA-phosphorylase kinase-glycogen phosphorylase-glycogen degradation, which was a breakthrough discovery in biochemistry showing that protein phosphorylation is a regulatory mechanism for enzyme activity. The 1992 Nobel Prize in Physiology or Medicine was awarded to Edwin Krebs and Edmond Fischer for their discovery of this phosphorylation cascade. The major receptors for cAMP in mammals are PKA regulatory (R) subunits and exchange proteins directly activated by cAMP (Epac). The role of Epac in the regulation of metabolism has been recently reviewed (Almahariq *et al.* 2014). The physiological role

of PKA in glucose metabolism is increasingly appreciated with the availability of genetically modified mice and pharmacological evidence.

In cells, cAMP is synthesized from adenosine triphosphate (ATP) catalyzed by adenylyl cyclase (AC), which is activated by stimulatory G α protein (G α) following the activation of Gs protein-coupled receptors (Fig. 1). The termination of cAMP signaling is mediated by the conversion of cAMP to AMP by phosphodiesterases (PDEs) (Furman *et al.* 2010). PKA is a serine/threonine kinase and the holoenzyme is inactive and formed by a dimer of two regulatory (R) subunits that each binds a catalytic (C) subunit (Krebs & Beavo 1979). Each R subunit contains two cAMP binding sites, and cAMP binding leads to conformational changes in the R subunits and release of the active C subunits. Four types of R subunits

Figure 1
cAMP/PKA signaling pathway in mammalian cells. The activation of cAMP/PKA signaling starts from ligand-dependent activation of the G protein-coupled receptors (GPCRs) and is followed by Gs α activation, adenylyl cyclase (AC) activation, and cAMP generation. cAMP activates PKA by binding to PKA regulatory subunits (RIs or RIIs). The PKA catalytic subunits (C) are then released from the PKA tetramer to phosphorylate targets in the cytoplasm, in the plasma membrane (Ca_v, K_{ATP} etc.), and in the nucleus (CREB). The subcellular localization of PKA is dependent on binding of R subunits to different A-kinase anchoring proteins (AKAPs). The termination of cAMP signaling is dependent on phosphodiesterases (PDEs)-mediated degradation of cAMP to AMP. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-15-0316>.

(RI α , RI β , RII α , and RII β) and two types of C subunits (C α and C β) have been identified in mice to be encoded by different genes. In human, a third C subunit (C γ) gene has been identified specifically in testes and postulated to be a retroposon of the C α gene (Beebe *et al.* 1990, Reinton *et al.* 1998). In general, the α isoforms of RI, RII, and C subunits are expressed in all tissues, whereas β isoforms show more restricted expression. Type I PKA holoenzymes contain homodimers of RI α or RI β subunits and are activated at a lower cAMP level compared with type II holoenzymes containing homodimers of RII α or RII β subunits. The subcellular localization of PKA is dependent on the binding of R subunits to A kinase anchoring proteins (AKAPs), which generally show higher affinity for type II holoenzymes (Tasken & Aandahl 2004). Binding to AKAPs is essential for the spatial and temporal regulation of cAMP/PKA signaling (Fig. 1) (Smith *et al.* 2006, Pidoux & Tasken 2010). Mice with global or conditional mutants of R subunits, C subunits, or AKAPs have been generated (Kirschner *et al.* 2009) and greatly enhanced our understanding of the role of cAMP/PKA in different physiological and pathological processes, such as neural synaptic plasticity (Rosenmund *et al.* 1994), cardiac hypertrophy (Antos *et al.* 2001, Enns *et al.* 2010), tumor progression (Kirschner *et al.* 2000), and glucose homeostasis (Niswender *et al.* 2005, Willis *et al.* 2011).

Blood glucose is regulated by coordinated actions of different tissues, especially the pancreatic islet, liver, skeletal muscle, fat, and the brain (Fig. 2). This review discusses how cAMP/PKA signaling in each of these tissues affects glucose homeostasis and potential strategies targeting this pathway for glycemic control.

cAMP/PKA in pancreatic islets

β cell

A hallmark of T2D is an early defect in glucose-stimulated insulin secretion from pancreatic β cells (Nolan *et al.* 2011). Following a meal, glucose levels in the circulation increase, leading to increased uptake of glucose into β cells via the glucose transporter GLUT2. Increased intracellular glucose then leads to production of ATP and inactivation of the ATP-sensitive potassium channel (K_{ATP}) and depolarization of the cell. Cell depolarization activates voltage-gated calcium channel (Ca_v) on the plasma membrane and calcium influx into β cells. Accumulation of Ca²⁺ in the cell then promotes insulin secretion via exocytosis. Subsequently, insulin circulates and acts on a variety of tissues, mainly fat, muscle, and liver, to increase glucose uptake and inhibit glucose production. This process is essential for postprandial glycemic control.

Although glucose is the primary regulator for insulin secretion, many hormones and neurotransmitters such as glucagon, glucagon-like peptide 1 (GLP1), epinephrine, and norepinephrine can either enhance or suppress insulin secretion through GPCRs on β cells (Ahren 2009). cAMP/PKA pathway acts downstream of the GPCRs and regulates the activities of key molecules involved in insulin secretion, including GLUT2, K_{ATP}, and Ca_v (Fig. 3A). It has been shown that GLUT2 is phosphorylated at multiple sites in the carboxyl-terminal domain by PKA following either forskolin or GLP1 stimulation of β cells (Thorens *et al.* 1996). PKA-dependent phosphorylation suppresses the catalytic activity of GLUT2 and reduces glucose uptake into β cells (Thorens *et al.* 1996). However,

Figure 2

Glucose homeostasis is regulated by the coordinated actions of various organs. Glucose inputs from food absorption and hepatic release can be either inhibited or increased by neural signals from the brain. Hepatic glucose production (HGP) is increased by glucagon during fasting and inhibited by insulin after food absorption. Insulin also lowers blood glucose by stimulating its uptake into various tissues, mainly muscle and fat tissue. Glucose uptake by liver and brain does not require insulin (not shown). The secretions of glucagon and insulin by pancreatic α and β cells, respectively, are regulated by glucose and hormones.

because activation of PKA potentiates glucose-induced insulin release, these results suggest that phosphorylation of GLUT2 may regulate insulin secretion independent of its transporter activity (Hughes *et al.* 1993).

K_{ATP} channels serve to couple cellular metabolism to electrical excitability in pancreatic β cells and are the targets for sulfonylureas, a group of antidiabetic drugs. K_{ATP} channels are regulated by receptor-mediated signals that modulate insulin release, including the cAMP/PKA pathway (Beguín *et al.* 1999, Chen *et al.* 2013). PKA-dependent phosphorylation of K_{ATP} channels at S¹⁴⁴⁸ in the SUR1 subunit inactivates the channels in an ADP-dependent manner and is required for GLP1 stimulation of insulin secretion (Light *et al.* 2002). In another scenario, however, leptin-induced PKA activation in β cells promotes K_{ATP} trafficking to the plasma membrane, increases potassium current, and inhibits insulin release (Chen *et al.* 2013). Thus, under different conditions, cAMP/PKA activation may have either positive or negative effect on insulin release through K_{ATP} regulation, although it remains unknown if leptin treatment also leads to increased phosphorylation of K_{ATP} channels by PKA. This discrepancy can be due to that GLP1 and leptin activate distinct PKA pools that have different subcellular localizations and downstream targets within β cells.

Multiple types of Cav channels have been identified in pancreatic β cells and play essential roles in insulin secretion as well as β cell development, survival, and growth (Yang & Berggren 2006). L-type Cav1.2 and Cav1.3 are the major mediators of Cav current in β cell from many species tested (Sinnegger-Brauns *et al.* 2004,

Yang & Berggren 2006). In human patients with Timothy syndrome, a G406R point mutation in Cav1.2 increases the channel activity and can lead to excessive insulin secretion and life-threatening hypoglycemia (Splawski *et al.* 2004). PKA-dependent phosphorylation of Cav1.2 increases channel activity although the exact PKA site responsible for the regulation remains elusive (Weiss *et al.* 2013). The potentiation of insulin secretion by cAMP is at least partially through PKA-mediated phosphorylation of Cav channels and increased Ca^{2+} influx (Ammala *et al.* 1993). More important, PKA can directly enhance insulin exocytosis through phosphorylating secretory granule-associated proteins such as snapin and synaptotagmin (Fig. 3A) (Seino & Shibasaki 2005, Song *et al.* 2011, Wu *et al.* 2015) to increase Ca^{2+} responsiveness, and this mechanism may account for the majority of PKA's effect on insulin release (Ammala *et al.* 1993).

RI α -PKA is the most abundant subtype of PKA in mouse pancreatic islets (Petyuk *et al.* 2008). Selective ablation of RI α in β cells leads to disinhibition of PKA and increased phosphorylation of the transcription factor cAMP-response element-binding protein (CREB) (Song *et al.* 2011). Mice with RI α ablation in β cells show greatly enhanced glucose-stimulated insulin secretion and increased glucose tolerance (Song *et al.* 2011, Hussain *et al.* 2012). More importantly, human patients with heterozygous RI α -inactivating mutation (Carney's complex) also showed increased insulin release during oral glucose tolerance test (Song *et al.* 2011). Another strategy to increase β cell PKA activity is the selective expression of a constitutively active PKA C α subunit in

Figure 3

Schematic illustration of the regulation of glucose homeostasis by cAMP/PKA pathway in pancreatic β cell, hepatocyte, and adipocyte. (A) Regulation of insulin production by cAMP/PKA signaling in pancreatic β cell. Upon stimulation by incretins such as glucagon-like peptide 1 (GLP1), PKA is activated to promote insulin transcription and secretion by phosphorylating the transcription factor CREB, ATP-sensitive potassium channel (K_{ATP}), glucose transporter GLUT2, L-type voltage-gated calcium channels (LTCC), and secretory vesicle-associated protein snapin. Activated CREB promotes the expression of MAFA, a transcription factor that stimulates the expression of insulin. In conditions of long-term hyperglycemia, insulin secretion is impaired at least partially due to the induction of the intrinsic PKA inhibitor B (PKIB). The effect of PKA on insulin release is also dependent on its subcellular localization that is determined by binding to A-kinase anchoring proteins (AKAPs). (B) Regulation of hepatic glucose production by cAMP/PKA. During a fasting, cAMP/PKA is activated in hepatocytes by glucagon and epinephrine binding to GPCRs. PKA inhibits glycogen synthesis and increases glycogenolysis and gluconeogenesis by phosphorylating glycogen synthase, glycogen phosphorylase kinase (PhK), and phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK2/F-2,6-BPase), respectively (see text for detail). At the transcription level, PKA promotes the expression of gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and PPAR γ coactivator-1 α (PGC1 α) by CREB phosphorylation. PKA also promotes the nuclear import of cAMP-regulated transcriptional coactivator 2 (CRTC2) by stimulating its dephosphorylation (through IP $_3$ receptor (IP $_3$ R)-calcium-calmodulin (CaN) pathway) and inhibiting its phosphorylation (through phosphorylation and inhibition of salt-inducible kinase SIK2). Insulin and metformin suppress hepatic glucose production at least partially by stimulating the activation of phosphodiesterase (PDE) and downregulation of cAMP signaling. Metformin also increases cellular AMP to inhibit adenyl cyclase (AC) and thus suppresses cAMP synthesis. (C) Regulation of lipolysis in adipocyte. Glucagon and epinephrine increase lipolysis in adipocytes by stimulating PKA-dependent phosphorylation of lipid droplet-associated protein perilipin A, hormone-sensitive lipase (HSL), and adipose triglyceride lipase (ATGL). Insulin promotes glucose uptake and *de novo* lipogenesis, and inhibits lipolysis by activating PDE3B to suppress cAMP/PKA signaling. Metformin inhibits lipolysis by downregulation of the cAMP/PKA pathway in adipocytes, probably also via AMP and PDE activation. TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; FFA, free fatty acid.

β cells. It has been shown that this enhances both acute and sustained glucose-stimulated insulin release (Kaihara *et al.* 2013) and improves glucose control in mouse models with diet-induced insulin resistance or streptozotocin-mediated glucose intolerance (Kaihara *et al.* 2015). Importantly, the enhanced insulin release by either R1 α ablation or constitutively active mutant C α expression in β cells is not associated with hypoglycemia, suggesting that pancreatic PKA can be a safe target for potential T2D drug development.

By contrast, β cell-selective deletion of Gs α in mice leads to severely impaired β cell proliferation and reduced insulin expression, accompanied by hyperglycemia and

glucose intolerance (Xie *et al.* 2007), suggesting that cAMP signaling is indispensable for β cell physiology. A recent study has shown that cAMP/PKA/CREB signaling promotes insulin transcription by stimulating the expression of transcription factor MAFA (Fig. 3A) (Blanchet *et al.* 2015). Under conditions of chronic hyperglycemia, PKA signaling is disrupted and insulin expression is suppressed due to the induction of PKA inhibitor B (PKIB) in β cells. Mice overexpressing PKIB in pancreatic islets are relatively glucose intolerant and have lower circulating concentrations of insulin during oral glucose tolerance test, whereas *Pkib*-knockout mice are protected from high-fat diet-induced glucose intolerance and

impairment in insulin release (Blanchet *et al.* 2015). These results suggested that dysfunctional PKA was involved in the deterioration of β cell function during transition from insulin resistance to T2D. Indeed, in patients with T2D, PKIs appear to be upregulated in pancreatic islets (Gunton *et al.* 2005), suggesting that reducing the expression of PKI can be therapeutically beneficial.

The subcellular localization of PKA in pancreatic β cells is also essential for its regulation of insulin secretion and several AKAPs have been identified in β cells (Tasken & Aandahl 2004). Disruption of PKA anchoring to AKAPs with inhibitory peptides suppresses GLP1- and cAMP-induced insulin secretion in pancreatic β cells concurrent with decreased Ca^{2+} accumulation in the cytoplasm (Lester *et al.* 1997), suggesting that PKA anchoring is required for the regulation of Cav channel activity, Ca^{2+} influx, or Ca^{2+} release from internal stores. Selective ablation of AKAP150 in β cells leads to reduced L-type Ca^{2+} influx, suppressed glucose-induced insulin secretion, and impaired glucose tolerance in mice (Hinke *et al.* 2012), indicating that AKAP150 is required for proper PKA localization in β cells. It should be noted that AKAP150 binds type II-PKA ($\text{RII}\alpha$ and $\text{RII}\beta$) with much higher affinity than $\text{RI}\alpha$ (Herberg *et al.* 2000), suggesting that, in addition to $\text{RI}\alpha$ -PKA (Hussain *et al.* 2012), type II PKA may also be involved in the process of insulin secretion. Although $\text{RI}\alpha$ is usually considered soluble, dual-specific AKAPs (Jarnaess *et al.* 2008) that bind both RI and RII subunits and RI-specific AKAPs (Means *et al.* 2011, Burgers *et al.* 2012) have been identified. It remains unknown if such an AKAP exists in pancreatic β cells to mediate the regulation of insulin secretion by $\text{RI}\alpha$ -PKA.

α cell

The whole-body glucose homeostasis is largely dependent on the coordinated secretion of insulin and glucagon by β cells and α cells, respectively, in pancreatic islets (Fig. 2). As opposed to insulin, glucagon secretion is increased during fasting to increase blood glucose. The hyperglycemia in diabetic patients has been associated with excessive glucagon levels in the circulation that lead to increased hepatic glucose production (Dunning & Gerich 2007). Glucagon expression and secretion are regulated by multiple factors including glucose, fatty acids, amino acids, hormones, and neurotransmitters (Quesada *et al.* 2008). At the transcription level, glucagon expression is promoted by cAMP/PKA/CREB pathway (Knepel *et al.* 1990, Miller *et al.* 1993). Somatostatin inhibits the

transcription of glucagon potentially by suppressing the cAMP/PKA pathway in a pancreatic cell line (Kendall *et al.* 1995). Through a positive autocrine feedback mechanism, glucagon increases its own transcription (Leibiger *et al.* 2012) and secretion (Ma *et al.* 2005) by stimulating the glucagon receptor/cAMP/PKA/CREB pathway in mouse and human pancreatic islets.

In contrast to its inhibitory effects on β cell proliferation and insulin production (Xie *et al.* 2007), $\text{Gs}\alpha$ deficiency promotes the proliferation of α cells without effect on the transcription and serum level of glucagon in mice with pancreas-selective deletion of $\text{Gs}\alpha$ (Xie *et al.* 2010). Normal glucagon expression in the presence of increased α cell numbers suggests that the expression of glucagon per cell is decreased due to $\text{Gs}\alpha$ deficiency, indicating that cAMP signaling may have opposite effects on α cell proliferation and glucagon expression.

Similar to insulin, glucagon secretion via exocytosis is also dependent on Ca^{2+} . At basal conditions, low glucose-induced glucagon secretion requires Ca^{2+} influx via N-type Ca^{2+} channels (Gromada *et al.* 1997). In isolated rat α cells, GLP1 stimulates glucagon secretion by activating cAMP/PKA (Ding *et al.* 1997). However, in whole animal and perfused islets, GLP1 inhibits glucagon secretion also in a PKA-dependent manner (Dunning *et al.* 2005), suggesting that the stimulation of glucagon secretion from isolated α cells is not a physiological effect of GLP1. Further study revealed that the inhibitory effect of GLP1 on glucagon secretion is dependent on PKA-induced phosphorylation and inactivation of N-type Ca^{2+} channels on α cells (De Marinis *et al.* 2010). Under conditions with higher cAMP production in the cell, such as during fasting or with adrenaline stimulation, both PKA and Epac are activated and L-type Ca^{2+} channels are activated and become the major Ca^{2+} conduit. As a result, glucagon secretion is enhanced (Gromada *et al.* 1997, De Marinis *et al.* 2010). This binary regulation of glucagon secretion by GLP1 and adrenaline-elicited cAMP signaling is likely related to the activation of PKA with distinct subcellular localizations within α cells.

cAMP/PKA in the liver

One essential function of liver is to store and produce glucose to meet the body's needs. Liver is one of the two major sites (another one is skeletal muscle) for glycogen storage and is the main site for glucose release into the circulation during a fasting (Fig. 2). Two main processes contribute to hepatic glucose

production (HGP): glycogenolysis and gluconeogenesis. cAMP/PKA signaling in hepatocytes regulates both processes and the synthesis of glycogen as well (Fig. 3B). As stated above, when blood glucose levels drop during an energy deficit, PKA is activated by the binding of glucagon and epinephrine to their Gs-coupled receptors on hepatocytes and acts as the upstream kinase for glycogen phosphorylase kinase (PhK) to promote glycogenolysis in the liver (Berthet *et al.* 1957). Meanwhile, PKA can phosphorylate glycogen synthase to inhibit its activity and thus suppress the synthesis of glycogen (Proud *et al.* 1977). When the state of energy deficiency continues, glycogen is depleted and gluconeogenesis is increased to meet the body's glucose demand. The master hormone that promotes gluconeogenesis is glucagon. In hepatocytes, glucagon-induced cAMP/PKA activation promotes gluconeogenesis at multiple levels. A rate-limiting enzyme for gluconeogenesis is fructose-1,6-bisphosphatase (F-1,6-BPase) whose activity is suppressed by the powerful negative allosteric effector fructose-2,6-bisphosphate (F-2,6-BP). The level of F-2,6-BP in hepatocytes is determined by the bifunctional enzyme phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK2/F-2,6-BPase). PKA phosphorylation of the enzyme turns on the F-2,6-BPase activity leading to dephosphorylation of F-2,6-BP and concomitant increases in F-1,6-BPase activity and gluconeogenesis (Rider *et al.* 2004). At the level of gene expression, activation of the cAMP/PKA/CREB pathway promotes the expression of several key enzymes and factors to increase gluconeogenesis, including phosphoenolpyruvate carboxykinase (PEPCK) (Quinn 1994, Leahy *et al.* 1999, Yang *et al.* 2009), glucose-6-phosphatase (G6Pase) (Streeper *et al.* 2001), and PPAR γ coactivator-1 α (PGC-1 α) (Herzig *et al.* 2001, Fernandez-Marcos & Auwerx 2011). PEPCK and G6Pase are two rate-limiting enzymes for gluconeogenesis. Overexpression of PEPCK in mice leads to increased hepatic glucose production and impaired insulin sensitivity in the liver (Sun *et al.* 2002). Increased *Pepck* mRNA levels have been observed in several animal models of T2D and also in human T2D patients (Hanson & Reshef 1997), although probably not in all T2D patients (Samuel *et al.* 2009). Increased expression of G6Pase in the liver is also associated with diabetes (Haber *et al.* 1995, van Schaftingen & Gerin 2002). PGC1 α is a transcriptional coactivator that plays a central role in the regulation of energy metabolism (Liang & Ward 2006). In hepatocytes, PGC1 α interacts with other factors such as FOXO1 (Puigserver *et al.* 2003) to increase the expression of gluconeogenic genes. On the other hand, insulin

inhibits hepatic gluconeogenesis at least partially through suppressing cAMP/PKA/CREB pathway to inhibit the expression of PEPCK, G6Pase, and PGC1 α (Fig. 3B) (Park *et al.* 1972, He *et al.* 2009).

Another PKA target that is involved in the regulation of hepatic gluconeogenesis is inositol-1,4,5-trisphosphate receptor (IP3R), which forms the main channels for Ca²⁺ release from endoplasmic reticulum (ER) (Fig. 3B) (Wang *et al.* 2012). PKA-dependent phosphorylation of IP3Rs increases Ca²⁺ release and activates the protein phosphatase calcineurin which in turn dephosphorylates cAMP-regulated transcriptional coactivator 2 (CRTC2). Dephosphorylated CRTC2 translocates to the nucleus, where it binds to CREB and stimulates the transcription of gluconeogenic genes. PKA can also promote the activity of CRTC2 by phosphorylation and inactivation of salt-inducible kinase 2 (SIK2) (Altarejos & Montminy 2011), which phosphorylates CRTC2 and promotes its translocation out of the nucleus.

Hyperglucagonemia-induced increase in liver gluconeogenesis is considered as the main cause of fasting hyperglycemia in T2D (Consoli 1992). Recently, it was shown that metformin, the most widely prescribed T2D medicine, suppresses gluconeogenesis by inhibiting hepatic cAMP/PKA pathway, in addition to the activation of AMP-activated protein kinase (AMPK) (Miller *et al.* 2013). The study showed that metformin increased cellular level of AMP, which directly inhibited the activity of adenylyl cyclase (Fig. 3B) to suppress glucagon-induced cAMP production and PKA activation. The phosphorylation of two key PKA substrates that promote gluconeogenesis, PFK2 and IP3R, were both reduced after metformin treatment (Miller *et al.* 2013). Moreover, a recent study has shown that hepatic AMPK can phosphorylate and activate PDE4B to suppress glucagon-mediated cAMP/PKA activation (Johanns *et al.* 2016), suggesting that metformin inhibits hepatic PKA via multiple pathways. Another study showed that selective inactivation of NF- κ B in mouse liver enhanced insulin sensitivity and suppressed hepatic gluconeogenesis also via inhibition of the cAMP/PKA/CREB pathway (Ke *et al.* 2015). These studies suggest that cAMP/PKA is the major positive regulator for hepatic gluconeogenesis.

Mice with selective expression of a constitutively active PKA C α R subunit in hepatic cells exhibit decreased levels of liver glycogen, F-2,6-BP content, and glucokinase expression in the fed state, indicating suppressed glycogen synthesis and increased glycogenolysis and gluconeogenesis (Niswender *et al.* 2005).

These mice exhibit fasting hyperglycemia, impaired glucose tolerance, and defective glucose-induced insulin release (Niswender *et al.* 2005), suggesting the presence of crosstalk between liver and pancreatic islets. By contrast, mice with selective expression of a dominant-negative PKA RI α B subunit in hepatic cells exhibit enhanced glucose disposal in glucose-tolerance test, suggesting improved insulin sensitivity in the liver (Willis *et al.* 2011). The expression of PGC1 α , PEPCCK, and G6Pase is unchanged despite of dramatically reduced PKA activity in the liver (Willis *et al.* 2011). The normal levels of expression of gluconeogenic genes in the liver with either C α R or RI α B expression suggest the existence of compensatory mechanisms during animal development. Temporally conditional manipulations of PKA activity in the liver would be ideal for studying the role of PKA in hepatic gluconeogenic gene expression at the physiological level. Consistent with the effect of RI α B, liver-specific knockout of Gs α in mice leads to improved glucose tolerance and increased insulin sensitivity in liver and skeletal muscle. These mice also had increased glycogen content and decreased expression of gluconeogenic genes in the liver, accompanied by hypoglycemia and more than 100-fold higher blood glucagon levels compared with controls (Chen *et al.* 2005b), indicating a condition of glucagon resistance. Recently, it was shown that mice with deficiency of the RI α subunit of PKA had decreased PKA activity in the liver and showed resistance to diet-induced insulin resistance and diabetes (London *et al.* 2014). Collectively, these studies indicate that cAMP/PKA inhibition is not only sufficient but also necessary for efficient insulin-induced synthesis of glycogen and suppression of HGP.

cAMP/PKA in skeletal muscle

Signals via cAMP/PKA pathway control the development (Chen *et al.* 2005a, Knight & Kothary 2011) and metabolism (Berdeaux & Stewart 2012) of skeletal muscle, which plays an essential role in the regulation of glucose homeostasis (Stump *et al.* 2006). As mentioned above, liver and skeletal muscle are the major sites for glycogen storage. In humans, about 80% of the glycogen is stored in skeletal muscles (Jensen *et al.* 2011) because of their much greater mass than the liver. Unlike liver, however, skeletal muscles are unable to release glucose into the blood because muscles lack glucose 6-phosphatase and muscle glycogen is mainly a local energy source for exercise. Exercise-induced glycogen depletion is largely induced by activation of the β -adrenergic receptor

(β AR)/cAMP/PKA signaling pathway (Goldfarb *et al.* 1989). Consistently, exercise is able to increase cAMP level and PKA activity in skeletal muscle (Mehrani & Storey 1993). Skeletal muscles mainly express β 2-AR and adrenaline promotes glycogen breakdown through PKA-dependent activation of glycogen phosphorylase and inactivation of glycogen synthase (Cohen 2002). Exercise-induced depletion of muscle glycogen can increase skeletal muscle insulin sensitivity and enhance glycemic control (Jensen *et al.* 2011).

Skeletal muscle is the major site for insulin-mediated glucose uptake in the postprandial state in humans (DeFronzo *et al.* 1981, Thiebaud *et al.* 1982). Skeletal muscle insulin resistance has been suggested as the primary defect in T2D and plays an essential role in the pathogenesis of the disease (DeFronzo & Tripathy 2009). Insulin stimulates skeletal muscle glucose uptake by increasing translocation of glucose transporter GLUT4 from intracellular vesicles to the plasma membrane and transverse tubules (Zisman *et al.* 2000). Insulin also increases the synthesis of muscle glycogen by activating glycogen synthase (Cohen 1993). These effects of insulin are at least partially dependent on suppression of muscle PKA activity, and diabetes has been associated with the failure of insulin to inhibit PKA activity in skeletal muscle (Ortmeyer 1997).

Thus, insulin signaling and β AR/cAMP/PKA signaling appear to reciprocally regulate each other and have opposite effects on glucose metabolism in skeletal muscle. This is supported by studies showing that epinephrine or norepinephrine inhibits insulin-induced glucose uptake in skeletal muscle (Chiasson *et al.* 1981, Lembo *et al.* 1994), while blockade of β AR activity increases GLUT4 expression in skeletal muscle and improves insulin sensitivity in diabetic animals (Alves-Wagner *et al.* 2015). Mice with β AR deficiency exhibit increased peripheral insulin sensitivity despite of systemic glucose intolerance because of defective insulin secretion (Asensio *et al.* 2005). Genetic deletion of adenylyl cyclase 5 (Ac5) also leads to improved glucose tolerance largely due to increased insulin sensitivity in skeletal muscle (Ho *et al.* 2015). Similarly, sustained stimulation of β 2-AR leads to cardiac insulin resistance by suppressing GLUT4 expression and glucose uptake in cardiomyocytes in a PKA-dependent manner, suggesting that the mechanism is also present in cardiac muscle (Mangmool *et al.* 2016).

However, other studies have shown that activation of the β 2-AR/cAMP/PKA pathway can also increase glucose uptake in skeletal muscle by promoting GLUT4

translocation to the plasma membrane in an insulin-independent manner (Ngala *et al.* 2013, Sato *et al.* 2014). The controversial results may be related to different physiological conditions: in the postprandial and rest state, when insulin level is increased, β AR/cAMP/PKA pathway is usually suppressed to facilitate anabolic processes in skeletal muscles; activation of the β AR/cAMP/PKA pathway at this time would antagonize the actions of insulin and lead to insulin resistance. In conditions of exercise or fasting, insulin level is low and β AR/cAMP/PKA system is activated to promote catabolic processes to meet the energy demands of skeletal muscles. The increased muscle glucose uptake by activation of β 2-AR/cAMP/PKA pathway could be a mechanism to provide energy substrate for muscle activity in the latter conditions. In T2D patients with insulin resistance, selective activation of PKA in skeletal muscle can serve as an alternative pathway for glucose disposal and glycemic control.

Consistent with the role of cAMP signaling in skeletal muscle development and systemic glucose homeostasis, $G\alpha$ deficiency in skeletal muscles leads to reduced skeletal muscle mass and impaired glucose tolerance, although muscle insulin sensitivity is intact (Chen *et al.* 2009a). PDE4 is a cAMP-selective hydrolase and is the predominant PDE subtype in skeletal muscle. The inhibition of muscle PDE4 at least partially accounts for the beneficial effects of resveratrol on metabolism, including improved glucose tolerance, by activation of cAMP/Epac/Sirt1 pathway (Park *et al.* 2012). Whether PKA is involved in this process remains unknown, but it should be noted that PKA can directly phosphorylate and activate SIRT1 in skeletal muscles (Gerhart-Hines *et al.* 2011). Consistently, PDE content in skeletal muscle is positively related to fasting plasma glucose level in diabetic patients (Szendroedi *et al.* 2011), suggesting a correlation between suppressed cAMP signaling and impaired glucose homeostasis. Generation of mouse models with conditional loss- or gain-of-function of cAMP signaling components such as PDE4, EPAC1, and PKA specifically in skeletal muscle will provide further insights into the physiological role of muscle cAMP signaling in glycemic control.

Insulin resistance are often associated with increased lipid accumulation and impaired lipid oxidation in skeletal muscle (Turcotte & Fisher 2008). Pharmacological or physiological stimulation of β -adrenergic signaling in skeletal muscle leads to PKA-dependent activation of SIRT1 which promotes fatty acid oxidation and energy expenditure (Gerhart-Hines *et al.* 2011). The monounsaturated fatty acid oleic acid is able to improve

skeletal muscle insulin resistance partially through a PKA-dependent mechanism (Coll *et al.* 2008). Specifically, oleic acid can activate PKA/Sirt1/PGC1 α pathway to stimulate fatty acid oxidation in skeletal muscle (Lim *et al.* 2013). These data suggest that PKA is positively associated with lipid oxidation and insulin sensitivity in skeletal muscle.

Taken together, these genetic and pharmacological studies have shown that appropriately regulated cAMP/PKA activity is essential for skeletal muscle insulin sensitivity and systemic glucose homeostasis.

cAMP/PKA in adipose tissues

Adipose tissues are crucial regulators for energy balance and glucose homeostasis through actions in glucose uptake, lipid metabolism, and hormone secretion (Rosen & Spiegelman 2006). Adipose tissues account for only about 10% of insulin-mediated glucose uptake; however, selective GLUT4 deletion in adipose tissues leads to glucose intolerance and insulin resistance in muscle and liver (Abel *et al.* 2001). By contrast, adipose-specific overexpression of GLUT4 enhances systemic insulin sensitivity and reverses diabetes in mice with muscle-specific GLUT4 deficiency (Carvalho *et al.* 2005), suggesting that adipose glucose uptake can compensate for defective muscle glucose uptake. In addition to insulin, norepinephrine can also increase glucose uptake in both white (WAT) and brown adipose tissues (BAT) (Liu *et al.* 1994), potentially by activation of the β 3-AR/cAMP/PKA pathway (Chernogubova *et al.* 2004).

cAMP/PKA signaling is the major positive regulator of lipolysis in WAT and BAT (Fig. 3C). PKA promotes lipolysis by phosphorylating several proteins including lipid droplet-associated protein perilipin A (Miyoshi *et al.* 2007), hormone-sensitive lipase (HSL) (Krintel *et al.* 2008), and adipose triglyceride lipase (ATGL) (Pagnon *et al.* 2012). In normal fed condition, insulin promotes lipogenesis and suppresses lipolysis partially through inhibition of cAMP/PKA signaling by activation of PDE3B in adipocyte (Eriksson *et al.* 1995, Kitamura *et al.* 1999). Obesity is associated with increased basal lipolysis and elevated levels of serum free fatty acids (FFAs) that contribute to insulin resistance (Bergman & Ader 2000). In diabetic patients with obesity, metformin improves insulin sensitivity by decreasing circulating FFAs (Abbasi *et al.* 1998). Further study showed that metformin inhibits lipolysis by suppressing cAMP production and PKA activation in adipocytes (Zhang *et al.* 2009). Thus, metformin exerts its antidiabetic effects by inhibiting

cAMP/PKA signaling in both adipose tissue and liver to suppress excessive lipolysis and glucose production, respectively.

R11 β is the major PKA subtype in adipose tissues in mouse (Cummings *et al.* 1996) and human (Mantovani *et al.* 2009, Peverelli *et al.* 2013). In mice, R11 β deficiency is associated with compensatory increase in R1 α PKA and increased basal PKA activity in WAT and BAT. β AR-stimulated lipolysis is impaired but the basal rate of lipolysis is increased in the WAT of R11 β -knockout mice (Planas *et al.* 1999). In BAT, R11 β deficiency leads to increases in mitochondrial content and thermogenesis (Nolan *et al.* 2004, Newhall *et al.* 2005). However in human, obesity is associated with reduced R11 β expression and PKA activity in WAT (Mantovani *et al.* 2009). β AR-stimulated lipolysis and mitochondrial respiration are also decreased in WAT of obese patients (Yehuda-Shnaidman *et al.* 2010). In 3T3-L1 adipocytes, it has been shown that the anchoring of PKA to AKAP is required for β AR-stimulated lipolysis (Pidoux *et al.* 2011). These results suggest that R11 β -PKA is required for β AR-stimulated lipolysis in the WAT of both mouse and human and impaired adipose R11 β -PKA signaling may contribute to human obesity.

Deletion of Gs α in mouse adipocytes leads to decreased lipogenesis and lipolysis, increased muscle insulin sensitivity, and improved glucose tolerance (Chen *et al.* 2010, Li *et al.* 2016). The increased insulin sensitivity is likely caused by decreased circulating lipids and lower fat contents in muscle and liver, because insulin-mediated glucose uptake in adipose tissues is suppressed by Gs α deficiency (Li *et al.* 2016). Interestingly, Gs α deletion in mature adipocytes has no overall effect on fat content and body weight despite of impaired BAT and WAT functions (Li *et al.* 2016). However, deletion of Gs α in preadipocytes from an earlier developmental stage leads to leanness with impaired adipocyte differentiation (Chen *et al.* 2010). These data suggest that cAMP signaling in mature adipocytes affects glucose homeostasis mainly by regulating lipid metabolism.

Glucose homeostasis is also closely regulated by adipokines produced by adipocytes, such as leptin and adiponectin (Rosen & Spiegelman 2006, Coppari & Bjorbaek 2012). Leptin expression and secretion is enhanced by insulin but suppressed by the activation of cAMP/PKA signaling (Szkudelski *et al.* 2005, Maeda & Horiuchi 2009). The expression of adiponectin has been shown to be positively regulated by PKA (Otani *et al.* 2015) and CREB signaling in 3T3-L1 adipocytes (Kim *et al.* 2010), whereas other studies suggested that

β AR/PKA signaling inhibits adiponectin expression in these cells (Fasshauer *et al.* 2001). Further studies are needed to determine if cAMP/PKA signaling regulates the expression of these adipokines *in vivo*.

cAMP/PKA in the brain

The brain regulates energy balance and glucose homeostasis through cooperation with peripheral tissues including fat, pancreatic islets, and liver (Morton & Schwartz 2011, Grayson *et al.* 2013, Schwartz *et al.* 2013, Rojas & Schwartz 2014, Scarlett & Schwartz 2015). Leptin, a fat-derived hormone, regulates food intake, energy expenditure, and glucose homeostasis mainly by its actions on hypothalamic neurons (Morton & Schwartz 2011). Insulin also acts in the brain to regulate food intake and HGP (Gray *et al.* 2014, Rojas & Schwartz 2014). In hypothalamic neurons, cAMP signaling is modulated by several hormones including leptin and insulin to affect food intake (Zhao 2005). A recent study found that glucagon acts in the hypothalamus to inhibit HGP by a PKA-dependent mechanism (Mighiu *et al.* 2013). Inhibition of PKA activity in the mediobasal hypothalamus (MBH) abolishes the suppressive effect of glucagon on HGP and leads to increased i.v. glucagon injection-induced HGP. By contrast, activation of PKA in MBH inhibits HGP and resembles the effects of glucagon injection to the hypothalamus. The glucagon-induced activation of hypothalamic PKA and inhibition of HGP are both attenuated in rats fed a high-fat diet, whereas activation of PKA by Sp-cAMPS infusion to MBH can still efficiently inhibit HGP in these rats (Mighiu *et al.* 2013). These findings suggest that hypothalamic glucagon resistance contributes to increased HGP and hyperglycemia in diabetes and obesity and that pharmacological activation of PKA in MBH can bypass the glucagon resistance with therapeutic potential for diabetes treatment.

The gene encoding Gs α (GNAS) shows parental-specific imprinting and patients with maternal inactive mutations exhibit obesity and insulin resistance (Weinstein 2014). Animal studies have shown that brain-specific maternal Gs α mutation leads to obesity, severe insulin resistance, and diabetes (Chen *et al.* 2009b). Especially, these mice developed insulin-resistant diabetes before the development of obesity, indicating that central Gs α signaling directly regulates peripheral glucose metabolism. Specific maternal Gs α deficiency in paraventricular nucleus of the hypothalamus leads to milder obesity and has marginal effects on glucose

homeostasis (Chen *et al.* 2012). By contrast, Gs α deletion selectively in ventromedial hypothalamic neurons leads to improved glucose homeostasis especially when the mice fed a high-fat diet (Berger *et al.* 2016). Thus, the specific neuronal populations that mediate the negative effects of Gs α mutations on glucose homeostasis remain to be determined. As discussed above, Gs α deficiency in glucagon-responsive neurons in MBH can lead to central glucagon resistance and contribute to the development of hyperglycemia and diabetes.

In mouse hypothalamus, multiple PKA R and C subunits are expressed with RII β -PKA as one of the major subtypes (Yang & McKnight 2015). Mice with global knockout of RII β subunit exhibit leanness, elevated insulin and leptin sensitivity, and resistance to diet-induced obesity and diabetes (Schreyer *et al.* 2001, Enns *et al.* 2009b, Yang & McKnight 2015). The lean phenotype and likely the improved glucose metabolism as well are caused by RII β -PKA deficiency in the hypothalamus (Zheng *et al.* 2013). RII β deficiency leads to reduced PKA C α and C β subunits and decreased total PKA activity in multiple brain regions (Brandon *et al.* 1998, Yang & McKnight 2015) that is accompanied by a compensatory increase in type I PKA. This shift from type II to type I PKA leads to increased sensitivity to activation by cAMP (Brandon *et al.* 1998) and likely altered the intracellular localization of PKA as well. Leanness and increased insulin sensitivity have also been observed in mice lacking PKA C β subunits, which are highly expressed in the hypothalamus (Guthrie *et al.* 1997, Enns *et al.* 2009a). C β deficiency reduces basal PKA activity, but has no significant effect on total PKA activity in some brain regions due to a compensatory increase in C α subunits (Howe *et al.* 2002). It remains unknown to what extent PKA activity is impaired by C β deficiency in hypothalamic regions that are essential for the regulation of energy balance. Generation of conditional mutant mice lacking C β in specific types of neurons will provide more mechanistic insights. Although the neuronal populations and molecular mechanisms responsible for the improved glucose metabolism in RII β KO and C β KO mice remain largely unknown, these findings suggest that PKA manipulations in the brain, most likely in hypothalamic neurons, has the potential to improve glycemic control in diabetes.

Strategies for cAMP/PKA pathway targeting

Recent findings have supported the feasibility of cAMP/PKA pathway as a target for treatment of a variety

of diseases, such as cancers (Chochung 1993, Tortora & Ciardiello 2003, Sapio *et al.* 2014), polycystic kidney disease (Torres & Harris 2014), cardiovascular diseases (Lee *et al.* 2013), and diabetes (Miller *et al.* 2013). Several strategies have been proposed for cAMP/PKA pathway targeting, including the following: (1) modulation of cAMP levels. As an example, metformin inhibits hepatic gluconeogenesis by suppressing glucagon-induced cAMP production in hepatocytes (Miller *et al.* 2013). Another class of drugs that have been clinically prescribed or in clinical trials for T2D treatment are GPCR agonists or antagonists (Ahren 2009). For example, the GLP1R agonists exenatide (Byetta) and liraglutide (Victoza) are FDA-approved antidiabetic drugs and are able to enhance insulin secretion by stimulating cAMP production in β cells. Small molecular antagonists for glucagon receptor are under preclinical or clinical investigations for their inhibitory effects on HGP (Sammons & Lee 2015). cAMP-degrading PDEs have been suggested as antidiabetic targets (Furman & Pyne 2006). Inhibitors for PDE4 has been recently shown to be able to improve glucose homeostasis in diabetic mice (Vollert *et al.* 2012) and human patients (Wouters *et al.* 2012), potentially by enhancing cAMP-mediated GLP1 secretion (Vollert *et al.* 2012); (2) inhibition of PKA. Small molecular inhibitors for PKA such as H89 and KT 5720 have exhibited beneficial effects in animal models of cardiac diseases (Oddis *et al.* 1996, Makaula *et al.* 2005). The specificity of these inhibitors, however, is questioned (Murray 2008). A more specific approach for PKA manipulation, antisense oligonucleotide-based knockdown of PKA R subunit, has shown anticancer effects in preclinical (Tortora *et al.* 1997) and clinical studies (Goel *et al.* 2006). It remains unknown if these strategies have any effect on glucose control in animal models or human patients with T2D; and (3) disruption of AKAP signaling complexes. AKAPs interact with PKA, PDEs, and protein phosphatases to ensure their appropriate intracellular localization and facilitate signal transduction, specificity, and termination. The signaling complexes have been suggested as potential drug targets for cancers, chronic heart failure, diabetes, etc. (Alto *et al.* 2002, Troger *et al.* 2012, Esseltine & Scott 2013). Although peptide disruptors for AKAP-PKA interaction have been extensively studied in research, validated small molecule disruptors suitable for therapeutic purposes are still lacking and under investigation (Troger *et al.* 2012). Another approach to suppress AKAP-mediated signaling is small interference RNA (siRNA)-mediated knockdown of AKAP expression (Josefsen *et al.* 2010).

Concluding remarks

In summary, cAMP/PKA signaling in different tissues has pleiotropic effects on whole-body glucose homeostasis and regulates glucose metabolism at multiple levels. Genetic and pharmacological studies in animal models suggest that selective activation of cAMP/PKA pathway in pancreatic β cells or selective inhibition of cAMP/PKA pathway in liver can be safe and effective in improving glycemic control in T2D. Activation of β AR/cAMP/PKA pathway in skeletal muscle is coupled to glycogen breakdown and lipolysis and positively regulates glucose homeostasis. However, chronic overactivation of the sympathetic nervous system is associated with increased basal lipolysis in adipose tissue and may contribute to insulin resistance in obesity and diabetes. Inhibition of cAMP/PKA in fat tissue has been suggested to contribute to the insulin-sensitizing effect of metformin via suppression of lipolysis and circulating FFAs. In the hypothalamus, cAMP/PKA pathway has profound effects on energy balance and glucose homeostasis that merit further studies. Although it is clinically challenging to yield tissue-specific manipulation of cAMP/PKA pathway, the findings that metformin exerts its antidiabetic effect by inhibiting hepatic cAMP/PKA/CREB pathway (He *et al.* 2009, Miller *et al.* 2013, Pernicova & Korbonits 2014) strongly support this signaling cascade as a target for the development of new T2D therapeutics.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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