REVIEW

PKA functions in metabolism and resistance to obesity: lessons from mouse and human studies

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Abstract

Both direct and indirect evidence demonstrate a central role for the cAMP-dependent protein kinase (PKA) signaling pathway in the regulation of energy balance and metabolism across multiple systems. However, the ubiquitous pattern of PKA expression across cell types poses a challenge in pinpointing its tissue-specific regulatory functions and further characterizing its many downstream effects in certain organs or cells. Mouse models of PKA deficiency and over-expression and studies in living cells have helped clarify PKA function in adipose tissue (AT), liver, adrenal, pancreas, and specific brain nuclei, as they pertain to energy balance and metabolic dysregulation. Limited studies in humans suggest differential regulation of PKA in AT of obese compared to lean individuals and an overall dysregulation of PKA signaling in obesity. Despite its complexity, under normal physiologic conditions, the PKA system is tightly regulated by changes in cAMP concentrations upstream via adenylate cyclase and downstream by phosphodiesterase-mediated cAMP degradation to AMP and by changes in PKA holoenzyme stability. Adjustments in the PKA system appear to be important to the development and maintenance of the obese state and its associated metabolic perturbations. In this review we discuss the important role of PKA in obesity and its involvement in resistance to obesity, through studies in humans and in mouse models, with a focus on the regulation of PKA in energy expenditure, intake behavior, and lipid and glucose metabolism.

Key Words:

- ▶ PKA
- ▶ obesity
- ▶ energy balance
- ▶ HPA axis
- ▶ metabolism

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Introduction

Since the early 1990s, obesity has been recognized as a complex, multifactorial disease in which genetics is one of the key factors (Bray 1992, Vogler *et al.* 1995). Our knowledge about the complex mechanisms underlying the imbalance between energy intake and expenditure continues to evolve, particularly with the use of genetic and optogenetic methodologies, yet it is still not well understood why some individuals are more susceptible to

obesity than others (Brownell & Wadden 1992, Kim *et al.* 2018). Rare monogenic forms of obesity have facilitated much of our understanding about key molecular pathways involved in maintaining energy balance. Most of these regulatory pathways involve cyclic AMP (cAMP) signaling.

The cAMP-dependent protein kinase (PKA) enzyme is essential for intracellular signal transduction and in the maintenance of cellular homeostatic processes

(Krebs 1972) by potentiating the signals from a wide range of ligands that bind G-protein coupled receptors (GPCRs) and activate the $G_{\alpha\beta\gamma}$ heterotrimer to release the G_{α} subunit that, in turn, binds to and activates adenylate cyclase and generates intracellular cAMP. PKA is therefore integral in regulating a great many cellular functions in diverse cell types.

The PKA holoenzyme is a tetramer assembled from two regulatory and two catalytic subunits comprised of different combinations of the PKA regulatory (RI α , RII α , RI β , and RII β) and catalytic (C α , C β , C γ , and PRKX) subunits, typically involving two identical pairs of a regulatory and a catalytic subunit. Each PKA subunit has a unique, tissue-specific pattern of expression (Corbin *et al.* 1975). Type I (RI α and RI β) regulatory subunits vs type II (RII α and RII β) regulatory subunit holoenzyme composition impacts PKA holoenzyme affinity for cAMP as well as its cellular localization, primarily via A-kinase anchoring proteins (AKAPs) (Feliciello *et al.* 2001) (Fig. 1). The ratio of type I to type II PKA activity can vary dramatically among tissues and species (Corbin & Keely 1977). Mouse studies have shown unequivocal specificity in PKA subunit expression across neural regions that suggest differences in PKA function that are likely mediated by the unique biochemical properties of the different isoforms (Cadd & McKnight 1989).

Figure 1

Diagram of intracellular PKA signaling. AC, adenylate cyclase; AKAP, A-kinase anchoring protein; AMP, adenosine monophosphate; ATP, adenosine triphosphate; C, PKA catalytic subunit; cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element binding protein; GPCRs, G-protein coupled receptors; PDE, phosphodiesterase; R, PKA regulatory subunit.

Activation of the PKA holoenzyme by cAMP binding at two sites on each regulatory subunit initiates the release of the catalytic subunits, thereby activating the catalytic subunits and enabling two distinct modes of regulation: cytoplasmic phosphorylation of its targets and translocation of the catalytic subunit to the nucleus where it exerts transcriptional regulation (Adams *et al.* 1991), largely through cAMP-response element binding protein (CREB) family phosphorylation (Gonzalez & Montminy 1989) (Fig. 1).

Defects or altered expression of one PKA subunit can lead to compensatory changes in the other subunits at the protein level. RI α is known to be the most critical mediator of regulated PKA activity in living systems (Amieux & McKnight 2002). Pulse-chase experiments in cell culture showed that the half-life of the RI α subunit increased over four times when assembled as part of the holoenzyme compared to unbound (Amieux *et al.* 1997) demonstrating the impact of subunit stability on enzymatic activity potential. Destabilization of the C α subunit upon its release from the holoenzyme has also been demonstrated to cause notable decreases in both protein concentration and PKA activity (Garrel *et al.* 1995). We have seen that the effects of subunit disruption vary from tissue to tissue, presumably because of the unique expression profiles of each PKA subunit and the subsequent abilities to compensate for these changes among different cell types. Considering its known role in regulating hormone secretion and hormone action downstream of PKA by phosphorylation, it is not surprising that the PKA-axis plays a central role in metabolism and energy balance.

Dynamic regulation of the PKA system

Molecular genetic techniques have enabled the generation of mouse models to investigate the specificity and relative importance of the different PKA subunits. Disruption of the genes coding for each PKA subunit have been individually studied in mice (Brandon *et al.* 1995, Cummings *et al.* 1996, Burton *et al.* 1997, Amieux *et al.* 2002, Howe *et al.* 2002, Skalhogg *et al.* 2002) (Table 1). Deletion of either the protein kinase cAMP-activated type 1 regulatory subunit alpha (*Prkar1a*) or protein kinase cAMP-activated catalytic subunit alpha (*Prkaca*) genes caused embryonic lethality (Amieux *et al.* 2002). Additionally, the concept that each PKA subunit has unique non-redundant functional properties has emerged.

Compensation for defects in PKA signaling is primarily accomplished by changes in PKA holoenzyme

Table 1 Summary of mouse models of altered PKA subunit expression and their associated phenotypes.

Genotype	Phenotype	Reference
<i>Prkaca</i> ^{-/-}	Perinatal lethality in 73% offspring, survival dependent on background and environment; surviving mice had growth deficits, male infertility	Skalhegg <i>et al.</i> (2002)
<i>Prkacb</i> ($\beta 1$) ^{-/-}	Deficiencies in contextual learning	Qi <i>et al.</i> (1996)
<i>Prkacb</i> ($\beta 1, \beta 2, \beta 3$) ^{-/-}	Resistant to DIO, sympathetic nervous system alterations with numerous sex-dependent characteristics	Enns <i>et al.</i> (2009), London <i>et al.</i> (2019)
<i>Prkaca</i> ^{-/-} : <i>Prkacb</i> ($\beta 1$) ^{-/-}	Embryonically lethal	Huang <i>et al.</i> (2002)
<i>Prkaca</i> ^{+/-} : <i>Prkacb</i> ($\beta 1$) ^{-/-}	Defects in neural tube development	Huang <i>et al.</i> (2002)
<i>Prkaca</i> ^{-/-} : <i>Prkacb</i> ($\beta 1$) ^{+/-}	Gestationally lethal; all pups had neural tube development defects, some had exencephaly	Huang <i>et al.</i> (2002)
<i>Adipoq</i> : <i>Prkaca</i>	Resistant to DIO, increased BAT UCP1 induction	Dickson <i>et al.</i> (2016)
<i>Prkar1a</i> ^{-/-}	Embryonically lethal	Amieux <i>et al.</i> (2002)
<i>Prkar1a</i> ^{+/-}	Tumorigenesis associated with CNC	Kirschner <i>et al.</i> (2005)
<i>Prkar1a</i> ^{-/-} : <i>Prkaca</i> ^{-/-}	Embryonically lethal with significant cardiac abnormalities	Amieux <i>et al.</i> (2002), Kirschner <i>et al.</i> (2005)
<i>Prkar1a</i> ^{-/-} : <i>Prkaca</i> ^{+/-}	Embryonically lethal with better (but still poor) cardiac status	Amieux <i>et al.</i> (2002)
<i>Prkar1b</i> ^{-/-}	Deficiencies in memory and synaptic plasticity	Brandon <i>et al.</i> (1995)
<i>Prkar2a</i> ^{-/-}	Resistant to DIO and steatohepatitis, improved glucose sensitivity	London <i>et al.</i> (2014a)
<i>Prkar2b</i> ^{-/-}	Resistant to DIO, insulin resistance and increased nocturnal activity	Brandon <i>et al.</i> (1995), Schreyer <i>et al.</i> (2001), Nolan <i>et al.</i> (2004)
<i>Prkar2b</i> ^{-/-} : <i>Ucp1</i> ^{-/-}	Maintenance of lean phenotype and increased nocturnal activity	Nolan <i>et al.</i> (2004)
<i>Prkar2b</i> ^{-/-} : <i>Lep</i> ^{-/-} (<i>Ob/Ob</i>)	Obese phenotype rescued	Newhall <i>et al.</i> (2005)
<i>Prkar2b</i> ^{-/-} : <i>A^Y</i> ^{-/-}	Obese phenotype rescued	Czyzyk <i>et al.</i> (2008)
<i>DARPP-32</i> ^{Cre} : <i>Prkar1a</i> ^{R1aB/WT}	Growth retardation; hypophagic, lean, resistant to DIO	Yang <i>et al.</i> (2014)

or catalytic subunit stability and not by transcriptional or translational modulation, which is often achieved by the enhanced half-life of PKA subunit RI α upon its incorporation into the holoenzyme (Amieux *et al.* 1997). More generally, regulation of intracellular PKA action in response to perturbations in the system occurs by changes in the stability of the holoenzyme and affinity for cAMP that are subunit specific (Otten *et al.* 1991). Further, we have seen that, in the presence of PKA subunit defects, one normal allele (heterozygosity) can be sufficient to maintain normal PKA enzymatic activity (Willis *et al.* 2011). Despite conserved domain organization among the PKA isoforms, small-angle x-ray scattering has revealed distinct quaternary structures for the different PKA holoenzyme isoforms (Heller *et al.* 2004, Vigil *et al.* 2006) that impact how the enzyme complex associates and dissociates (Zhang *et al.* 2012). The notion of non-redundancy among the isoforms is evidenced by the differential metabolic impacts of defects in the different PKA subunits that are later described in this review.

Dysregulation of PKA signaling in obesity: observations in humans and mice

Human obesity

Though sparse, a few studies in humans have shown differences in the PKA signaling system in obese compared to lean individuals. *PRKAR2B* mRNA was decreased in visceral and s.c. adipose tissue (AT) from obese compared to lean individuals, and *PRKAR2B* levels were inversely related to circulating insulin and homeostasis model assessment of insulin resistance as well as BMI and waist circumference (Mantovani *et al.* 2009). Another study of lean and obese, non-diabetic, non-hyperlipidemic males revealed the downregulation of genes involved in lipolysis in s.c. AT, including *PRKAR2B* and A-kinase anchoring protein 1 (*AKAP1*) (Marrades *et al.* 2010) that were independent of differences in intake or activity level. In another investigation, PKA-dependent skeletal muscle lipolysis was blunted via hormone-sensitive lipase (HSL) phosphorylation on PKA sites Ser⁵⁶³ and Ser⁶⁵⁹ in a cohort of obese compared to lean men (Jocken *et al.* 2008).

The role of PKA signaling in Cushing syndrome (CS)

PKA activation is the primary mediator of cortisol secretion in adrenocortical cells (Fig. 2). Somatic or germline mutations in the genes that code for molecules in the PKA signaling pathway are frequently associated with hypercortisolemia. CS is caused by elevated circulating glucocorticoids and leads to central obesity. Clinical features of CS include weight gain, hypertension, diabetes mellitus, osteoporosis, proximal muscle weakness, striae, and decreased growth velocity in children. There have been several thorough reviews of the genetics of CS that reported on the discovery of many gene variants associated with cortisol-producing adenomas and hyperplasias (Espiard *et al.* 2014, Lodish & Stratakis 2016). Briefly, activating mutations in PKA signaling molecules or inactivating mutations in molecules that inhibit the PKA-signaling pathway cause hypercortisolemia. For example, activating mutations in guanine nucleotide-binding protein G(s) subunit alpha (*GNAS*) are associated with cortisol-secreting tumors in McCune–Albright syndrome (Weinstein *et al.* 1991). Somatic activating mutations in *PRKACA* were found in cortisol-producing adenomas

(Beuschlein *et al.* 2014), and inactivating mutations of *PRKAR1A* cause Carney complex (Kirschner *et al.* 2000) and a rare form of adrenocortical hyperplasia called primary pigmented nodular adrenocortical disease (Groussin *et al.* 2002). Inactivating mutations in phosphodiesterases (PDEs), specifically *PDE8B* and *PDE11A*, lead to elevated cAMP levels and aberrant PKA activity (Horvath *et al.* 2008a,b).

Despite the common endpoint of hypercortisolemia, the various PKA subunit mutations yield a distinct phenotype in line with the specificity of function inherent in each PKA subunit and are associated with differences in adiposity and distribution of AT (London *et al.* 2014b). CS occurs much more frequently in women than in men, which highlights the existing sexual dimorphism in PKA regulation of energy balance and adiposity that is also a feature of PKA mouse models (Stratakis 2008).

Obesity in rodents

Mouse studies have provided significant mechanistic insight into PKA dysregulation in the obese state. In obesity, AT remodeling and expansion vary and impact the

Figure 2

Overview of the integrated central and peripheral regulation of metabolism and energy balance by the PKA signaling system. *The hypothalamus and other brain regions regulate neural systems important to energy balance as well as peripheral organs such as AT, liver, and gut through multiple GPCRs linked to PKA signaling; the graph is simplified given the many factors and complicated interactions that include but are not limited to POMC, NPY, BDNF, and their receptors from the melanocortin family (MC2R, MC3R, and MC4R). ACTH, adrenocorticotrophic hormone; BAT, brown adipose tissue; cAMP, cyclic adenosine monophosphate; CRH, corticotropin-releasing hormone; CORT, cortisol; TG, triglycerides; WAT, white adipose tissue.

susceptibility to metabolic disease. Ultimately, differences in distribution of AT and adipogenesis impact AT function and physiology, beyond the expansion of adipocytes and increased lipid content, and can be a key determinant of metabolic health (Vishvanath & Gupta 2019). In WAT from diet-induced obese rats, cAMP concentration, PKA and HSL activity, and perilipin phosphorylation were all decreased. Further, isoproterenol-stimulated lipolysis was decreased and β 1- and β 3-adrenergic receptors were downregulated in obese compared to WT animals (Ding *et al.* 2016). There is increasing evidence that depot specific WAT-brain circuitry is crucial for regulating lipolysis, energy expenditure, and the maintenance of energy homeostasis (Bartness *et al.* 2014).

The PKA signaling response to the fasted state is significantly altered in obese compared to lean mice. In WAT, fasting-induced activation of PKA was impaired in obese rats and this was associated with altered lipid and carbohydrate metabolism in AT (Flores-Opazo *et al.* 2019). In the fasted state, CREB phosphorylation was impaired in the dorsomedial hypothalamus (DMH) of obese mice compared to the robust induction of CREB observed in lean-fasted mice. In the arcuate nucleus, phosphorylated CREB (pCREB) levels were high in both fed and fasted states in obese mice, whereas lean mice had substantial CREB induction only during fasting, which is likely to impact feeding behavior (London *et al.* 2017). Others have reported that high-fat diet (HFD) feeding caused decreased hypothalamic PKA signaling that was accompanied by elevated PDE4A levels in obese mice that exhibited a depression-like behavioral phenotype (Vagena *et al.* 2019).

Resistance to diet-induced obesity (DIO) in mouse models of PKA subunit deficiency

The *Prkar2b* knockout (RII β KO) mouse

The regulatory subunit IIR is expressed highly in brain and in AT and is modulated throughout embryonic development in several other tissues (McKnight *et al.* 1988). This expression pattern has led to extensive characterization of the role of RII β in the regulation of adiposity, thermogenesis, and energy balance in mice. See Table 1 for a summary of PKA mouse models. Disruption of the *Prkar2b* gene that codes for PKA RII β caused a lean phenotype and resistance to DIO in mice (Cummings *et al.* 1996). RII β KO mice had fat-pad weights half that of WT littermates and decreased adipocyte size and number in white AT (WAT). In brown AT (BAT), the disruption

of RII β initiated an isoform switch from RII β to RI α , thereby causing a five-fold increase in basal PKA activity and similar increases in uncoupling protein-1 (UCP1) (Cummings *et al.* 1996, Planas *et al.* 1999). Alterations in glucose regulation and neural control of energy balance in RII β KO mice are discussed in detail later in this review.

The *Prkar2a* KO (RII α KO) mouse

Deficiency of the PKA type 2 regulatory subunit alpha conferred resistance to DIO obesity despite the lack of phenotype when mice were maintained on a normal chow diet that was low in fat (~6% fat by weight) (Burton *et al.* 1997, London *et al.* 2014a). RII α KO mice had decreased hepatic PKA activity after HFD feeding and decreased hepatic steatosis, but unlike the RII β KO mouse, RII α KO mice did not have marked increases in oxygen consumption that could explain the observed DIO resistance. Instead, it appears that the low, but very specific, brain expression of RII α in habenula originally identified by McKnight and colleagues is likely responsible for alterations in intake behavior specifically of rewarding sweet and high-fat foods (Cadd & McKnight 1989; London E, Bloyd M & Stratakis CA, unpublished observations). RII α KO mice also resisted diet-induced glucose dysregulation and maintained superior insulin sensitivity compared to that of WT littermates after chronic HFD feeding (London *et al.* 2014a).

The *Prkarcb* KO (C β KO) mouse

The protein kinase cAMP-activated catalytic subunit beta (*Prkarcb*) has three isoforms (C1, C2, and C3), the last two of which are transcribed exclusively from neural promoters (Guthrie *et al.* 1997, Desseyn *et al.* 2000). Mice deficient for all three isoforms of C β had decreased PKA enzymatic activity in brain (Howe *et al.* 2002), were genetically lean, and resisted DIO (Enns *et al.* 2009, London *et al.* 2019). C β KO mice also resisted hepatic steatosis, dyslipoproteinemia, and had improved glucose and insulin sensitivity (Enns *et al.* 2009). Growth curves revealed sex-specific differences in body mass as male but not female C β KO mice maintained on a normal chow diet had decreased body weight compared to WT mice from young adulthood that appeared to be primarily due to decreased lean body mass (London *et al.* 2019). Male C β KO mice had a significantly elevated oxygen consumption rate and female mice exhibited increased home cage locomotor activity. A major feature of the lean, DIO-resistant C β KO mouse phenotype was elevated

heart rate and in females elevated s.c. body temperature that was due to centrally mediated increased sympathetic outflow. In line with this finding, C β KO mice had denser mitochondria and increased UCP1 in brown adipocytes, evidence of increased β 3-adrenergic activation of BAT (London *et al.* 2019).

Central regulation of energy balance by PKA: hunger, satiety, and energy expenditure

PKA and regulation of the neuropeptide Y (NPY)/agouti-related protein (AgRP) and proopiomelanocortin (POMC) system

The leptin-deficient *ob/ob* mouse is a well-studied model of monogenic obesity characterized by hyperphagia, hyperinsulinemia, decreased locomotor activity, defective BAT activation, and excessive adiposity (Zhang *et al.* 1994). The *ob/ob* mouse has enabled deeper insight into the central regulation of energy homeostasis and the NPY/AgRP and POMC system. The adipocyte-derived hormone leptin, dubbed a 'satiety factor', is a sensor of lipid stores that exerts control over intake behavior, perceived hunger, and endocrine function via the melanocortin system in the arcuate nucleus of the hypothalamus. Leptin acts via inhibition and stimulation of the *OBRb* receptors on two sets of opposing neurons, NPY/AgRP orexigenic neurons and pro-opiomelanocortin (POMC)/cocaine and amphetamine-related transcript (CART) anorexigenic neurons, that stimulate or inhibit feeding, respectively (Elias *et al.* 1999, Elmquist *et al.* 1999). For an in-depth review see Schwartz *et al.* (2000).

PKA is an integral mediator of this dichotomous hypothalamic system at multiple junctions because GPCRs mediate the actions of the primary targets of NPY and melanocortin: thyrotropin-releasing hormone (TRH), melanin-concentrating hormone (MCH), and gamma-aminobutyric acid (GABA) neurons (Fig. 2). TRH, melanocortin 4 receptor (MC4R), and MC3R act via G α , while the GABA receptor acts through Gi/Go. The TRH gene is regulated by overlapping thyroid response and cAMP response elements, and enhanced CREB phosphorylation caused robust TRH promoter stimulation that was further amplified by co-expression of a constitutively activated PKA construct (Wilber & Xu 1998). MC4R internalization in response to an agonist was shown to be dependent upon PKA or G-protein-coupled receptor kinase and was partially inhibited by pretreatment with H89, a specific PKA inhibitor (Shinyama *et al.* 2003). MCR stimulation

leads to increased cAMP concentrations and activation of PKA (Kim *et al.* 2002).

Intact hypothalamic PKA/CREB signaling is necessary to maintain energy homeostatic processes in mice (Fig. 2). Inhibition of CREB binding protein (Cbp) by AAV-mediated ventromedial hypothalamic Cre expression in *Cbp^{flox/flox}* mice increased food intake, decreased body temperature, and led to decreased *Pomc* and brain-derived neurotrophic factor (*Bdnf*) mRNA expression (Moreno *et al.* 2016). Also, important to the regulation of energy and glucose homeostasis are orexin peptides A and B that signal through the GPCRs orexin receptors 1 and 2 (OX1R and OX2R) and therefore are regulated downstream by PKA signaling and CREB transcriptional activity. Orexin deficiency in mice results in obesity (Hara *et al.* 2001), age-related glucose intolerance, and insulin insensitivity (Tsuneki *et al.* 2008). OX1R and OX2R in lateral hypothalamus (LH) project throughout the CNS including major projections to septal nuclei, bed nucleus of the stria terminalis, paraventricular and reuniens nuclei of the thalamus, zona incerta, subthalamic nucleus, central gray, substantia nigra, raphe nuclei, parabrachial area, medullary reticular formation, and nucleus of the solitary tract (Peyron *et al.* 1998, Xu *et al.* 2013). This complex connectivity suggests roles for orexin signaling, that is in part, mediated by downstream PKA signaling, in physiologic functions such as sleep, feeding, and addiction as well as in the regulation of neuroendocrine systems.

Crossing the RII β KO mouse with well-characterized mouse models of obesity such as the *ob/ob* mouse has helped elucidate the mechanisms by which PKA and other 'obesity' genes function in regulating energy balance (Nolan *et al.* 2004, Newhall *et al.* 2005). Double KO of *Prkar2b* and *Ob* partially rescued the obese *ob/ob* phenotype of the leptin-deficient mouse. Body weight and energy intake were significantly decreased, while both night-time locomotor activity and basal oxygen consumption were increased. Deletion of RII β activated BAT in the double (RII β /*ob*) KO and partially restored the ability to maintain body temperature in sub-thermoneutral conditions (Newhall *et al.* 2005). Similarly, deletion of NPY in the *ob/ob* mouse attenuated the obesity and glucose dysregulation of the *ob/ob* mouse, but did not entirely reverse the *ob/ob* phenotype (Erickson *et al.* 1996). Inhibition of NPY by amphetamine has an anorectic effect that increases both PKA and CREB in hypothalamus of rats (Hsieh *et al.* 2007). These studies reaffirm the importance of PKA RII β in hypothalamus and specifically in leptin-responsive cells in neuronal- and AT regulation of energy balance.

Agouti lethal yellow mice (A^y) express the *agouti* gene next to a constitutively active promoter causing high expression of the agouti peptide which is a powerful antagonist of both MC3R and MC4R (Bultman *et al.* 1992, Miller *et al.* 1993). A^y mice have hyperphagia, hypoactivity, and increased fat mass. Agonism of MC4R directly enhances intracellular hypothalamic cAMP levels through its activation of G_s that in turn activates PKA to enable normal energy homeostatic control. The MC4R KO mouse was hyperphagic and had a severe obese phenotype (Huszar *et al.* 1997). PKA RII β deletion in the A^y mouse successfully reversed the obesity of A^y mice and heterozygosity of RII β KO led to partial rescue of the obese phenotype (Czyzyk *et al.* 2008), confirming the essential regulatory role of RII β in the melanocortin system downstream of MC4R in hypothalamus.

Intriguingly, the use of specific Cre drivers to re-express RII β in specific neuronal populations of the hypothalamus illustrated that it was not a single population of cells mediating the hyperactive, lean phenotype of the RII β KO mouse. While neuronal re-expression of RII β using a Synapsin-Cre transgenic line rescued both the hyperactive and lean phenotypes, re-expression in striatum via DARPP-32-Cre reversed only the hyperactivity, and AAV-Cre-mediated hypothalamic re-expression rescued the lean phenotype (Zheng *et al.* 2013).

In medium spiny neurons (MSNs) and hippocampus, PKA RII β localizes to dendrites by its association with AKAP5 and mice lacking these associations displayed defects in both synaptic plasticity and operant tasks with a food reward that manifest behaviorally (Weisenhaus *et al.* 2010). PKA RI α mediates the effects of dopamine in striatal MSNs and is necessary for appropriate MSN cytoplasmic function (Yang *et al.* 2014). Expression of a dominant negative form of RI α in striatal MSNs in mice caused growth retardation, hypophagia, and impaired locomotor activity.

cAMP/PKA signaling performs numerous functions throughout the CNS and its roles are well-established in the molecular pathways governing fear and fear memory. PKA mouse models have also demonstrated a role for PKA signaling in anxiety. Increased cAMP signaling has been associated with an anxiety phenotype in mice that were haploinsufficient for either *Prkar1a* (Keil *et al.* 2012) or *Prkaca* (Briassoulis *et al.* 2016). Similarly, in mice over-expressing $G_{s\alpha}$, enhanced striatal PKA enzymatic activity was associated with an anxiety phenotype (Favilla *et al.* 2008). While current data on the relationship between anxiety and obesity is mixed (Gariepy *et al.* 2010), anxiety disorders are the most prevalent mental disorders

in the developed world (Kessler & Wang 2008). Better understanding the relationship between psychological disorders and behaviors that impact energy balance are key to identifying therapeutic approaches to some forms of obesity.

The role of *Prkar2b* in thermogenesis and regulation of adiposity

Studying the RII β KO and Ucp1 KO mouse provided novel mechanistic information about BAT activation and the role of RII β in the central regulation of energy balance. In a thermoneutral environment, RII β KO mice had four times more UCP1 protein in BAT than WT mice. Double KO of RII β and UCP1 in mice, however, resulted in BAT cellularity that more closely resembled that of the Ucp1 KO mouse, confirming that RII β disruption affects BAT activity upstream of UCP1 (Nolan *et al.* 2004) (Fig. 2). The elevated oxygen consumption rate characteristic of the RII β KO mouse was dependent on UCP1, yet leanness was conveyed in the double KO mouse despite small increases in adiposity compared to the single RII β mutant. The evidence that enhanced β 3-adrenergic stimulation and activation of UCP1 was not the primary component of the RII β KO lean phenotype pointed to a non-adipose tissue driver controlling energy balance.

PKA signaling plays a central role in the regulation of AT function

Lipolysis and non-shivering thermogenesis

Using mouse models and *in vitro* cell studies, McKnight and others have demonstrated how PKA activity, and specifically, the subunits RII β and RI α , can regulate lipolysis through dynamic compensatory mechanisms in the PKA system (Cummings *et al.* 1996, McKnight *et al.* 1998, Planas *et al.* 1999). While multiple PKA subunits including RII β , RI α , C α , and C β 1 are expressed in adipocytes, under normal conditions the major holoenzyme comprises RII β and C α . Studies exploiting the unique binding properties of cAMP analogs that bind specifically to one of the two cAMP-binding sites (site 1, C-8 analogs; site 2, C-6 analogs) have demonstrated that the type II regulatory subunit is responsible for the lipolytic response and that when site-1- and site-2-specific cAMP analogs are incubated in combination, they work synergistically (Beebe *et al.* 1984).

PKA RII β is the most abundant regulatory subunit in AT and its deletion impacts lipolytic activation in both

BAT (Cummings *et al.* 1996) and WAT (Planas *et al.* 1999) via an isoform switch from RII β to RII α . The net result was increased basal PKA activity and dramatically increased UCP1. Lipolytic stimulation by isoproterenol (β 1, β 2, and β 3) or selective stimulation of β 3-adrenergic receptors with CL 316,243, however, was severely blunted in RII β KO mice despite unchanged PKA-mediated transcriptional regulation in AT (Planas *et al.* 1999). Interestingly, PKA inhibition of acetyl-CoA carboxylase (ACC), lipoprotein lipase (LPL), and glucose transporter type 4 (GLUT4) (and enhancement of phosphoenolpyruvate carboxykinase (PEPCK) in RII β KO mice did not differ from WT mice under both fed and fasted states, despite altered PKA enzymatic activity initiated by the isoform switch. This differential response in AT to β -adrenergic stimulation further supports the finding that type II PKA activity is responsible for the lipolytic response.

Along a similar line of investigation, constitutive activation of PKA in WAT by expressing an activated $C\alpha$ allele under the adiponectin (*Adipoq*) promoter in mice increased energy expenditure, AT PKA activity, and UCP1 induction (Dickson *et al.* 2016). These changes to AT physiology were sufficient to prevent DIO and preserve insulin sensitivity after HFD challenge in mice. *In vitro*, the stimulation of normal human and mouse adipocytes with forskolin, isoproterenol, or dibutyryl-cAMP caused increased oxidative and glycolytic respiration, and as expected, the increased oxygen consumption rate was dependent on PKA-induced lipolysis (Yehuda-Shnaidman *et al.* 2010).

PKA regulation of lipolysis downstream of β -adrenergic receptors (β -adr Rs) in AT relies on phosphorylation of several key lipolytic players. Elevated cAMP levels activate PKA that in turn phosphorylates the two primary lipolytic proteins, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL), that account for almost all triglyceride lipase activity in adipocytes (Schweiger *et al.* 2006). β -adr R activation induces the PKA-mediated phosphorylation of ATGL at Ser⁴⁰⁶ (Ser⁴⁰⁴ in humans) to generate a moderate increase in ATGL-mediated lipolysis (Pagnon *et al.* 2012), and this specific phosphorylation also occurs during fasting or moderate intensity exercise (Haemmerle *et al.* 2006, Huijsman *et al.* 2009).

Hormone-sensitive lipase (HSL) is a major phosphorylation target of PKA at three serine residues: Ser⁵⁶³, Ser⁶⁵⁹, and Ser⁶⁶⁰ (Stralfors & Belfrage 1983, Anthonsen *et al.* 1998). HSL phosphorylation is also regulated by perilipin in a PKA-dependent manner (Brasaemle 2007). Perilipin, a protein that is integral in organizing the interaction of lipolytic proteins on the

surface of lipid droplets, is phosphorylated by PKA on Ser⁴⁹² and Ser⁵¹⁷. This PKA-mediated phosphorylation enables the dissociation of the ATGL coactivator, CGI-58, that can then associate with ATGL on lipid droplets to permit its PKA-dependent activation to enable basal and stimulated lipolysis (Granneman *et al.* 2009). HSL also relies on the phosphorylation of perilipin-1 at Ser⁵¹⁷ by PKA to facilitate its access to lipid droplets (Londos *et al.* 1999, Miyoshi *et al.* 2007). These phosphorylations by PKA are the hallmark of β -adr R-stimulated lipolysis that generate free fatty acids for use as fuel by other tissues and maintain healthy nutrient partitioning, lipid storage and utilization, and permit non-shivering thermogenesis (Fig. 2).

Adipogenesis

Although the mechanism is unknown, adipogenesis requires the availability of intracellular cAMP for the differentiation of 3T3L1 preadipocytes into adipocytes that is concomitant with the programmed expression of the genes that comprise an adipocyte-specific profile which is stimulated by phosphodiesterase inhibitors including IBMX (Spiegelman *et al.* 1993). More recently, we have seen that IBMX permits CREB phosphorylation through the PKA signaling pathway and upregulates C/EBP β to stimulate adipogenesis in 3T3-L1 preadipocytes (Zhang *et al.* 2004, Lee *et al.* 2018).

Involvement of the PKA pathway in hepatic glucose metabolism and lipid metabolism and storage

Hepatic PKA signaling in the fasted state initiates gluconeogenesis as a nutrient-sensing response. In obesity, impairment of this system leads to the inappropriate initiation of gluconeogenesis that is a key part of the associated metabolic dysregulation (Fig. 2). Activation of the PKA pathway in hepatocytes increased the gene expression of gluconeogenic gene expression through the de-phosphorylation CREB-regulated transcription coactivator 2 (CRTC2) (Altarejos & Montminy 2011). It has also been demonstrated that biguanides, the most widely used drug to treat type II diabetes mellitus (T2DM), acts by antagonism of glucagon and the subsequent accumulation of AMP and decrease of cAMP that in turn reduces PKA signaling (Miller *et al.* 2013). Along a similar line of investigation, metformin was unable to reduce hepatic glucose production or hyperglucagonemia in

mice that expressed a constitutively active form of PKA (He *et al.* 2016).

PKA is intimately involved in hepatic fatty acid uptake and trafficking and lipogenesis through the modification of downstream proteins. PKA has been shown to activate CREBH transcriptional activity through the phosphorylation of Ser¹³³ in the fasted state (Shaywitz & Greenberg 1999, Nakagawa & Shimano 2018). Lee *et al.* determined that, once activated, CREB H (CREBH) can constrain plasma triglyceride levels to a normal range through the downstream activation of a variety of genes involved in triglyceride metabolism in *CREB3L3*^{-/-} mice. KO of CREBH led to downregulation of *Fads1*, *Fads2*, *Elovl2*, *Cidec*, *Apoc2*, *Apoa5*, hepatic *Egf21*, *Apoa4*, *Elov5*, and *G0s2*, while overexpression caused significant upregulation of *Cidec*, *Apoc2*, *Egf21*, and *Apoa4* (Lee *et al.* 2011). PKA is also involved in the regulation of CREBH through the phosphorylation of peroxisome proliferator-activated receptor alpha (PPAR α) at its DNA-binding domain, resulting in activation of PPAR α that in turn upregulates CREBH by binding to the PPRE region of the CREBH promoter (Nakagawa & Shimano 2018). PPAR α has further implications in hepatic lipid metabolism through its ability to promote fatty acid β oxidation, ketogenesis, and lipid transport. Kersten and others showed that the deletion of PPAR α in mice caused development of fasting-induced hepatic steatosis due to reduced hepatic fatty acid uptake and oxidation, thus linking PKA regulation to proper maintenance of fasting-fatty-acid regulation (Kersten *et al.* 1999).

PKA is crucial to hepatic lipogenesis through the regulation of sterol regulatory element-binding protein (SREBP), an integral ER membrane protein involved in stress-induced lipogenesis, and carbohydrate regulatory element-binding protein (ChREBP), a glycolytic enzyme involved in stimulating the expression of numerous lipogenic genes (Kawaguchi *et al.* 2001, Kammoun *et al.* 2009). PKA can inhibit SREBP both directly and through the activation of farnesoid X receptor (FXR) or sirtuin 1 (SIRT1). Lee *et al.* conducted experiments on *SREBP1c*^{-/-} mice that proved PKA directly inhibits SREBP through phosphorylation of its Ser³⁰⁸ residue that promotes its sumoylation and ultimate degradation (Lee *et al.* 2014). Studies conducted in WT and FXR mutant cells that lack Ser³²⁵ and Ser³⁵⁷ but had intact protein function revealed that PKA phosphorylation of FXR at Ser³²⁵ and Ser³⁵⁷ was necessary and sufficient to stimulate FXR transcription and protein function in WT cells, as this effect was absent in mutant cells (Ploton *et al.* 2018). Other studies have suggested that FXR then proceeds to suppress the

ability of LXR, a reverse cholesterol transport protein, to stimulate SREBP (Watanabe *et al.* 2004). In fasting conditions, PKA also activates SIRT1, a NAD⁺-dependent protein deacetylase, through phosphorylation of Thr⁵²². Once phosphorylated, SIRT1 deacetylated SREBP at Lys²⁸⁹ and Lys³⁰⁹, suppressing its ability to conduct lipogenesis (Ponugoti *et al.* 2010, Guo *et al.* 2012). ChREBP is altered both directly and indirectly by PKA. ChREBP is inhibited through phosphorylation by PKA and activated protein phosphatase 2 (PP2A)-mediated dephosphorylation at Ser¹⁹⁶ (Kawaguchi *et al.* 2001).

Mouse models of PKA deficiency have also indirectly implicated PKA in the regulation of hepatic lipid metabolism, in some cases, likely as a secondary effect of DIO resistance. One such model is the RII β KO mouse, that had enhanced lipolysis (Cummings *et al.* 1996), maintained superior glucose sensitivity after chronic HFD, and had significantly decreased hepatic lipid accumulation compared to WT littermates (Cummings *et al.* 1996, Planas *et al.* 1999). The C β KO mouse similarly resisted DIO, glucose intolerance, and insulin insensitivity as well as excessive hepatic lipid accumulation after exposure to HFD feeding (Enns *et al.* 2009, London *et al.* 2019). These various defects in PKA signaling all had the similar end point of altered nutrient partitioning and a decrease in the hepatic lipid accumulation that is a common feature of obesity. A third knockout mouse model of RII α deficiency had reduced hepatic steatosis after chronic HFD due to, in part, decreased hepatic PKA activity that occurred only after the challenge of over-nutrition, suggesting the ability of the PKA system to respond to changes in nutritional status (London *et al.* 2014a).

Regulation of insulin secretion in pancreatic β cells

Pancreatic PKA dysfunction links obesity to the impairment of glucose-stimulated insulin secretion. Proper functioning of the cAMP signaling pathway in pancreatic β -cells is necessary for glucose-stimulated insulin secretion, one of the first processes disrupted in the development of T2DM (Gerich 2002, Nolan *et al.* 2011) (Fig. 2). In T2DM, impaired glucose-stimulated insulin secretion can be ameliorated by the incretin, glucagon-like peptide-1 (GLP-1) (Egan *et al.* 2002). GLP-1 enhances insulin secretion in part by cAMP-stimulated PKA-dependent phosphorylation of snapin, a component of a larger complex that mediates insulin exocytosis from pancreatic β cells (Song *et al.* 2011).

PKA directly phosphorylates CREB and de-phosphorylates cAMP-regulated transcriptional coactivators (CRTC) to promote cellular gene expression. CREB phosphorylation upregulates insulin receptor substrate 2 (IRS2) in β cells and promotes proper islet function. Expression of a dominant-negative form of A-CREB that blocks all family members (CREB1, ATF1, and CREM) led to hyperglycemia (Jhala *et al.* 2003). In the state of hyperglycemia, protein kinase A inhibitor β is induced in β cells to inhibit the PKA signaling pathway upstream of CREB and CRTC2 (Blanchet *et al.* 2015). Insulin resistance causes disruption of β cell CREB activity via a decrease in expression of the transcription factor MafA that can be attenuated by disruption of the PKIB gene.

Conclusions

The PKA system exerts tight control over the regulation of metabolism and energy balance in many organ systems through an impressive network of GPCR ligands and downstream phosphorylation targets, including CREB which regulates transcription of CRE-containing genes. Studies in mice and cell systems have illuminated many of the mechanisms that govern WAT lipolysis, BAT activation, and the central regulation of both, the modulation of leptin sensitivity in hypothalamus, specifically through the subunit RII β , and hypothalamic regulation of the melanocortin system. With respect to a role for PKA in glucose metabolism and the development of T2DM, mouse and cell studies have illuminated the details of PKA function in pancreatic β -cell insulin secretion as well as facets of hepatic glucose and lipid metabolism regulation. While human studies are few, we have seen differences in the PKA system between lean and obese individuals and identified PKA signaling genes that can cause dysregulation of the HPA axis in CS. While there is a growing body of evidence that demonstrates the translation of this basic research in humans, the exploration of potential therapeutics, including specific small molecule agonists and inhibitors of the PKA systems, is nascent.

Transgenic and KO mouse models enabled the manipulation of genes with central roles in energy homeostasis that improved our understanding of this complex system of integrated central and peripheral controls. However, the *ob/ob* mouse is an example of a break in the chain of translation of obesity research from mouse to human. While leptin replacement in the *ob/ob* mouse was effective in rescuing the obesity of the *ob/ob*

mouse and generated hope for a possible therapeutic for human obesity, leptin replacement was not a viable 'cure' for obesity, in part, because of developed leptin resistance. Similarly, the prospect of enhancing BAT activation and browning of WAT depots has become a popular line of research for its potential therapeutic value in humans. While it is likely that constitutive activation of PKA in AT can prevent DIO in mice, WAT and BAT activation in humans is not likely to be a viable preventive therapeutic for DIO, in part, because of species-specific physiologic differences such as the dramatic differences in body surface area. Despite the potential incongruities, the body of work in mouse models described here have enriched our knowledge of how the PKA-regulated systems are integrated to govern whole body energy homeostasis and maintain proper nutrient sensing and glucose metabolism.

Declaration of interest

Dr Stratakis holds patents on the *PRKAR1A*, *PDE11A*, and *GPR101* genes and/or their function and has received research funding from Pfizer Inc. on the genetics and treatment of abnormalities of growth hormone secretion. The other authors have nothing to disclose.

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