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Targeting PKA Signaling to Prevent Metabolic Syndrome and Delay Aging

Enns, Linda C and Ladiges, Warren C
University of Washington, USA

1. Introduction

Protein kinase A (PKA) is a ubiquitous serine-threonine kinase that is activated by adenyl cyclase (AC)-mediated cAMP (Niswender et al., 1975). Canonically, the PKA signaling pathway is triggered when G-coupled receptors, a family of seven transmembrane domain proteins, are bound by extracellular hormones. The resultant dissociation of the Gs complex allows the stimulatory Gsα protein to bind to and activate membrane-bound adenylyl cyclases (ACs), which convert ATP to cAMP. The PKA holoenzyme has four subunits: 2 of which are catalytic (PKA C) and two of which are regulatory (PKA R). When associated, the heteroteramericenzyme is inactive. When cAMP binds to the regulatory subunits, the PKA C monomers are released, becoming catalytically active (Kirschner et al., 2009) (Fig. 1).

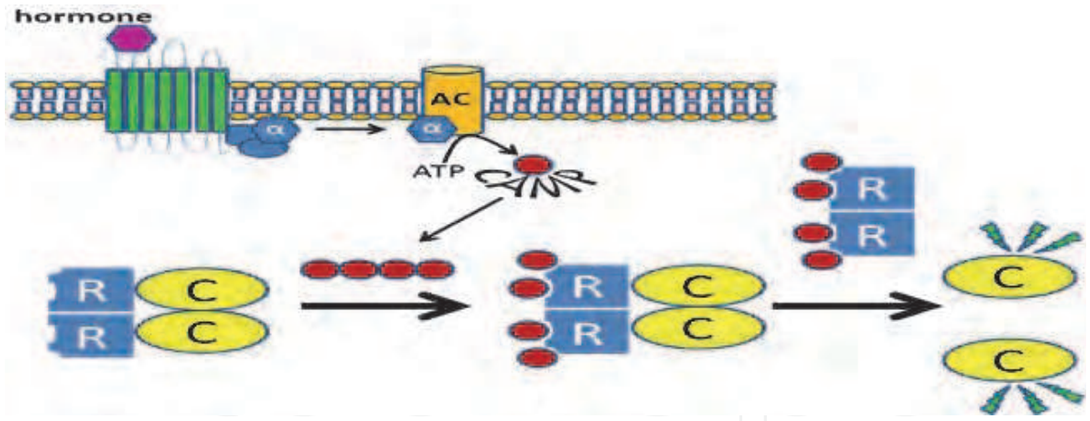

Fig. 1. cAMP activation of PKA.

PKA plays a role in numerous biological functions, with hundreds of PKA substrates identified in both the cytoplasm as well as the nucleus (Budovskaya et al., 2005; Huang et al., 2007; Neuberger et al., 2007; Gao et al., 2008). PKA signaling is known to influence cellular differentiation, ion channel activity, and plays a key role in the regulation of metabolism and triglyceride storage (Enns and Ladiges, 2010). This single enzyme is able to regulate such numerous and diverse processes by having isoforms and splice variants for both the catalytic and regulatory subunits, each of which has its own spatial and temporal patterns of expression, and each of which confers a different mutant phenotype when knocked out in mice (Table 1).

	Subunit	Expression	KO Phenotype
R	RIα	widespread	embryonic lethal (Amieux et al., 2002)
	RIIα	widespread	no obvious phenotype (Burton et al., 1997)
	RIβ	brain	deficits in neural plasticity (Brandon et al., 1995)
	RIIβ	**brain, adipose**	**obesity resistant** (Cummings et al., 1996)
C	Cα	widespread	early postnatal lethality, growth retardation, sperm dysfunction (Skalhegg et al., 2002)
	Cβ	**brain**	**obesity resistant** (Enns et al., 2009b)

Table 1. Different isoforms of regulatory and catalytic subunits of PKA.

The C and R subunits are encoded by three and four different isoforms, respectively: Cα, Cβ and Cγ for the catalytic subunit, and RIα, RIβ, RIIα and RIIβ for the regulatory subunit (Burton et al., 1997). Generally speaking, the α isoforms are constitutively expressed in most tissues, whereas the β isoforms are expressed at highest levels in the brain (Cadd and McKnight, 1989). Specific responses to different hormones and neurotransmitters is also believed to be achieved by the subcellular compartmentalization of PKA (Harper et al., 1985). RII subunits are thought to contribute to this type of regulation, by anchoring PKA in close proximity to its substrates through the binding of a family of A-kinase anchor proteins (AKAPs) (Lohmann et al., 1984; Rubin, 1994; Dell'Acqua et al., 1997). AKAPs anchor PKA by interacting with specific R subunits, and thus determine the subcellular localization of the PKA holoenzyme by what type of R subunit is present. The type-I PKA holoenzyme contains RI subunits (RIα and RIβ) and is primarily cytoplasmic, while the type II holenzyme contains RII subunits (RIIα and RIIβ) and is associated with particulate subcellular fractions (McConnachie et al., 2006).

Different and specific roles for the various subunits of PKA have been verified using conventional gene knockout mouse models. Deletion of the Cα subunit causes perinatal lethality, or, in the case of survivors, severe growth retardation (Skalhegg et al., 2002), while up until recently, it was believed that knockout (KO) mice for Cβ are phenotypically indistinguishable from their WT littermates (Qi et al., 1996). Complete loss of function of the R1α subunit leads to embryonic death early during gestation caused by abnormal mesodermal development (Amieux et al., 2002), while mice heterozygous for the knockout allele are predisposed to develop the myoxymas and endocrine tumors associated with Carney Complex (CNC) in humans (Kirschner et al., 2005). RIβ KO mice have deficiencies in synaptic plasticity (Brandon et al., 1995).

Yet loss of function of various elements of the PKA signaling pathway have also been shown to have health and lifespan benefits. Some of these beneficial PKA functions have been conserved evolutionarily from yeast to mammals, as evidenced by lifespan studies in yeast, worms, flies and mice. In yeast, loss of function of CYR1, an adenylyl cyclase, increases lifespan (Longo, 2003) as do mutations in the GTP-GDP exchange factors CDC35 and CDC25. Reduced function of TPK1, 2 and 3, functionally redundant yeast PKA catalytic

subunits which are homologous to those in both mouse and human, also promotes longevity (Lin et al., 2000). PKA activity also mediates age-related decline in flies (Yamazaki et al., 2007; Laviada et al., 1997), and recent studies in mice have described delayed cardiac aging and extended lifespan by deletion of the adenylyl cyclase, AC5 (Yan et al., 2007) and obesity resistance, increased lifespan and healthy aging by disruption of specific PKA subunit genes (Cummings et al., 1996; Enns et al., 2009a, 2009b). This chapter will focus on recent studies describing the health benefits of disruption of two different PKA subunits, the regulatory isoform RIIβ, and the catalytic subunit Cβ. The proposed mechanisms behind these effects will be discussed as well as future work required to further investigate the potential of these subunits as pharmaceutical targets for the treatment of aging and age-related disease in humans.

2. Disruption of subunits of PKA leads to obesity resistance and leptin sensitivity

Most mammals maintain their body composition within a narrow range of fat mass. For example, following caloric restriction and a subsequent weight loss, rats increase food intake and decrease energy expenditure until they return to their original body weight (Mitchel and Keesey, 1977). Likewise, obese rats which have been induced to overeat by electrical stimulation of the lateral hypothalamus, return to original body weights and blood glucose levels when the stimulus is removed (Steffens, 1975). In order to accomplish energy homeostasis, an animal must be able to sense the amount of energy available in adipose tissue as well as sense and integrate opposing signals; it also must be able to regulate both energy intake and expenditure in response to this information. The main tissues responsible for both the sensing of as well as the response to nutrient status are the hypothalamus, which controls body weight and appetite, brown adipose tissue (BAT), which controls thermogenesis and energy expenditure, and white adipose tissue (WAT), which is involved in energy storage (Cypess & Kahn, 2010). The AC/cAMP/PKA pathway plays a major role in the genetic regulation of obesity and energy balance, as evidenced by mouse studies showing that disruption of specific PKA subunits leads to a more lean phenotype under 'normal' conditions, as well as to obesity resistance when challenged with either a high fat/high carbohydrate diet or with age-induced obesity.

2.1.1 PKA and obesity resistance

Disruption of the regulatory RIIβ subunit of PKA causes obesity resistance (Cummings et al., 1996). RIIβ is known to play a role in energy homeostasis. The RIIβ regulatory isoform of PKA is abundant in brown and white adipose tissue and the brain, with limited expression elsewhere. As mentioned earlier, these three tissues are the key players in the coordination of adiposity through regulation of energy storage, energy expenditure, and feeding behaviour. Disrupting the RIIβ gene in these tissues does not cause any overt abnormalities, but RIIβ null mutants are remarkably lean, with fat pad weights about half that of their wild-type littermates. Body composition differences are only a result of a reduction in fat; these mutants do not suffer from decreases in muscle mass. In addition, disrupting the RIIβ gene protects the obesity-susceptible C57/BL6 strain of mice from high fat/high carbohydrate (HF/HC) diet-induced both obesity and fatty livers (Cummings et al., 1996). Obesity resistance caused by deletion of the RIIβ subunit has also been observed

in aging C57/BL6 mice, the WT which we have found to gain body weight post-maturity due to the accumulation of fat (Enns et al., 2009a). At the peak of obesity, aging male WT mice, maintained on a regular diet, had 25% body fat, while RIIβ null mice only had about 15% body fat; aging WT females and RIIβ null mice showed average maximum body fat percentages of about 30% and 15%, respectively. This same study also found that WT mice, with age, developed livers up to twice their original size due to an accumulation of fat, and that disruption of RIIβ prevented this from occurring. RIIβ thus represents a potential pharmacological target for the treatment of diet and age-induced obesity and fatty liver.
Our studies have indicated that the Cβ catalytic subunit of PKA also plays a role in maintaining a set point of adiposity (Enns et al., 2009b). Young Cβ null mice appear overtly normal when maintained on a regular chow diet, but when challenged with a HF/HC diet, show resistance to the obesity and fatty liver disease suffered by their WT littermates. This obesity resistance is not due to reduced food intake, which is similar between genotypes, nor to increased locomoter activity, which also shows no differences between genotypes. In addition, knocking out Cβ protects aging mice on a regular diet from developing age-related obesity and fatty livers (Enns, In Press).

2.1.2 PKA and thermogenesis

The leanness of RIIβ null mutants had been thought in the past to be due to changes in PKA activity in brown adipose tissue (BAT). BAT is a major contributor to non-shivering, diet-induced thermogenesis, or heat production (Rothwell and Stock, 1979). Thermogenesis is caused by the uncoupling of oxidative phosphorylation from ATP production in the mitochondria by uncoupling protein-1 (UCP1), which results in the energy from the proton motive force being dissipated as heat. Increases in thermogenesis occur in response to marked increases in energy intake, such as those that occur in HF/HC diet-challenged mice. Non-shivering thermogenesis is in part regulated by the sympathetic nervous system, and can be stimulated by hormones such as norephinephrine and leptin. It is known that PKA plays a mediating role in this process, phosphorylating perilipin, the main regulator of lipolysis, in response to these hormones (Souza et al., 2007). Loss of the RIIβ subunit in BAT is associated with a compensatory increase in the RIα isoform, which has a higher affinity for binding cAMP. The resultant increase in basal PKA activity in the BAT of RIIβ null mutants leads to an increase in UCP, an elevated metabolic rate and an increase in body temperature, suggesting that mutants are metabolically inefficient and waste food calories as heat (Cummings et al., 1996). The hypothesis that metabolic inefficiency is the cause of the RIIβ null lean phenotype is, however, confounded by data showing that disrupting UCP1 in RIIβ null mice reduces basal oxygen consumption but does not prevent the lean phenotype (Nolan et al., 2004). Regardless, the brain is believed to regulate adiposity in part through modulating sympathetic stimulation of PKA in BAT, resulting in changes in UCP expression and facultative energy expenditure (Himms-Hagen, 1990), and chronic activation of PKA in adipose tissue through β-adrenergic stimulation is being investigated for its potential in obesity therapy. While UCP1 induction does not appear to be required for the maintenance of the lean phenotype in RIIβ null mice, it is still essential to sustain their increased basal oxygen consumption, a process important to the regulation of energy expenditure and metabolic setpoint. Understanding the role that RIIβ may play in this process is important for determining pharmaceutical targets that may also be useful for the development of anti-obesity drugs.

Altered thermogenesis does not appear to play a role in the PKA Cβ null obesity-resistant phenotype. We did not find body temperature differences between Cβ null mice and their WT littermates, nor did we observe differences in UCP1 levels in the BAT between genotypes maintained on either a regular chow or a HF/HC diet. Taken together, the RIIβ and Cβ mutant data indicates that while RIIβ is involved in regulating energy expenditure through induction of thermogenesis, upregulation of this process in particular is not essential in either of these mutants for obesity resistance to occur.

2.1.3 PKA and WAT signaling

In white adipose tissue (WAT), PKA is known to integrate a number of hormonal signals in order to regulate the lipolysis, or the catabolism of stored triglycerides into fatty acids and glycerol by hormone-sensitive lipase (HSL) (Planas et al., 1999). Lipolysis is in part increased by β-adrenergic agonists, which stimulate PKA to both activate HSL (Stralfors et al.,1984; Anthonsen et al., 1998) as well as promote its translocation to lipid droplets (Egan et al., 1992; Hirsch & Rosen, 1984). PKA also inhibits the expression of a number of lipogenic genes. In RIIβ mutant WAT, there is an elevated basal rate of lipolysis when measured *in vitro*, and a blunted lipolytic response to β-AR stimulation that is observed both *in vitro* and *in vivo*. It is unknown if these changes in WAT metabolism could play a role in the lean phenotype and obesity resistance observed in the RIIβ null mutants. We have not yet characterized the WAT metabolism of Cβ null mutants.

2.1.4 PKA and leptin sensitivity

Studies on RIIβ using the leptin-deficient, obese *ob/ob* mouse (*ob*) indicate an important role for this particular PKA subunit in the leptin-dependent regulation of energy homeostasis (Newhall et al., 2005). Leptin is a well-known peptide hormone that is produced by adipose tissue. It plays a key role in the regulation of energy intake and energy expenditure, including appetite and metabolic rate. The level of circulating leptin is directly proportional to the amount of fat stored in the body, and acts on receptors in the hypothalamus of the brain where it regulates the activity or synthesis of many neuropeptides that are important in appetite and metabolic control. The region of hypothalamus which serves to integrate signals controlling feeding and energy expenditure is called the arcuate nucleus region (ARC). Two distinct populations of leptin-responsive neurons exist here: one that expresses the anabolic neuropeptides, NPY and agouti-related protein (AgRP) and one that expresses a precursor protein for the catabolic neuropeptide α-MSH, called proopiomelanocortin (POMC) (Cone 2005; Morton et al., 2006). Both sets of neurons project into the paraventricular hypothalamus (PVH) where the Gs-coupled melanocortin receptor MC4R is activated by α-MSH; this activation is antagonized by AgRP. Activation of the MC4R receptor is believed to decrease food intake and increase energy expenditure. PKA is a downstream mediator for many of these neuropeptides, (Schwartz et al., 2000; Flier, 2004). Generally speaking, catabolic and anabolic neuropeptides signal through pathways that increase and decrease PKA activity, respectively. Leptin inhibits NPY and AgRP release from the paraventricular nucleus, which normally leads to the activation of anabolic pathways through a decrease in PKA activity. Conversely, leptin stimulates the release of α-MSH, leading to an increase in PKA activity and the activation of catabolic pathways (Fig. 2).

Studies on RIIβ null mice indicate that this mutation may confer leptin sensitivity. Even when fed only standard rodent chow, leptin serum levels in mutants were found to be

threefold lower than for WT mice (Schreyer et al., 2001). When maintained on the HF/HC diet, serum leptin levels increased differently between genotypes. Both genotypes experienced elevations in serum leptin levels, but these increases were delayed in the mutants compared to WT, consistent with their delayed weight gain. In spite of lower leptin levels, when standardized to body weight, food intake was actually slightly higher in the mutants, indicative of leptin sensitivity.

This hypothesis was verified by knocking out the RIIβ subunit in other mouse mutants known to have problems with leptin signaling. The *ob* mouse is hyperphagic, hypoactive, hypothermic and hyperinsulinemic (Bray & York, 1979), due to decreased expression of β-adrenergic receptors (β-ARs) and UCP in BAT (Reichling et al., 1988; Collins et al., 1994). The obese phenotype can be rescued by administration of leptin, which decreases their food intake and increases their metabolic rate in addition to restoring normal expression levels of adipose β-AR and UCP1 (Weigel et al., 1995; Mistry et al., 1997; Phelleymounter et al., 1995; Halaas et al., 1995; Campfield et al., 1995; Breslow & Berkowitz, 1997; Commins et al., 1999). The phenotype of the *ob* mouse can also be rescued by knocking out RIIβ. The double mutant shows decreased body weight, increased energy expenditure, and activation of BAT resulting in increased thermogenesis (Newhall et al., 2004). RIIβ is expressed in high levels in the hypothalamus (Planas et al., 1999). An increase in basal PKA activity here, due to the disruption of the RIIβ subunit could lead to increased stimulation of leptin sensitive catabolic pathways.

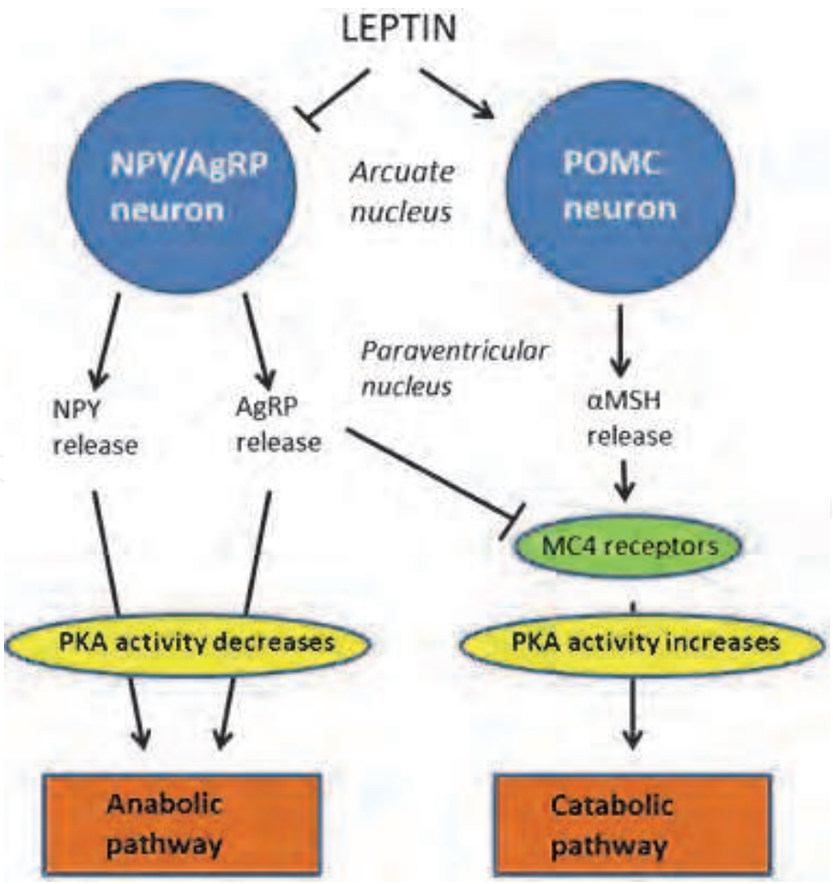

Fig. 2. Leptin signaling pathway as mediated by PKA.

Disruption of RIIβ also reverses the obesity syndrome found in agouti lethal yellow mice (A^y), which express agouti-related protein (AgRP) ectopically due to a genetic rearrangement at the agouti locus (Czyzyk et al., 2007). Constitutive expression of AgRP, such as occurs in A^y mice, leads to hypoactivity, hyperphagy, hyperglycemia and hyperinsulinemia (Yen et al., 1994; Manne et al., 1995). Disruption of RIIβ reduces both food intake and adiposity in these mice, indicating that the signaling pathway downstream of the agouti antagonism has been modified. It has been suggested that the known compensatory increase in the more cAMP-affinitive type I PKA, observed in RIIβ KO tissues, results in an overall increase in the basal activity of PKA that is downstream and thus independent of AgRP antagonism in hypothalamic neurons.

We have also found that Cβ null mice are leptin sensitive in addition to being obesity resistant (In Press). After several weeks of being maintained on a high fat diet, WT mice had elevated leptin serum levels that were 2.5-fold higher than Cβ null mutants. In spite of lower serum leptin, mutants were found to be hypophagic and hypermetabolic, indicative of leptin sensitivity. Although mutants maintained on a regular diet showed metabolic rates similar to WT, as determined using indirect calorimetry to measure the rate of O_2 consumption (VO_2), we did observe a higher metabolic rate in the Cβ null mutants compared to WT when both genotypes were maintained on a HF/HC diet (Enns, In Press). To directly test leptin sensitivity in the mutants, at the end of the HF/HC dietary challenge, both WT and mutants were injected with 4.0 ug leptin/g mouse, twice daily, for a period of a week. Mutants, but not WT mice, showed significant weight loss, indicating that the WT mice had become leptin resistant on the HF/HC diet, while the Cβ null mice had not. Leptin sensitivity in the Cβ null mice was verified by injecting young both mutants and WT mice, maintained on a regular diet, with leptin twice daily. While weight loss and food intake were not affected differently between genotypes, by the end of the week-long injections, Cβ null mice were showing significantly higher rates of oxygen consumption compared to WT mice. Thus it is possible that Cβ, like RIIβ, acts downstream of leptin signaling in the hypothalamus. The Cβ gene encodes three isoforms: Cβ1 is expressed in most tissues, while Cβ2 and Cβ3 are neural-specific (Guthrie et al., 1997). Measurements of brain PKA activity in the PKA Cβ null mutant have shown that knocking out all Cβ isoforms does not result in changes in total PKA activity, at least in the amygdala and hippocampus, as Cα protein levels are upregulated in order to compensate. It does, however, result in a 26% decrease in basal activity (without added cAMP) that may affect kinase activity at low cAMP concentrations (Howe et al., 2002). This data would appear to be at odds with that showing that increased PKA activity is responsible for leptin sensitivity and obesity resistance in the RIIβ null mutant. It is unknown how PKA activity is affected in the hypothalamus of Cβ null mice, but there are a number of possibilities. It may be affected differently than in other regions of the brain (ie. it may increase). Conversely, Cα1 and Cβ1 have diverged by about 10% in amino acid sequence in the mouse, but these sequence differences are strictly maintained with almost perfect fidelity across mammalian species (Uhler et al., 1986), suggesting that each has an important and unique function. If Cα plays a specific role in the leptin signaling pathway compared to Cβ, it may be that the compensatory increase in Cα activity in the Cβ null mutant is what is increasing signaling downstream of the MC4R receptor. Regardless, this leptin sensitivity is likely the cause of the obesity resistant phenotype observed in both age and HF/HC diet-challenged Cβ null mutants.

3. Disruption of subunits of PKA protects against diet-induced insulin resistance and dyslipidemia

In addition to inducing obesity, the HF/HC diet used in our studies is known to induce diabetes in C57/BL6 mice (Surwit et al., 1988; Surwit et al., 1991). It is also common for mice fed this diet to develop hyperlipidemia, or an elevation of lipids in the blood, reflected by serum increases in low density and very low density lipoproteins (LDL and VLDL) (Kirk et al., 1995; Srivastava et al., 1991; Ishida et al., 1991; LeBoeuf et al., 1993). Studies on HF/HC diet-fed PKA mutants have clearly illustrated an important role for PKA in the mediation of diet-induced insulin resistance and lipid disorder.

3.1.1 PKA and insulin sensitivity

RIIβ null mutants are resistant to HF/HC diet-induced insulin resistance. Knocking out the RIIβ subunit of PKA resulted in mice with 26% lower serum insulin levels than WT when maintained on a regular diet. When maintained on a HF/HC diet for 15 weeks, both genotypes developed hyperinsulinemia, however insulin levels were much higher for WT mice (Schreyer et al., 2001). Blood glucose levels increased similarly for both genotypes when challenged with the HF/HC diet, but the observation that RIIβ null mice achieve similar glucose levels with less insulin indicates improved insulin sensitivity. In keeping with this, loss of the RIIβ subunit resulted in improved glucose disposal in mice maintained on a regular chow diet, with lower blood glucose levels in the mutants compared to WT at all time points following a glucose injection. Also, while there were no differences in blood glucose between genotypes raised on a regular chow diet, RIIβ null mice were resistant to the marked reduction suffered by WT mice in the percentage of blood glucose cleared following an insulin injection after 15 weeks on the HF/HC diet.

It is believed that since RIIβ expression is absent from pancreatic islets, these effects are not directly due to changes in insulin secretion in response to circulating glucose. It was proposed by Shreyer et al. that RIIβ null mice are at least in part protected from diet-induced insulin resistance due to their resistance to obesity under a HF/HC dietary challenge. They were unable to test the null hypothesis due to a lack of sufficient number of RIIβ null mice that had white adipose tissue weights similar to those of their WT littermates; however, when insulin-mediated glucose disposal was corrected for differences in body weight, it was found that the HF/HC diet-fed RIIβ null mice cleared glucose in response to insulin at a similar rate to regular chow-fed mutants, while WT mice on the HF/HC diet showed decreased glucose disposal per gram mouse weight when compared with those on the regular diet. This suggests that loss of RIIβ improves insulin sensitivity at least in part via a mechanism independent of adiposity. One proposed mechanism is a reduction in PKA's known ability to antagonize insulin's activation of the mitogen-activated protein kinase (MAPK) cascade in adipose tissue (Sevetson et al., 1993). Although it is known that in this particular tissue, the compensation for RIIβ by the more cAMP-affinitive RIα causes a four- to fivefold increase in basal PKA activity, is is possible that inhibition of this particular cascade, probably at the level of ras or raf, is dependent on an RIIβ-containing PKA holoenzyme.

Similarly, we have found that PKA Cβ also plays a role in insulin sensitivity. As with RIIβ null mice, Cβ null mice are significantly protected against HF/HC diet-induced insulin resistance, showing improved glucose dispersal in response to insulin, compared to WT (Enns et al., 2009). Interestingly, we have found that Cβ null mice are extremely sensitive to insulin compared to their WT littermates, even when maintained on a regular chow and

without major differences in adiposity levels. In fact, on a regular diet, at least for females, young Cβ null mice show insulin sensitivity in spite of having slightly higher body fat percentages than WT. In other words, insulin sensitivity of the Cβ null mutant is independent of adiposity, and the loss of the Cβ null mutant has direct effects on insulin sensitivity. Given the similarity between the Cβ and the RIIβ null phenotypes, it would be logical to propose that these two mutations are acting on insulin sensitivity via a similar mechanism. This would support the hypothesis of Shreyer et al. that PKA directly affects insulin sensitivity of adipose tissue, and that this particular mechanism is dependent on a specific subunit composition of PKA, specifically one containing either RIIβ or Cβ.

3.1.2 PKA and dyslipidemia

Dyslipidemia, another problem often observed in conjunction with obesity and diabetes, was found to be reduced in both RIIβ and Cβ null mutants when challenged with a HF/HC diet. Plasma both total cholesterol as well as very low density and low density lipoproteins (VLDL and LDL) were significantly lower in RIIβ null mice compared to WT (Schreyer, 2001). Cβ null mutants, at least for males, when challenged with the HF/HC diet were resistant to the increases in LDL and VLDL and partially resistant to the increases in high density lipoprotein (HDL) observed in the serum of WT mice (Enns et al., 2009b). Because insulin inhibits the assembly and release of VLDLs from the liver (Koo & Montminy, 2006), it is possible that the reduced serum VLDL levels seen in both types of mutants is an indirect effect of their insulin sensitivity. Whether the marked loss of lipoproteins from the VLDL/LDL fraction of the diet-challenged RIIβ null mice and of lipoproteins from both the HDL and VLDL/LDL fraction of the diet-challenged Cβ null mice is due to direct effects of the mutations on lipoprotein production or clearance, or is a result of indirect influences due to their insulin sensitivity or obesity resistance, is unknown.

4. Disruption of PKA protects against cardiac hypertrophy and dysfunction

Cardiac hypertrophy is an increase in the mass of the heart in response to and to compensate for an increased workload. Prolonged stress leads to impaired diastolic and eventually systolic properties of the left ventricle, leading to heart failure (Shapiro & Sugden, 1996). Altered PKA signaling has been implicated in cardiomyopathy by many previous studies (Enns et al., 2010; Lohse & Engelhardt, 2001). For example, it is believed that the muscle-specific A-kinase Anchoring Protein (mAKAP) targets PKA to the perinuclear region of the cell where it can modulate cardiomyocyte size. Inhibiting mAKAP expression suppresses the ability of leukemia inhibitory factor (LIF), which acts by increasing ERK5 activity, to induce cardiac hypertrophy (McConnachie et al., 2006). Deficiencies in PKA signaling have been linked to human cardiomyopathy due to reduced phosphorylation of downstream targets such as cardiac troponin I (Zakhary et al., 1999) and to preservation of cardiac function against pressure overload in mice (Okumura et al., 2003a; Okumura et al., 2003b). We have found that the Cβ subunit of PKA plays an important role in the development of cardiac hypertrophy and dysfunction in response to both angiotensin II-induced as well as age-induced hypertension.

Cβ null mice are resistant to angiotensin II- and age-induced cardiac hypertrophy and dysfunction (Enns et al., 2010; Enns et al., In Press). Angiotensin (ang) II is the effector of the renin-angiotensin system (RAS) and increases blood pressure by causing potent

vasoconstriction through stimulation of angiotensin receptors in the vascular system (Ito et al., 1995). When ang II was administered to Cβ null mice and their WT littermates at a continuous rate and over a period of 4 weeks, both genotypes experienced similar and significant increases in both systolic and diastolic blood pressure. In spite of experiencing similar hypertension, the hearts of the Cβ null mice were smaller and showed improved cardiac function in 4 of 5 echocardiographical parameters measured including left ventricular mass index (a measure of the thickness of the ventricular wall), fractional shortening (a measure of contractility of the left ventricle), ratio of early to late diastolic filling (a measure of compensation by the left atrium for left ventricle failure), and ratio of aortic to left atrial diameter (a measure of left atrial enlargement due to overcompensation for left ventricular failure). We have also recently shown that as C57/BL6J mice age, they have a natural tendency to develop hypertension (Enns et al, In Press). As with angiotensin II-challenged mice, aged (24 month-old) WT mice of this strain also experience significant cardiac hypertrophy, some showing hearts twice the size of those found in young (4 month-old) mice. In addition to enlarged hearts, aged WT mice, like those challenged with ang II, show thickened ventricular walls, reduced fractional shortening of the left ventricle, reduced early to late diastolic filling ratios, and enlarged left atria. An additional parameter of global left ventricular function, myocardial performance index (MPI) was also found to worsen in aging WT mice. Disruption of the Cβ subunit did not protect aging C57/BL6 mice from hypertension, but did make mice resistant to both the cardiac hypertrophy experienced by the aging WT mice, as well as to their decline in cardiac performance in all parameters measured. Effects of disruption of RIIβ on age and ang II-induced cardiac decline have not yet been assessed.

PKA Cβ thus appears to play an important role in the mediation of hypertension and its myopathological effects. The β-adrenergic (β-AR)/adenylyl cyclase/PKA pathway, central to stimulating cardiac function, is known to be dysfunctional in heart failure (Bristow et al., 1982). Blockade of β-AR receptors improves survival in heart failure patients (Bristow, 2000) and transgenic mouse studies have shown that chronic activation of the cAMP-PKA pathway by cardiac-specific overexpression of β-AR, Gsα, and the α-catalytic subunit of PKA result in cardiomyopathy (Lohse & Engelhardt, 2001; Antos et al., 2001). PKA is known to cause cardiac hypertrophy in response to elevation of cAMP by β-adrenergic agonists (Rockman et al., 2002). There are, however, conflicting data in the literature to support the idea that activation of the β-AR/cAMP/PKA pathway may play a protective role in response to hemodynamic overload. In humans, phosphorylation of troponin I (TnI) by PKA is reduced in dilated cardiomyopathy (Zakhary et al., 1999), and in mice, overexpression of two types of cardiac adenylyl cyclases results in improved cardiac function (Lipskaia et al., 2000; Gao et al., 1999). The RIIβ mutant is thought to be sensitive to β-AR activation (McKnight et al., 1998; Montovani et al., 2009), an idea supported by their exaggerated response to amphetamine (Brandon et al., 1998). Given the other phenotypic similarities between the RIIβ and Cβ mutants, it is possible that the Cβ null mouse has a similar sensitivity, and that an overactive β-AR pathway is protecting their hearts against pressure overload.

PKA plays many other roles in cardiac signaling, and any of these may play a role in cardiac hypertrophy and dysfunction. For example, activation of cAMP/PKA signaling in the heart has been shown to inhibit smooth muscle proliferation (Indolfi et al., 1997). Calcium signaling pathways also play a role in cardiac hypertrophy (Passier et al., 2000; Minamisawa

et al., 1999), supported by the finding that in the presence of hypertension, its development can be prevented by L-type calcium channel blockers (Zou et al., 2002). PKA has multiple downstream targets involved in calcium signaling in the heart, including the L-type Ca^{2+} channel in the sarcolemma, the ryanodine receptor (RyR2), and phospholamban in the sarcoplasmic reticulum (Antos et al., 2001). The Cβ subunit of PKA may play a specific role in the activation of one or more of these substrates.

5. PKA and longevity

Given that disruption of either the RIIβ or Cβ subunit of PKA in mice confers resistance to a number of health problems associated with aging, including obesity, leptin and insulin resistance, and cardiac hypertrophy and dysfunction, it was of interest to determine whether or not knocking out either of these genes would also lengthen the murine lifespan. Lifespan studies revealed an increase in both the median and maximum lifespans for RIIβ null males with an increase in median lifespan from 884 days to 1005 days, and an increase in the 80% lifespan (80% deaths of the cohort) from 941 to 1073 days. There was no difference in either median or 80% lifespan between genotypes in females (Enns et al., 2009a). Lifespan cohorts for Cβ null mice showed no effect on either the median or maximium lifespan for females, and a reduced lifespan for Cβ null males.

Whether or not the attenuation of an age-related health problem translates to an increase in lifespan for a particular strain of mouse depends on its contribution to that strain's probable cause of death. We have determined that adiposity plays a significant role in the lifespan of the male, but not the female C57BL/6J WT mouse (Enns et al., 2009a). As mentioned earlier, this strain of mouse is susceptible to age-related obesity, and individuals continue to put on body weight in the form of body fat for many months post-maturity. This gain in adiposity was found to be variable between individuals, however, and when the lifespan of individual WT mice was plotted against their maximal body weight, a strong correlation was found for males (R^2=0.4795), but not for females (R^2=0.0369). Age-related obesity is thus a strong risk factor for mortality in male C57BL/6J mice, and it is not surprising that disruption of a gene such as RIIβ, that removes this risk factor would also lengthen their lifespan .

Lifespan analyses can be an indicator for whether or not disrupting a gene also confers detrimental effects. For example, the shortened lifespan of the Cβ null male mouse implies that this PKA subunit plays a role in other necessary functions. When mice heterozygous for the Cβ null mutation are bred , male homozygous nulls are born at a lower than expected frequency, indicating that the Cβ subunit of PKA may be important to males during their embryonic development. That disruption of either RIIβ or Cβ does not lengthen the female lifespan is not necessarily surprising, given that we found no correlation between adiposity and lifespan for C57BL/6J females. However, it can also be said for females that there appear to be no detrimental effects, at least those which would impact lifespan, of disrupting either of these PKA subunits. This is important for validating either of these subunits as a potential pharmaceutical target for the treatment of age-related disease in humans.

6. Conclusions

The Cβ and RIIβ subunits of PKA represent promising pharmaceutical targets for the treatment of metabolic syndrome, a name for a group of risk factors that together increase

the risk of coronary disease, stroke, and type II diabetes and a problem which is rapidly becoming the predominant cause of poor health and reduced lifespan in industrialized nations. Mouse mutants lacking either of these subunits display a number of health benefits, including resistance to age and diet-induced obesity, protection against age and diet-induced leptin and insulin sensitivity, and resistance to cardiac hypertrophy and dysfunction.

The potential of a protein or protein subunit as a pharmaceutical target depends on whether or not its disruption also causes negative effects. Lifespan analyses show that in C57BL/6J mice, there appear to be no major detrimental health effects either on males and females from disrupting the RIIβ subunit, or on females from disrupting the Cβ subunit. Disrupting the Cβ subunit in males appears to carry some detriment, possibly during embryonic development, that affects the overall lifespan of the mouse, but pharmaceutical treatment of obesity and aging in humans would presumably occur beyond the age of maturity. Conditional mouse mutants need to be constructed to determine if knocking out the gene later in life removes the detrimental effects on the male PKA Cβ null lifespan.

Future work needs to address the many unanswered questions that have arisen from these studies. Are the phenotypes we are observing the result of the loss of the RIIβ and Cβ subunits, or of the known compensation by other PKA isoforms? Do these subunits affect the nature of the downstream targets of PKA, and if so, what are those targets, and what is their potential for pharaceutical targeting? Is leptin sensitivity the sole cause of the obesity resistance in the RIIβ and Cβ null mutants, or are there other mechanisms? By what direct mechanism is RIIβ and Cβ influencing insulin sensitivity, and what is its specific contribution to healthy aging and lifespan? What are the mechanisms behind the resistance to cardiac hypertrophy and dysfunction? Finally, how can we discover or develop pharaceuticals that will specifically target these PKA isoforms? Answering these questions will both validate the potential of these subunits as pharmaceutical targets, as well as identify new potential targets for the treatment of age-related metabolic syndrome in humans.

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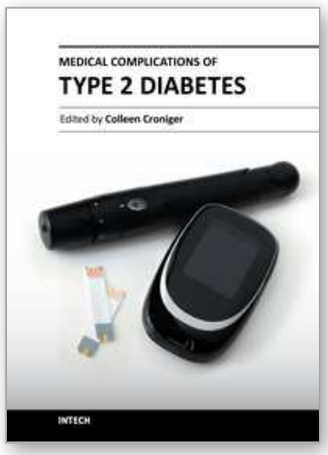

Medical Complications of Type 2 Diabetes

Edited by Dr. Colleen Croniger

ISBN 978-953-307-363-7
Hard cover, 412 pages
Publisher InTech
Published online 12, September, 2011
Published in print edition September, 2011

Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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