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Resveratrol Ameliorates Aging-Related Metabolic Phenotypes by Inhibiting cAMP Phosphodiesterases

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SUMMARY

Resveratrol, a polyphenol in red wine, has been reported as a calorie restriction mimetic with potential antiaging and antidiabetogenic properties. It is widely consumed as a nutritional supplement, but its mechanism of action remains a mystery. Here, we report that the metabolic effects of resveratrol result from competitive inhibition of cAMP-degrading phosphodiesterases, leading to elevated cAMP levels. The resulting activation of Epac1, a cAMP effector protein, increases intracellular Ca^{2+} levels and activates the CamKKβ-AMPK pathway via phospholipase C and the ryanodine receptor Ca^{2+}-release channel. As a consequence, resveratrol increases NAD^+ and the activity of Sirt1. Inhibiting PDE4 with rolipram reproduces all of the metabolic benefits of resveratrol, including prevention of diet-induced obesity and an increase in mitochondrial function, physical stamina, and glucose tolerance in mice. Therefore, administration of PDE4 inhibitors may also protect against and ameliorate the symptoms of metabolic diseases associated with aging.

INTRODUCTION

Calorie restriction (CR) is the most robust intervention demonstrated to extend life span and delay the physiological deterioration associated with aging (McCay et al., 1935). Because CR involves a number of overlapping and interconnected signaling pathways, it is difficult to identify with certainty the mechanism(s) underlying the beneficial effects of CR. Based on studies of the budding yeast *Saccharomyces cerevisiae*, it was initially proposed that CR extends life span via the activity of Sir2 (Lin et al., 2000), the founding member of the conserved sirtuin family of NAD^+-dependent protein deacetylases (Guarente, 2006). Although it remains unclear whether Sir2 plays a direct role in the antiaging effects of CR (e.g., Kaeberlein et al., 2004), overexpression of Sirt1, the mammalian homolog of Sir2, has been reported to protect mice from aging-related phenotypes that are similar to type 2 diabetes (Banks et al., 2008; Bordone et al., 2007; Pfluger et al., 2008), cancer (Herranz et al., 2010), and Alzheimer's disease (Donmez et al., 2010). Suggesting that Sirt1 activity does not protect against aging-related diseases by delaying the aging process, overexpression of Sirt1 does not extend life span in mice (Herranz et al., 2010).

The positive health effects of CR and sirtuin activity in animal models have provoked intense interest in the development of small-molecule activators of Sirt1 to prevent or delay aging-related diseases. An in vitro screen performed using a fluorophore-tagged substrate identified resveratrol as an activator of Sirt1 deacetylase activity (Howitz et al., 2003). Resveratrol is a natural polyphenol produced by plants in response to environmental stress (Signorelli and Ghidoni, 2005) and is present in many plant-based foods, most notably red wine. Subsequent work has shown that resveratrol extends the life spans of lower eukaryotes (Gruber et al., 2007; Viswanathan et al., 2005; Wood et al., 2004). These studies set the stage for testing resveratrol as a CR mimetic in mammals. In mice, long-term administration of resveratrol induced gene expression patterns that resembled those induced by CR and delayed aging-related deterioration, even though it did not extend life span (Pearson et al., 2008). Resveratrol protected against obesity and development of insulin resistance in rodents fed a high-calorie

diet (Baur et al., 2006; Lagouge et al., 2006). Resveratrol also decreased insulin resistance in type 2 diabetic patients (Brasnyó et al., 2011), suggesting that the pathway targeted by resveratrol might be important for developing therapies for type 2 diabetes.

An important mediator of the metabolic effects of resveratrol (Lagouge et al., 2006; Um et al., 2010) is peroxisome proliferator-activated receptor γ coactivator, PGC-1α (Puigserver et al., 1998). It is a coactivator that controls mitochondrial biogenesis and respiration and can contribute to fiber-type switching in skeletal muscle (Lin et al., 2002) and increase adaptive thermogenesis in brown adipose tissue (Puigserver et al., 1998). Consistent with the known ability of Sirt1 to deacetylate and activate PGC-1α (Gerhart-Hines et al., 2007; Rodgers et al., 2005), resveratrol increased Sirt1 and PGC-1α activity in mice fed a high-fat diet (HFD) (Lagouge et al., 2006; Um et al., 2010).

Two findings have raised doubt that resveratrol is a direct Sirt1 activator. First, although resveratrol activates Sirt1 in vivo, it activates Sirt1 to deacetylate fluorophore-tagged substrates but not native substrates in vitro (Beher et al., 2009; Borra et al., 2005; Kaeberlein et al., 2005; Pacholec et al., 2010), suggesting that resveratrol activates Sirt1 indirectly in vivo. Second, resveratrol activates AMP-activated protein kinase (AMPK) in vivo (Baur et al., 2006; Cantó et al., 2010; Dasgupta and Milbrandt, 2007; Park et al., 2007; Um et al., 2010). AMPK is a trimeric complex that senses nutrient deprivation by sensing the AMP/ATP (Carling et al., 1987) and ADP/ATP (Xiao et al., 2011) ratios. AMPK, which is emerging as a key regulator of whole-body metabolism, has been shown to increase NAD^+ levels and activate Sirt1 and PGC-1α (Cantó et al., 2009, 2010; Fulco et al., 2008; Um et al., 2010). However, a causal link between the increase in NAD^+ and Sirt1 activation has not been established. We and others have shown that AMPK-deficient mice are resistant to the metabolic effects of resveratrol, providing evidence that AMPK is a key mediator of the metabolic benefits produced by resveratrol (Cantó et al., 2010; Um et al., 2010). These findings demonstrated that activation of Sirt1 and PGC-1α by resveratrol is downstream of AMPK activation.

Studies on how resveratrol activates AMPK have led to different and often conflicting mechanisms. Hawley et al. reported that at a high concentration (100–300 μM), resveratrol decreased ATP; and in a cell line expressing a mutated γ subunit of AMPK that made AMPK insensitive to AMP, resveratrol did not activate AMPK (Hawley et al., 2010). This suggested that resveratrol, at high concentrations, activated AMPK by decreasing energy and increasing the AMP/ATP or ADP/ATP ratios. However, resveratrol can activate AMPK at a concentration less than 10 μM (Dasgupta and Milbrandt, 2007; Feige et al., 2008; Park et al., 2007). At low concentrations (≤50 μM), resveratrol appears to activate AMPK without decreasing energy (Dasgupta and Milbrandt, 2007; Suchankova et al., 2009). As the plasma level after oral administration of resveratrol is low (Crowell et al., 2004), the mechanism by which resveratrol activates AMPK at physiologically relevant concentrations most likely does not involve decreasing energy.

For this report, we attempted to find the direct target of resveratrol and to elucidate the biochemical pathway by which it activates AMPK and produces metabolic benefits. We found that resveratrol directly inhibits cAMP-specific phosphodiesterases (PDE) and identified the cAMP effector protein Epac1 as a key mediator of the effects of resveratrol, which leads to the activation of AMPK and Sirt1.

RESULTS

Resveratrol Activates AMPK in an Epac1-Dependent Manner

A hint that cAMP may mediate the effects of resveratrol was suggested by a previous study reporting that resveratrol increased cAMP production in breast cancer cells (El-Mowafy and Alkhalaf, 2003). In this study, we found that cAMP levels increased significantly with a low dose (≤50 μM) of resveratrol in C2C12 myotubes (Figures 1A and 1B). To confirm that resveratrol increased cAMP levels in vivo, we administered resveratrol to mice by oral gavage and measured cAMP levels in skeletal muscle and white adipose tissue (WAT) (Figure S1A available online). We found that resveratrol increased cAMP levels in both tissues. In contrast, resveratrol did not significantly increase cGMP levels in myotubes (Figure S1B). The possibility that the increase in cAMP production was responsible for the activation of AMPK by resveratrol was examined by treating myotubes and HeLa cells with resveratrol in the presence of the adenylyl cyclase (AC) inhibitor MDL-12,330A (Figure 1C). In both cell types, MDL-12,330A prevented resveratrol (50 μM) from increasing the phosphorylation of both AMPK (T172) and the AMPK substrate acetyl-CoA carboxylase (ACC) (S79), which are markers of AMPK activity. These findings indicate that cAMP signaling is essential for resveratrol to activate AMPK.

In most cells, intracellular cAMP signaling is mediated by two groups of effectors that bind cAMP: protein kinase A (PKA) and cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs, Epac1 and Epac2) (de Rooij et al., 1998; Kawasaki et al., 1998). To determine which of these two effector groups activate AMPK in response to resveratrol, we treated cells with siRNA specific for either the PKA catalytic subunit (PKAc) or Epac1, which is more widely expressed than Epac2, prior to resveratrol treatment. For this purpose, we used HeLa cells instead of myotubes because Epac-regulated pathways affect the myogenesis process (Pizon et al., 1999). As shown in Figures 1D and 1E, resveratrol increased the phosphorylation of AMPK and ACC in the presence of PKA siRNA but not in the presence of Epac1 siRNA, suggesting that Epac1 is essential for resveratrol to activate AMPK in HeLa cells.

Epac proteins function as GEFs for Rap1 and Rap2, members of the Ras family of small G proteins that cycle between an inactive GDP-bound state and an active GTP-bound state (de Rooij et al., 1998; Kawasaki et al., 1998). Epac proteins catalyze the exchange of GDP for GTP and thereby activate Rap1 and Rap2. As a result, Epac activity increases the fraction of Rap that is GTP bound, which can be detected by a pull-down assay using the immobilized Ras association (RA) domain of RalGDS (van Triest et al., 2001). We found that Rap1•GTP level in myotubes is increased by resveratrol, indicating that resveratrol increases Epac activity (Figure 1F). Treating myotubes with