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Metabolic benefits of inhibiting cAMP-PDEs with resveratrol

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Calorie restriction (CR) extends lifespan in species ranging from yeast to mammals. There is evidence that CR also protects against aging-related diseases in non-human primates. This has led to an intense interest in the development of CR-mimetics to harness the beneficial effects of CR to treat aging-related diseases. One potential CR-mimetic that has received a great deal of attention is resveratrol. Resveratrol extends the lifespan of obese mice and protects against obesity-related diseases such as type 2 diabetes. The specific mechanism of resveratrol action has been difficult to elucidate because resveratrol has a promiscuous target profile. A recent finding indicates that the metabolic effects of resveratrol may result from competitive inhibition of cAMP-degrading phosphodiesterases (PDEs), which increases cAMP levels. The cAMP-dependent pathways activate AMP-activated protein kinase (AMPK), which is essential for the metabolic effects of resveratrol. Inhibiting PDE4 with rolipram reproduces all of the metabolic benefits of resveratrol, including protection against diet-induced obesity and an increase in mitochondrial function, physical stamina and glucose tolerance in mice. This discovery suggests that PDE inhibitors may be useful for treating metabolic diseases associated with aging.

Keywords: resveratrol, Sirt1, cAMP, phosphodiesterases, AMP-activated protein kinase, Epac1, metabolic, calorie-restriction, aging

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CR is the most robust non-genetic intervention for life extension in many species, including rodents and lower eukaryotes.[1] If started early in life, decreasing calorie intake by 30% or more below ad libitum intake increases maximum lifespan by 30–60% in rodents. In general, CR not only extends the maximal lifespan but also decelerates many aging-related physiological changes and chronic diseases in rodents. Whether CR extends the maximal lifespan by delaying aging and/or by decreasing incidence or progression of aging-related diseases is controversial. Nevertheless, the beneficial effects of CR observed across a wide range of animals, suggests that CR may also be beneficial for primates, including humans. Although studies of CR in non-human primates have not yet concluded, evidence seems to be pointing in that direction. Long-term, moderate CR decreases aging-related mortality and diseases in rhesus monkeys.[2] As in rodents, CR decreases adiposity, improves insulin sensitivity and lipid profile and decreases inflammation. Aging-related diseases such as cardiovascular disease, type 2 diabetes, sarcopenia and cancer are significantly lower in the CR rhesus monkeys.[2] Whether CR protects against aging-related diseases by delaying the aging process remains unanswered as these monkeys have not reached their maximum lifespan.

Despite the health benefits of CR, countless past experiences with therapies that rely on reducing food intake indicate that CR is not a viable long-term therapy except for the most disciplined few. Therefore, CR research has triggered an intense interest in the development of CR-mimetics, drugs that produce the biochemical, cellular and physiological changes that are critical for the CR benefits without limiting food intake. One potential CR-mimetic that has received a great deal of attention is resveratrol, a polyphenol belonging to a group of compounds called stilbenes, which is produced in plants in response

to stress and is present in many plant-based foods, most notably red wine. A number of studies have found that resveratrol increases lifespan in lower eukaryotes,[3-5] although other studies have disputed these findings.[6,7] Resveratrol also delays aging-related deterioration and produces a transcriptional profile that overlaps that of CR in mice, but without extending lifespan.[8,9] In mice fed a high-fat diet, resveratrol protects against obesity, type 2 diabetes[10] and premature death.[11] Several clinical trials have been conducted to study the metabolic effects of resveratrol. Although these trials have used different subject groups (e.g., obese healthy, type 2 diabetics or older adults with glucose intolerance) and different resveratrol doses (150 mg–2 g per day), they suggest that resveratrol may improve insulin sensitivity[12,13] and mimic some aspects of CR.[14]

Although the beneficial effects of resveratrol are widely accepted, the mechanism by which resveratrol confers these benefits was hotly debated. The notion that resveratrol may be a potential CR-mimetic was first proposed by Howitz et al.[15] who reported that resveratrol is an activator of the protein deacetylase Sirt1. Whether resveratrol is a direct activator of Sirt1 came into question when several groups showed that resveratrol activated Sirt1 to deacetylate fluorophore-tagged, but not native substrates.[16-19] Interestingly, resveratrol decreased acetylation of Sirt1 substrates in vivo.[10,11,20,21] The Sirt1-dependency of some resveratrol effects (reviewed in ref. 22) raises the possibility that resveratrol activates Sirt1 indirectly in vivo via another target. However, the acetylation status of a protein is determined not only by the rate of deacetylation but also by the rate of acetylation, which may also be affected by resveratrol.[23] Therefore, until a direct marker of Sirt1 activity becomes available, it will be difficult to distinguish whether Sirt1 activity is merely needed for the effects of resveratrol or whether Sirt1 activity is directly or indirectly induced by resveratrol.

The first clue regarding the possible alternate mechanism of action of resveratrol came from the observation that resveratrol activates AMP-activated protein kinase (AMPK) in vivo.[11,20,21,24,25] AMPK senses nutrient deprivation by sensing the AMP/ATP[26] and ADP/ATP[27] ratios and has been shown to increase NAD^+ levels and to decrease acetylation of Sirt1 substrates.[20,21,28,29] We and others have shown that AMPK is required for the metabolic effects of resveratrol,[20,21] suggesting that AMPK is the key mediator of and is upstream of Sirt1 in the resveratrol response. However, the epistasis between AMPK and Sirt1 may be more complicated because AMPK activation is suppressed in Sirt1 knockout mice treated with a low dose of resveratrol but not with a high dose of resveratrol.[30] Although the glucose lowering effect of resveratrol is AMPK-dependent,[20] it is not Sirt1-dependent.[30]

Since resveratrol does not directly activate AMPK, what is the upstream target(s) that directly binds to resveratrol? The target protein(s) should satisfy two conditions: it should be upstream of AMPK and it should be able to mediate the CR-mimetic effects of resveratrol. In response to conditions that decrease serum glucose, serum levels of glucagon and catecholamines rise. These hormones, which stimulate adenylate cyclases and cAMP production, act to increase glucose production and to increase fat utilization. Our observation that resveratrol increased cAMP levels in myotubes[31] led to the discovery that resveratrol increased cAMP levels by competitively inhibiting a number of cAMP phosphodiesterases (PDEs), which degrade cAMP. Increased cAMP levels activate AMPK by increasing intracellular Ca^{2+} levels and the activity of the AMPK kinase calcium/calmodulin-dependent protein kinase , processes that are dependent on the cAMP effector protein Epac1 (cAMP guanine-nucleotide exchange factor).[32,33] In certain conditions or other cell types, another cAMP effector protein kinase A appears to contribute to resveratrol-mediated activation of AMPK via AMPK kinase LKB1.

PDE4 comprises most of the PDE activity in skeletal muscle, the tissue where the metabolic effects of resveratrol are best elucidated. If the metabolic effects of resveratrol are mediated by inhibiting cAMP PDEs, inhibiting PDE4 should reproduce, at least qualitatively, the effects of resveratrol. Indeed the PDE4 inhibitor rolipram activated AMPK and reduced acetylation of the Sirt1 substrate PGC-1α as well as increasing mitochondrial content and respiration in myotubes.[31] In mice fed a high-fat diet, rolipram increased metabolic rate, protected against obesity and improved glucose tolerance.[31]

Inflammatory signaling contributes to development of type 2 diabetes, and suppressing it with anti-inflammatory drugs improves insulin sensitivity.[34] Resveratrol may improve insulin sensitivity partly by suppressing inflammatory signaling because resveratrol has been reported to attenuate inflammatory signaling in primary and 3T3-L1 adipocytes.[35] Consistent with the notion that resveratrol inhibits PDE4, inhibitors of PDE4 suppress inflammation,[36] and mice deficient in PDE4B have reduced obesity-induced inflammation in adipose tissue.[37] The anti-inflammatory effect of PDE4 inhibition is most likely related to the ability of cAMP-induced signals to interfere with the function of the proinflammatory transcription factor nuclear factor-κB.[38]

It is unlikely that PDE inhibition will reproduce all of the effects of resveratrol because resveratrol binds to many other proteins[19] and also because the target of its action may differ depending on the tissue or cell type and the effects under study. However, the therapeutic potential of resveratrol is complicated by such a promiscuous target profile.[19] Clinical trials using resveratrol have shown modest metabolic benefits without obvious toxicity, but they used small sample sizes and the trials lasted no more than four weeks.[12-14] Whether resveratrol can be useful as a drug for chronic diseases such as type 2 diabetes will depend on the robustness of its efficacy long-term as well as its possible toxicity, which are currently unknown. A more promising strategy may be to use a PDE4 inhibitor to reproduce the metabolic benefits of resveratrol. Indeed, the PDE4 inhibitor roflumilast,[39] which was recently approved by the FDA for the treatment of chronic obstructive pulmonary disease, was unexpectedly found to significantly lower glucose in individuals with type 2 diabetes.[40] Therefore, the potential utility of PDE4 inhibitors for treating type 2 diabetes and other aging-related diseases is worth investigating.

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