Diabetol Int (2012) 3:65–67
DOI 10.1007/s13340-012-0076-9

COMMENTARY

Red wine acts through a familiar drug target

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Received: 18 April 2012 / Accepted: 19 April 2012 / Published online: 5 June 2012

Resveratrol, a natural polyphenol first identified and named by a Japanese chemist in 1940 [1], is present in many plant products, including grape skin. It has been the focus of much attention recently because it provides a variety of potential health benefits, in particular protection from various age-related conditions (such as diabetes mellitus, vascular diseases, cognitive disorders, and cancer), and increases life span [2–4]. Given that red wine is rich in resveratrol, and that moderate wine consumption has been shown to lower cardiovascular risk [5], the intake of resveratrol through the drinking of red wine may contribute to the "French paradox"—which refers to the fact that the mortality due to cardiovascular diseases is lower in France than in other European countries despite similar lifestyle-related risk factors [6]. Sirt1, an NAD^+-dependent protein deacetylase, is thought to play an important role in the action of resveratrol [2]. Resveratrol stimulates the activity of Sirt1, and overexpression of Sirt1 protects mice from age-related diseases [2]. Moreover, orthologs of Sirt1 appear to contribute to life span extension by resveratrol in worms and flies [2]. Whereas an early study suggested that resveratrol directly stimulates the activity of Sirt1 [7], this notion has been challenged by more recent studies [8, 9]. Resveratrol was found to stimulate the activity of AMP-activated protein kinase (AMPK) [10], which can increase cellular NAD^+ levels and thereby stimulate the activity of Sirt1 [11], but the effect of resveratrol on AMPK is also likely not direct. The direct target of resveratrol for its health-promoting effects has thus remained a mystery. Park et al. [12] have now revealed that a group of enzymes that serve as common drug targets are also targeted by resveratrol.

The cyclic nucleotides cAMP and cGMP are both important messenger molecules in intracellular signaling. An increase in the cellular levels of cAMP and cGMP is achieved through their synthesis by adenylyl cyclase and guanylyl cyclase, respectively. Conversely, the levels of these molecules are reduced through their hydrolysis by phosphodiesterases (PDEs). Mammalian PDEs constitute a large family of enzymes that are categorized into 11 subtypes (PDE1 to PDE11) encoded by 21 different genes [13]. Individual PDEs differ in their affinity for substrates, tissue distribution, and mode of regulation, and together they share a broad range of biological tasks [13]. Various pharmacological inhibitors of PDEs have been developed, some of which are marketed as drugs for a wide variety of conditions, including bronchial asthma (theophylline), peripheral artery disease (cilostazol), heart failure (amrinone), cognitive disorders (vinpocetine), depression (rolipram), and erectile dysfunction (sildenafil, vardenafil, and tadalafil) [13].

Park et al. [12] have now shown that resveratrol inhibits the activity of several PDEs (PDE1, -3, and -4, but not -2 or -5) in vitro by directly competing with cAMP to bind to these isozymes (Fig. 1). Inhibition of PDEs results in an increase in cellular cAMP levels and the consequent activation of two signaling pathways: the cAMP-dependent protein kinase (PKA) pathway and the Epac1 (exchange protein directly activated by cAMP 1) pathway [14]. Park et al. [12] found that both Sirt1 and AMPK were activated by resveratrol in a manner dependent on Epac1, consistent with the notion that the cellular accumulation of cAMP, likely due to PDE inhibition, is the primary cellular event triggered by resveratrol. Calcium- and calmodulin-

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dependent kinase kinase β (CaMKKβ), which is activated in response to an increase in the intracellular Ca^{2+} concentration, is an upstream regulator of AMPK. Park et al. [12] have shown that a Ca^{2+}-chelating agent or pharmacological inhibitor of CaMKKβ prevented resveratrol-induced activation of AMPK. These findings are consistent with the previous observations that both resveratrol and an active form of Epac1 were capable of increasing intracellular Ca^{2+} levels [14, 15]. Park et al. [12] further analyzed the signaling pathways initiated by resveratrol, and found that the resveratrol-induced activation of Epac1 leads to stimulation of Ca^{2+}- and calmodulin-dependent kinase II (CaMKII) in a phospholipase C (PLC)-dependent manner, which in turn results in the phosphorylation of the ryanodine receptor [12]. The ryanodine receptor, which resides on the surface of the endoplasmic reticulum, then facilitates Ca^{2+} influx into the cytosol.

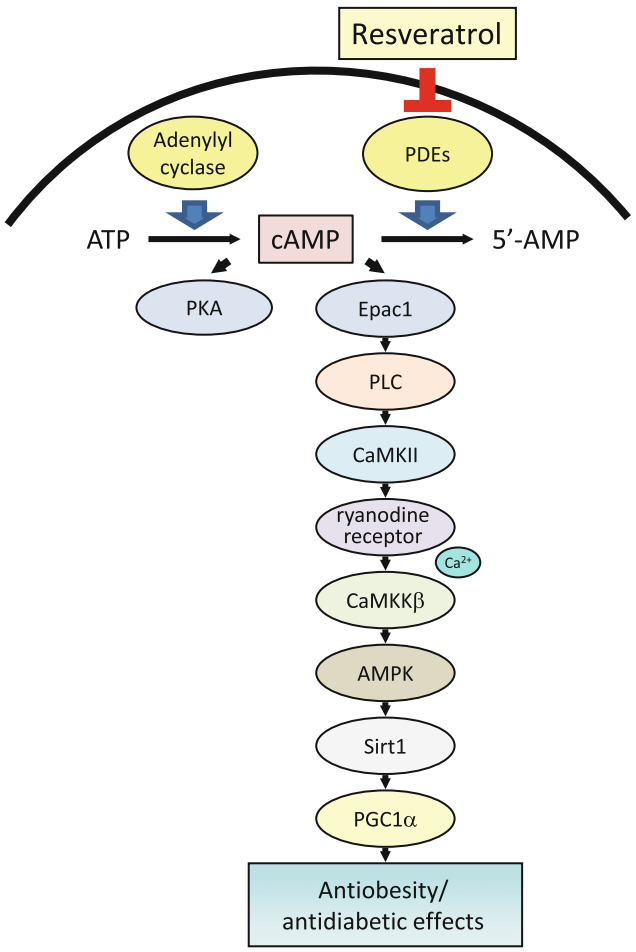

Fig. 1 Intracellular mechanism of resveratrol action. Resveratrol increases the intracellular concentration of cAMP by directly inhibiting PDEs. The accumulation of cAMP results in the activation of Epac1, which in turn triggers the Ca^{2+} and CaMKKβ signaling pathway. CaMKKβ activates Sirt1 in an AMPK-dependent manner, and Sirt1 deacetylates and activates the transcriptional coactivator PGC1α, which is thought to be an important mediator of the antiobesity and antidiabetic effects of resveratrol

If the biological effects of resveratrol are attributable to PDE inhibition, known pharmacological inhibitors of PDEs might be expected to exert similar effects. Indeed, Park et al. [12] found that the treatment of mouse cultured myotubes, in which PDE4 accounts for most of the PDE activity, with a specific inhibitor of PDE4 (rolipram) stimulated AMPK activity as well as deacetylation of the transcriptional coactivator PGC1α, a well-known substrate of Sirt1. Moreover, administration of rolipram to mice increased both the amount of mitochondria in skeletal muscle as well as whole-body energy consumption, effects that were associated with protection from both diet-induced obesity and glucose intolerance. Resveratrol has previously been shown to exert similar actions in both mice [10] and humans [16].

The study of Park et al. has substantially improved our knowledge of the signaling pathways triggered by resveratrol. It has shown that the two known major mediators of resveratrol action, Sirt1 and AMPK, are activated in response to an increase in the intracellular concentration of cAMP and in a manner dependent on Epac1. Moreover, the observations that resveratrol directly inhibited PDE activity and that a specific PDE inhibitor mimicked the effects of resveratrol strongly suggest that PDEs are direct targets of resveratrol. Given that rolipram exerted antidiabetic and antiobesity effects in mice, such effects of resveratrol are likely attributable to the inhibition of PDE4. Park et al. showed that not only PDE4 but at least two other PDE isozymes, PDE1 and PDE3, were inhibited by resveratrol. Vinpocetine, a PDE1 inhibitor, is used clinically for the treatment of cognitive impairment, a condition for which resveratrol has been shown to exert a beneficial effect in animal models [3]. Resveratrol exhibits anticancer activities in cell lines and experimental animals [4], and rolipram as well as cilostazol, a PDE3 inhibitor, seem to have similar activities [13]. These findings favor the notion that multiple biological effects of resveratrol are mediated through the inhibition of multiple isozymes of PDE (Fig. 2).

Although the study of Park et al. has clearly shown that PDEs serve as a primary site of action for resveratrol, it remains to be determined whether all of the actions of resveratrol are triggered by PDE inhibition. Further studies are thus required to elucidate whether other cellular targets contribute to the biological effects of resveratrol. Despite the large body of evidence obtained with cell systems and animal models in support of the potential health benefits of resveratrol, information regarding the clinical utility of this compound remains limited. The

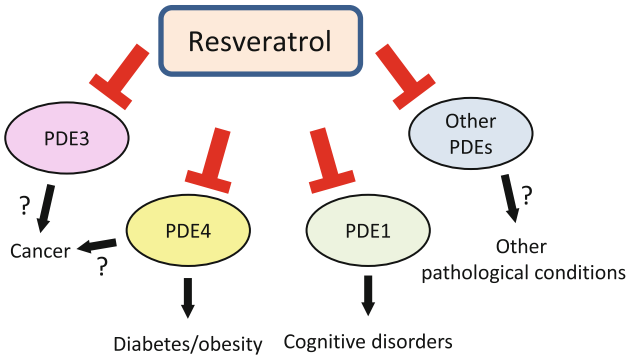

Fig. 2 Multiple biological effects of resveratrol may be mediated through the inhibition of multiple isozymes of PDE

findings of Park et al. should prove useful for the design of clinical studies by providing insights into the potential applications as well as possible adverse effects of resveratrol in humans.

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