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ACCELERATED COMMUNICATION

Resveratrol Suppresses Angiotensin II-Induced Akt/Protein Kinase B and p70 S6 Kinase Phosphorylation and Subsequent Hypertrophy in Rat Aortic Smooth Muscle Cells

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ABSTRACT

Resveratrol (RV), a polyphenolic substance found in grape skin, is proposed to account in part for the protective effect of red wine in the cardiovascular system. Angiotensin II (Ang II)-induced hypertrophy of vascular smooth muscle cells (VSMCs) is a pivotal step in the development of cardiovascular disease. The aims of this study were to test the hypothesis that RV may alter Ang II-mediated hypertrophic VSMC growth and to identify the putative underlying signaling pathways. We show that RV indeed potently inhibits Ang II-induced [^{3}H]leucine incorporation in a concentration-dependent manner (50 μM RV, 71% inhibition). Western blot analysis reveals that phosphorylation of Akt/protein kinase B (PKB) and to a lesser extent the mitogen-activated protein kinase extracellular signal-regulated kinase (ERK) 1/2, both essentially involved in Ang II-mediated hypertrophy, is dose dependently reduced by RV. Consistent with these results, we show that RV attenuates phosphorylation of the p70 ribosomal protein S6 kinase (p70^{S6K}), a kinase downstream of the ERK 1/2 as well as the Akt pathway, that is implicated in Ang II-induced protein synthesis. Upstream of Akt/PKB RV seems to mediate its antihypertrophic effect by inhibiting phosphorylation of the phosphatidylinositol 3-kinase (PI$_3$K) rather than by activating phosphatases. In summary, we demonstrate for the first time that RV inhibits Ang II-induced VSMC hypertrophy, possibly by interfering mainly with the PI$_3$K/Akt and p70^{S6K} but also with the ERK 1/2 signaling pathway. Thus, this study delivers important new insight in the molecular pathways that may contribute to the proposed beneficial effects of RV in cardiovascular disease.

Hypertrophy and hyperplasia of vascular smooth muscle cells (VSMCs) are hallmarks of vascular disorders such as atherosclerosis, restenosis, and hypertension (Takahashi et al., 1997). A pivotal stimulus for VSMC hypertrophy is angiotensin II (Ang II), the main effector of the renin-angiotensin system. In the absence of other growth factors, Ang II induces hypertrophy but not hyperplasia in VSMC through the G protein-coupled angiotensin type I (AT$_1$) receptor (Geisterfer et al., 1988; Berk et al., 1989; Ushio-Fukai et al., 1996; Zafari et al., 1998; Braun-Dullaeus et al., 1999). The importance of Ang II in the pathogenesis of vascular disease is reflected by the efficacy of angiotensin-converting enzyme inhibitors and Ang II receptor blockers in the treatment of atherosclerosis and hypertension. Stimulation of the AT$_1$ receptor in VSMC leads to the activation of multiple protein kinase pathways. Of these, the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase (ERK) 1/2 (Servant et al., 1996) and p38 (Zafari et al., 1998; Ushio-Fukai et al., 1998) have been shown to be implicated in the hypertrophic response of VSMC to Ang II. More recently, the serine/threonine kinase Akt/protein kinase B (PKB) was demonstrated to be activated in VSMC after stimulation with Ang II (Takahashi et al., 1999) and to play a significant role in Ang II-mediated VSMC hypertrophy (Ushio-Fukai et al., 1999; Hixon et al., 2000). The Ang II-initiated activation of the more downstream p70 ribosomal protein S6 kinase

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ABBREVIATIONS: VSMC, vascular smooth muscle cells; Ang II, angiotensin II; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; AT$_1$, angiotensin type 1; PKB, protein kinase B; p70^{S6K}, p70 ribosomal protein S6 kinase; RV, *trans*-resveratrol; PI$_3$K, phosphatidylinositol 3-kinase; PD98059, 2′-amino-3′-methoxyflavone; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; LDH, lactate dehydrogenase; ANOVA, analysis of variance.

($p70^{S6K}$) in VSMC was shown to require both the Akt/PKB and the ERK signaling cascade (Giasson and Meloche, 1995; Eguchi et al., 1999). Active $p70^{S6K}$ plays a critical role in regulating the translation of mRNAs containing an oligopyrimidine tract at their transcriptional start sites and encoding for many of the components of the protein synthetic apparatus (Pullen and Thomas, 1997). Thus, the MAPKs ERK1/2 and p38, as well as the survival kinase Akt/PKB and the $p70^{S6K}$, play pivotal roles in Ang II-induced VSMC protein synthesis and cellular hypertrophy.

Interestingly, many epidemiological studies correlate a low incidence of coronary heart disease and atherosclerosis with a moderate consumption of red wine (Goldberg et al., 2001; Yoshizumi et al., 2001). *trans*-Resveratrol (RV; *trans*-3,5,4′-hydroxystilbene), a phytoalexin found in grape skin, is one of the substances proposed to be responsible for this effect that is commonly referred to as the *French paradox* (Frankel et al., 1993; El Mowafy and White, 1999; Mizutani et al., 2000). RV was shown to exert biological effects consistent with a putative protective effect on the cardiovascular system: The stilbene-derivative has been demonstrated to inhibit the cholesterol and triglyceride deposition in the liver of rats and mice fed a high cholesterol diet, to modify the eicosanoid biosynthesis by interfering with the cyclooxygenase as well as the 5-lipoxygenase pathway, and to prevent platelet aggregation and human low-density lipoprotein oxidation (Soleas et al., 1997). More recent studies suggest that RV inhibits nuclear factor-κB regulated gene expression (Ferrero et al., 1998; Pendurthi et al., 1999; Chan et al., 2000; Holmes-McNary and Baldwin, Jr., 2000; Manna et al., 2000), the advanced glycation end-products–induced proliferation of VSMC from stroke-prone spontaneously hypertensive rats (Mizutani et al., 2000), and the intimal hyperplasia after endothelial denudation in a rabbit model (Zou et al., 2000).

However, no studies exist that address the effect of RV on VSMC hypertrophy or the interference of RV with Ang II-induced signaling pathways. As pointed out above, Ang II is an important contributing factor to many vascular diseases, in part through its effects on VSMCs. The aims of this study were, therefore, to investigate the effect of RV on the Ang II-induced VSMC protein synthesis and to identify signaling protein kinase cascades that may be responsible for the putative effect of RV.

Materials and Methods

Reagents. Materials were obtained from the following suppliers: L-[4,5-^{3}H]leucine (1.0 mCi/ml) was from Amersham Biosciences (Freiburg, Germany). Antibodies against phospho-Akt (Ser^{473}), phospho-$p70^{S6K}$ (Thr^{421}/Ser^{424}), phospho-ERK 1/2 MAPK (Thr^{202}/Tyr^{204}), and phospho-p38 (Thr^{180}/Tyr^{182}) were from Cell Signaling Technology (Frankfurt, Germany). The anti-phosphatidylinositol 3-kinase (PI_3K) p85 antibody was from Upstate Biotechnology (Lake Placid, NY), the anti-phosphotyrosine antibody was from BD Biosciences (clone, PY20; Heidelberg, Germany). Complete was from Roche Applied Science (Mannheim, Germany). PD98059 and wortmannin were from Alexis (Gruenberg, Germany), and okadaic acid was from Acros (Schwerte, Germany). Ang II, Hoechst 33342 dye, and *trans*-resveratrol were from Sigma (St. Louis, MO). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was purchased from Dianova (Hamburg, Germany). Liquiscint was from Roth (Karlsruhe, Germany). Phenol red-free DMEM was obtained from Pan Biotech GmbH (Aidenbach, Germany). Calf Serum was from Invitrogen (Karlsruhe, Germany).

Cell Culture. VSMCs were isolated from male Sprague-Dawley rat thoracic aortas by enzymatic digestion as described previously (Ushio-Fukai et al., 1996). Cells were grown in phenol red-free DMEM supplemented with 10% calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and were passaged twice a week by harvesting with trypsin/EDTA and seeding into 75-cm^2 flasks. For experiments, cells between passages 7 and 15 were used at 70 to 95% confluence.

Western Blotting. VSMCs at 70 to 95% confluence in 60-mm dishes were rendered quiescent by incubation with DMEM containing 0.1% calf serum overnight. Cells were preincubated with RV, wortmannin, or PD98059 at the indicated concentrations or DMSO only for 30 min before stimulation with 100 nM Ang II for 10 min. When the phosphatase inhibitor okadaic acid was used, cells were pretreated with RV, wortmannin, or DMSO for 15 min, treated with or without okadaic acid for another 30 min, and finally stimulated with 100 nM Ang II for 10 min. After treatment, cells were harvested on ice and Western blot was performed as described previously (Ushio-Fukai et al., 1999). Phosphorylated forms of proteins were detected and quantified by enhanced chemiluminescence with a Kodak Digital Science image station 440 cf (PerkinElmer, Köln, Germany).

[^{3}H]Leucine Incorporation. To measure hypertrophy of VSMCs, cells were made quiescent by 48 h in DMEM containing 0.1% calf serum. After pretreatment with RV at the indicated concentrations or with DMSO only for 30 min, cells were incubated with [^{3}H]leucine (1 μCi/ml) in the presence or absence of 100 nM Ang II for 24 h and the amount of incorporated [^{3}H]leucine was assessed as described previously (Zafari et al., 1998).

Immunoprecipitation. Cell lysates were prepared in 60-mm dishes by the addition of lysis buffer (50 mM HEPES, 50 mM NaCl, 5 mM EDTA, 10 mM sodium pyrophosphate, 50 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1× Complete). The lysates were cleared by centrifugation, and protein concentrations were determined by the bicinchoninic acid assay method (Pierce, Rockford, IL). Anti-PI_3K p85 antibody (3.5 μl) was added to a 200-μg aliquot (1 μg/μl) and mixed overnight at 4°C. Protein A-agarose beads (50 μl) were added for an additional 2 h and subsequently collected by centrifugation. The beads were washed three times with 500 μl of lysis buffer and resuspended in 25 μl of 3× sample buffer containing 1.5% β-mercaptoethanol. After addition of 25 μl of 1× sample buffer, beads were boiled for 5 min at 95°C and subsequently removed by centrifugation. Thirty microliters (for anti-phosphotyrosine) or 10 μl (for p85) of the lysate were separated on a 7.5% polyacrylamide gel and transferred to a nitrocellulose membrane (90 min, 100 V). Membranes were blocked for 60 min with 5% bovine lacto transfer optimizer in Tris-buffered saline-Tween 20. Anti-p85 or anti-phosphotyrosine antibody (1:4000 and 1:1000 dilutions, respectively) was added overnight at 4°C. Horseradish-peroxidase conjugated secondary antibodies were added for 60 min at room temperature. Proteins were detected and quantified by enhanced chemiluminescence with a Kodak Digital Science image station 440 cf (PerkinElmer).

Staining of Apoptotic Nuclei with Hoechst 33342. Cells were grown to 80% confluence, serum-starved overnight in DMEM supplemented with 0.1% calf serum, preincubated for 30 min with or without RV, and stimulated with Ang II (100 nM) for 24 h. Culture medium was replaced by PBS containing 1 μg/ml Hoechst 33342. After incubation at 37°C for 10 min cells were visualized by fluorescence microscopy (Axiovert 25; Carl Zeiss, Jena, Germany).

Measurement of LDH Release. Cells were grown in 60-mm dishes to 80% confluence, serum-starved overnight in 0.1% calf serum, and treated with RV in different concentrations or DMSO only for 30 min before stimulating with or without Ang II. After 24 h, lactate dehydrogenase (LDH) activity in the supernatant was assessed.

Results and Discussion

RV Inhibits Ang II-induced VSMC Hypertrophy. Increased vascular hypertrophy is a critical determinant of vascular disease. Ang II is a pivotal stimulus in this process, inducing protein synthesis but not DNA synthesis (Takahashi et al., 1997; Schmidt-Ott et al., 2000). To test whether RV is able to attenuate Ang II-mediated [^{3}H]leucine incorporation, cells were inactivated for 48 h and preincubated with different concentrations of RV or vehicle only for 30 min before stimulation with 100 nM Ang II, a concentration previously shown to be effective in VSMCs (Ushio-Fukai et al., 1998; Takahashi et al., 1999). After 24 h, hypertrophy was determined. As expected, Ang II strongly induced [^{3}H]leucine incorporation (~60%). However, in RV-pretreated cells, Ang II-mediated hypertrophy was markedly reduced (Fig. 1A). The inhibition observed was concentration-dependent and reached significance by RV 25 μM. At 50 μM RV, protein synthesis was reduced to almost basal levels (71% reduction of Ang II stimulated [^{3}H]leucine incorporation). RV (50 μM) also reduced basal (0.1% calf serum without Ang II) levels of [^{3}H]leucine incorporation (Fig. 1B). To the best of our knowledge, this is the first study to show a reduction in VSMC hypertrophy by RV.

RV Inhibits the Activation of the Survival Kinase Akt/PKB, the MAPK ERK 1/2, and p70^{S6K}. The exact signaling mechanisms leading to VSMC hypertrophy are only partially understood. However, it is known that phosphorylation and dephosphorylation of protein kinases play an important role in regulating overall protein synthesis (Servant et al., 1996). Of the protein kinases activated by Ang II in VSMCs, the MAPKs ERK1/2 and p38, the serine/threonine kinase Akt/PKB, as well as one of its downstream effector kinases, p70^{S6K} (Eguchi et al., 1999), have been shown to mediate Ang II-induced hypertrophy (Servant et al., 1996; Ushio-Fukai et al., 1998, 1999). In our system, both PD98059 (20 μM), an inhibitor of the ERK 1/2 kinase MEK 1/2 (Servant et al., 1996), as well as wortmannin (40 nM), an inhibitor of the Akt/PKB pathway at the level of the PI$_3$K), attenuated Ang-II-stimulated [^{3}H]leucine incorporation in VSMCs to an extent similar to that of RV 50 μM (Fig. 1A). Wortmannin also inhibited basal leucine incorporation (Fig. 1B).

To gain insight into the mechanism of action of RV, we thus examined whether RV affects these protein kinase signaling pathways. As demonstrated in Fig. 2, A and B, RV inhibits Ang II-induced Akt/PKB and p70^{S6K} phosphorylation in concentrations as low as 10 μM (although statistically not yet significant). Inhibition of Ang II-induced ERK 1/2 phosphorylation was less pronounced but still evident (35% inhibition by RV 50 μM) (Fig. 2C). Phosphorylation of p38 MAPK was not affected (Fig. 2D). It has been shown recently that both the ERK 1/2 and the Akt/PKB pathway contribute to p70^{S6K} activation in VSMCs (Eguchi et al., 1999). Interestingly, our results show that inhibition of the downstream kinase p70^{S6K} by RV is less potent than that of Akt/PKB. This is probably caused by signaling from the ERK 1/2 pathway to the p70^{S6K}, because RV reduced ERK 1/2 phosphorylation far less effectively than it did Akt/PKB phosphorylation. Presumably, inhibition of both Akt/PKB and, to a lesser extent, the ERK 1/2 pathway contribute to reduction of hypertrophy by RV; others have shown that inhibition of either pathway alone only partially inhibits hypertrophy (Servant et al., 1996).

To gain insight into whether RV inhibits Akt/PKB phosphorylation via inhibition of the upstream PI$_3$K pathway or by activation of phosphatases, we examined whether the phosphatase inhibitor okadaic acid is able to override the observed RV effect. Western blot analysis clearly shows that both RV (50 μM) and the PI$_3$K inhibitor wortmannin (50 nM) reduce Akt/PKB phosphorylation. This effect is not abrogated by okadaic acid (1 μM), although both effects are less pronounced (Fig. 3A). The slightly increased Akt/PKB phosphorylation in the presence of okadaic acid is most likely caused by okadaic acid itself, because okadaic acid alone enhances

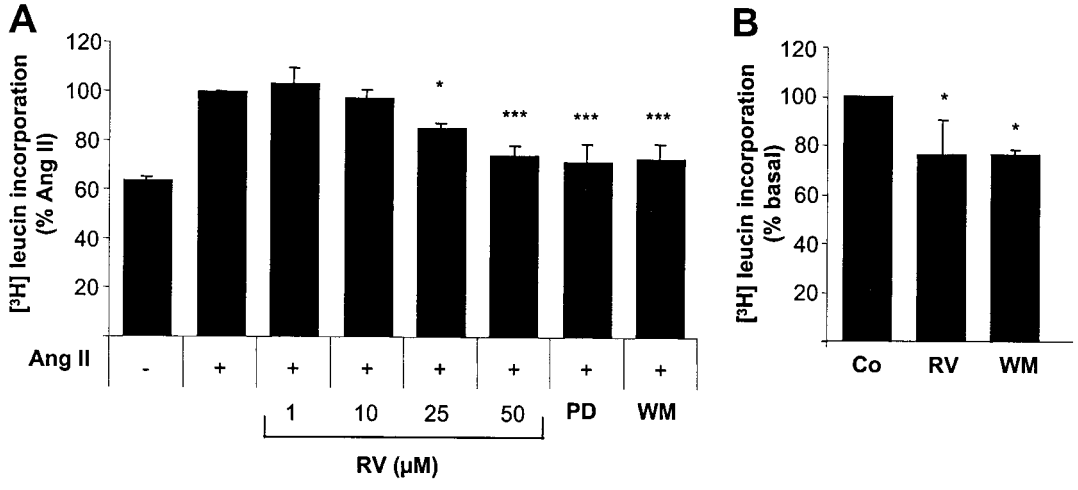

Fig. 1. Effect of RV on [^{3}H]leucine incorporation in rat aortic VSMCs. A, VSMCs in 0.1% calf serum were treated with RV in the indicated concentrations, PD98059 (PD, 20 μM) or wortmannin (WM, 40 nM), or vehicle only for 30 min and subsequently stimulated with (+) or without (−) 100 nM Ang II for 24 h. Data represent the percentage of [^{3}H]leucine incorporation after treatment with or without RV, PD or wortmannin, and Ang II compared with Ang II-stimulated cells only. B, VSMCs in 0.1% calf serum were treated with RV (50 μM), WM (40 nM), or vehicle only (Co) for 24 h. Data represent the percentage of [^{3}H]Leucine incorporation after treatment with or without RV or WM compared with vehicle only. [^{3}H]Leucine incorporation was assessed as described under *Materials and Methods*. Values are the mean ± S.E. of four independent experiments performed in triplicate. *, $P < 0.05$; ***, $P < 0.001$ (ANOVA/Dunnett).

phospho-Akt/PKB levels to a similar extent (Fig. 3B). Thus, RV seems not to act via phosphatases. This leaves the possibility that RV, similar to wortmannin, acts via the PI_3K signaling pathway. Class 1A PI_3K are heterodimeric proteins composed of an 85-kDa regulatory subunit that is tightly associated with a 110-kDa catalytic subunit. Activation occurs by translocation to the plasma membrane by targeting the SH2 domain of p85 to pYXXM motifs of receptor protein tyrosine kinases or their substrates (for review, see Wymann and Pirola, 1998). Saward and Zahradka (1997) have shown that Ang II stimulates tyrosine phosphorylation of the p85 subunit and, accordingly, PI_3K activity in porcine coronary artery VSMCs. As shown in Fig. 3C, tyrosine phosphorylation of the p85 subunit peaks at 5 min of Ang II stimulation in our VSMCs; this effect is dramatically reduced by 30 min of pretreatment with 50 μM RV (Fig. 3D), suggesting that RV indeed acts via the PI_3K signaling pathway.

Interestingly, no inhibition of p38 phosphorylation was detected (Fig. 2D). This is in contrast to recent findings that RV inhibits phosphorylation of p38 in endothelin-1-treated coronary artery smooth muscle (El Mowafy and White, 1999). This discrepancy most probably results from the different stimulus and the differences in the experimental settings.

RV is known to induce apoptosis in various cancer cells (Clement et al., 1998). However, no chromatin condensation or DNA fragmentation in response to Ang II (100 nM) and RV (1–50 μM) treatment could be detected after VSMC staining with Hoechst 33342 (data not shown). To verify that RV mediated effects on VSMCs are not a consequence of cytotoxicity, we determined LDH activity as a parameter of necrosis

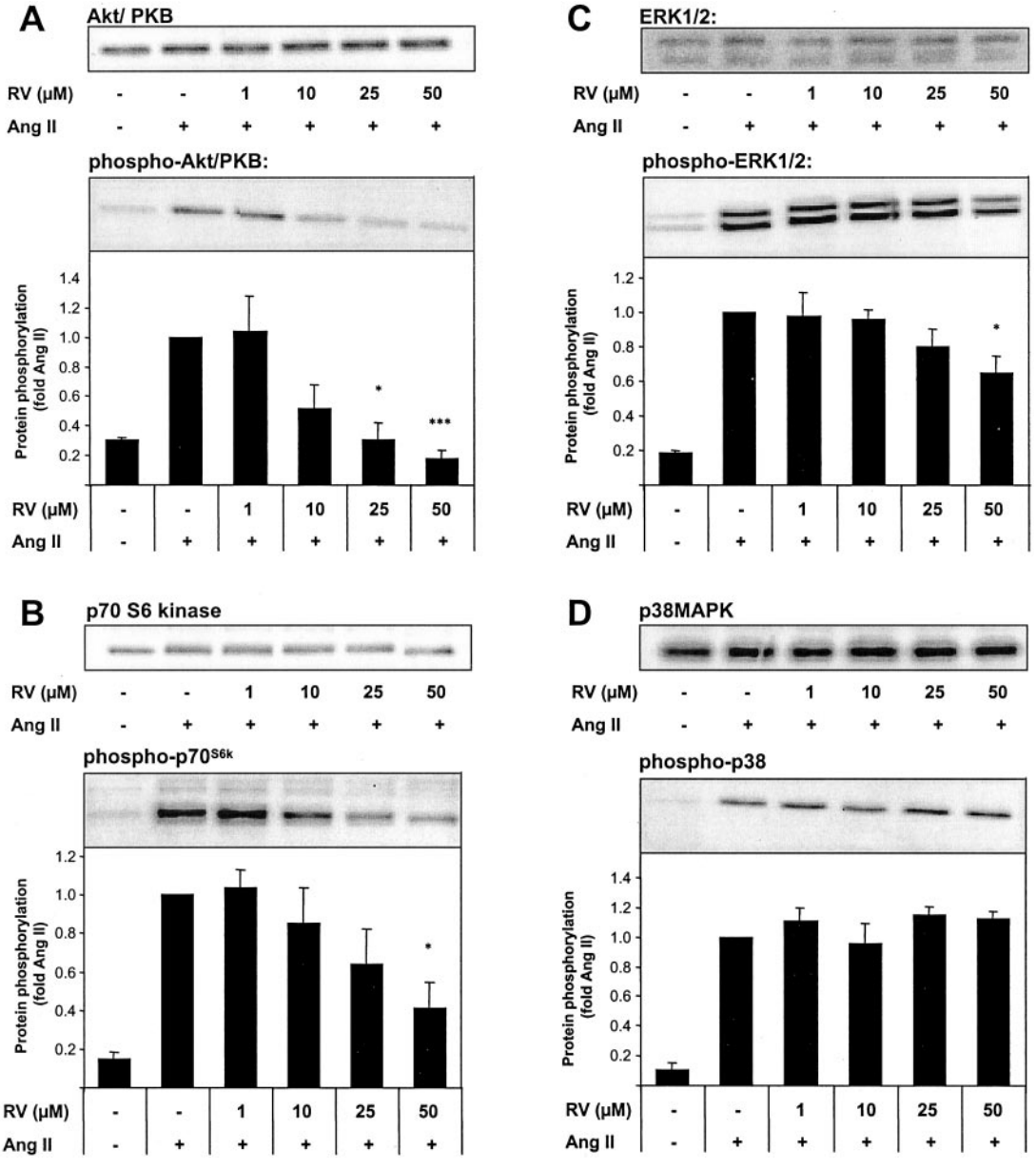

Fig. 2. Effect of RV on Ang II-induced phosphorylation of different protein kinases. A, Akt/PKB; B, $p70^{S6K}$; C, ERK 1/2; and D, p38. VSMCs in 0.1% calf serum were treated with RV in the indicated concentrations or vehicle only (−) for 30 min and subsequently stimulated with (+) or without (−) 100 nM Ang II for 10 min. Top, representative Western blots demonstrating expression of total protein kinases. Bottom, representative Western blots demonstrating the amount of phosphorylated kinases and the mean signal intensities obtained by densitometric analysis of three immunoblots. Values are mean ± S.E. of three independent experiments. *, $P < 0.05$; ***, $P < 0.001$ (ANOVA/Dunnett)

in supernatants of RV (1–50 μM) and Ang II-treated cells. Consistent with light microscopic observations, LDH activity in supernatants of treated and untreated cells remained unchanged (data not shown). The chemical mechanism by which RV inhibits hypertrophy-related signaling is unclear. In concordance with its polyphenolic structure, RV has been shown to act as an antioxidant (Frankel et al., 1993). Upon Ang II stimulation, reactive oxygen species are generated via a membrane bound NAD(P)H oxidase and act as second messengers within VSMCs (Zafari et al., 1998; Berk et al., 1989; Griendling et al., 2000; Griendling and Ushio-Fukai, 2000). In particular, it has been demonstrated that activation of p38 and Akt/PKB and, variably, ERK 1/2 are redox-sensitive (Ushio-Fukai et al., 1998, 1999; Frank et al., 2000) and that suppression of reactive oxygen species inhibits Ang II-induced hypertrophy (Ushio-Fukai et al., 1998; Zafari et al., 1998; Irani, 2000). One possible explanation for the antihypertrophic effect of RV may thus be its ability to act as an antioxidant. However, in our experiments, RV failed to inhibit phosphorylation of the redox-sensitive MAPK p38, suggesting that its antioxidant properties may not be its major inhibitory mechanism. Alternatively, RV may be antihypertrophic by virtue of its estrogen-like activity. RV is a stilbene-derivative and has been suggested to act as a phytoestrogen (Gehm et al., 1997). Indeed, VSMCs bind estrogen with high affinity and estrogen directly inhibits the migration and proliferation of smooth muscle cells in vitro (Mendelsohn and Karas, 1999). However, the effect of RV on ERK 1/2, Akt/PKB, and $p70^{S6K}$ phosphorylation could not be abrogated by pretreatment of cells with the estrogen receptor blocker ICI 182,780 (1 μM, 30 min preincubation, data not shown). Thus, further experiments will be necessary to identify the mechanisms by which RV exerts its antigrowth effects.

So far, the putative benefits of RV for cardiovascular dis-

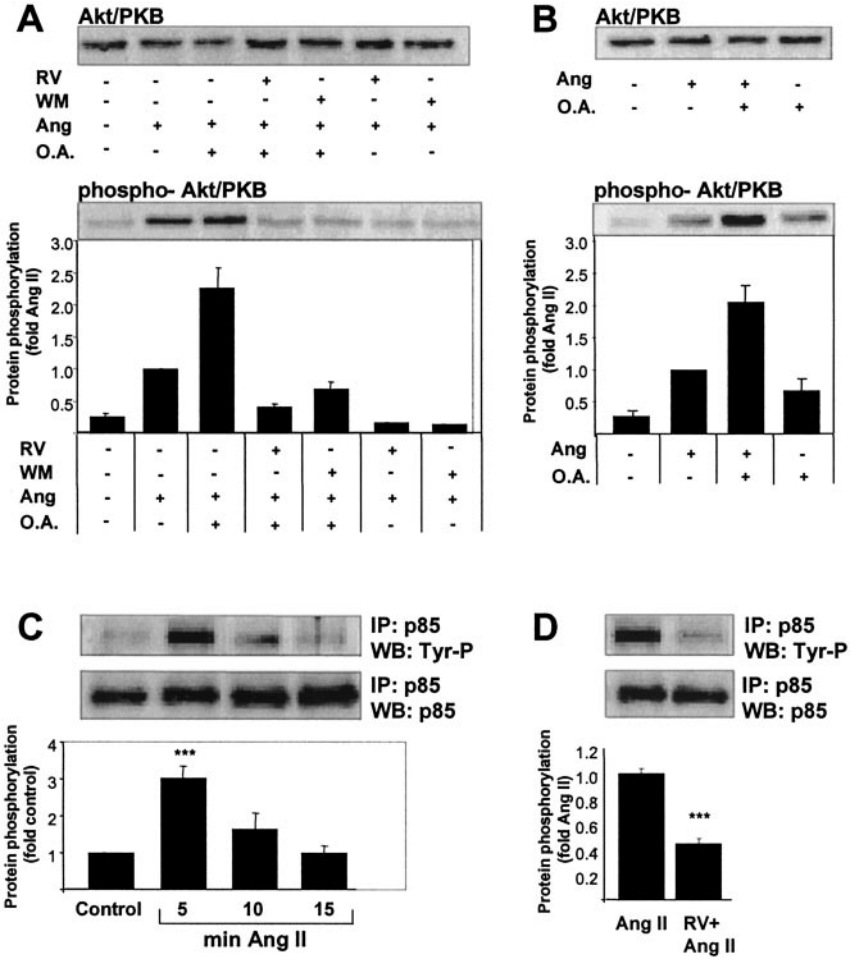

Fig. 3. RV does not activate phosphatases but inhibits tyrosine phosphorylation of the p85 PI_3K subunit. A, okadaic acid (O.A.) does not override the effects of RV and wortmannin on Akt phosphorylation. VSMCs in 0.1% calf serum were preincubated with RV (50 μM), wortmannin (WM, 50 nM), or vehicle only for 15 min, subsequently treated with (+) or without (−) O.A. (1 μM) for an additional 30 min before stimulation with (+) or without (−) Ang II (100 nM) for 10 min. B, okadaic acid itself enhances phospho-Akt/PKB levels. VSMCs in 0.1% calf serum were treated with or without okadaic acid (1 μM) for 30 min before stimulation with (+) or without (−) Ang II (100 nM). A and B, top, representative Western blots demonstrating expression of total Akt/PKB kinase. Bottom, representative Western blots demonstrating the amount of phosphorylated kinase and the mean signal intensities obtained by densitometric analysis of four immunoblots. C, assessment of the p85 PI_3K subunit tyrosine phosphorylation in response to Ang II (5–15 min). VSMCs in 0.1% calf serum were stimulated with Ang II for the indicated times (5–15 min). Cell lysates were prepared and immunoprecipitation (IP) was performed using an anti-p85 antibody. Immunoprecipitated protein was analyzed by Western blot (WB) using an anti-phosphotyrosine antibody (top) or, as control, an anti-p85 antibody (bottom). D, RV prevents Ang II-induced p85 phosphorylation. VSMCs in 0.1% calf serum were pretreated with RV (50 μM) or vehicle only for 30 min and then stimulated with Ang II for 5 min. Immunoprecipitation and Western blot analysis was performed as in C. C and D, top, representative Western blots. Bottom, mean signal intensities obtained by densitometric analysis of three immunoblots. Values are mean ± S.E. of at least three independent experiments. ***, $P < 0.001$ (C, ANOVA/Dunnett; D, unpaired two-tailed t test).

ease have been linked to its abilities to inhibit oxidation of human low-density lipoprotein (Frankel et al., 1993), to interfere with the cyclooxygenase and 5-lipoxygenase pathway, to inhibit platelet aggregation (Soleas et al., 1997), and to suppress cultured endothelial cell and VSMC proliferation, as reported more recently (Hsieh et al., 1999; Mizutani et al., 2000). Consistent with our results, effective doses of RV in most studies range between 10 and 100 μM, depending on the target studied. Unfortunately, little is known about RV bioavailability in vivo. However, concentrations in red wine vary between 0.4 and 60 μM (Wu et al., 2001), with concentrations up to 100 μM being reported (Pace-Asciak et al., 1995). Soleas et al. (1997) have shown that RV-enriched grape juice (17.5 μM) is absorbed from the intestine in amounts sufficient to attenuate platelet aggregation in healthy male volunteers (Pace-Asciak et al., 1996). In this context it is noteworthy that there is evidence that RV, when administered regularly to rats, accumulates in certain tissues (Soleas et al., 1997; Wu et al., 2001).

The present study delivers important new insights to the molecular mechanisms of action of RV in VSMC. Our results for the first time clearly show that RV remarkably influences important Ang-II activated pathways in VSMC. Moreover, they suggest that RV acts predominantly via the PI_3K/Akt pathway to reduce Ang II-mediated VSMC hypertrophy. These observations may have important implications for understanding the molecular basis for the therapeutic benefits of red wine consumption in vascular disease.

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