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European Journal of Pharmacology 442 (2002) 195–203

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Interleukin-1 inhibits angiotensin II-stimulated protein kinase B pathway in renal mesangial cells via the inducible nitric oxide synthase

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Received 3 December 2001; received in revised form 12 March 2002; accepted 19 March 2002

Abstract

Exposure of rat renal mesangial cells to angiotensin II and angiotensin III leads to a rapid phosphorylation and activation of the protein kinase B (PKB) pathway. The angiotensin II analogs angiotensin-(1–7), angiotensin-(1–6) and angiotensin-(3–8) were unable to activate PKB. The angiotensin II and III effects are mediated by the angiotensin type 1 receptor as documented by the inhibitory action of valsartan. Furthermore, angiotensin II-induced activation of PKB involves neither a pertussis toxin-sensitive pathway nor the small G proteins of the Rho/Rac/cdc42 family, but is completely blocked by inhibitors of the PI 3-kinase. Moreover, angiotensin II-stimulated PKB activation is inhibited by long-term pretreatment with interleukin-1β, an effect that is reversed by the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA). Similarly, the nitric oxide donor (*Z*)-1-[2-Aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (Deta-NO) blocks the angiotensin II-induced PKB activation. The NO-mediated inhibition of PKB activation in turn is reversed by the phosphatase inhibitor calyculin A but not by ocadaic acid, implying the induction of a protein phosphatase 1 activity by NO.

Keywords: Protein kinase B/Akt; Interleukin-1; Nitric oxide (NO); Angiotensin II; Mesangial cell

1. Introduction

Mesangial cells are a major determinant in the regulation of the glomerular filtration rate. Morphologically, they resemble smooth muscle cells, able to contract upon stimulation by vasoactive hormones like angiotensin II or arginine vasopressin (Pfeilschifter, 1989). In addition, mesangial cells are crucially involved in most pathological processes of the renal glomerulus (Pfeilschifter, 1989, 1994; Kashgarian and Sterzel, 1992).

Angiotensin II is involved in several physiological functions and regulates vascular tone, glomerular haemodynamics, tubular transport, chemoattraction of cells, processing of macromolecules, immunomodulation, angiogenesis and cell growth (Bottari et al., 1993; De Gasparo and Levens, 1994; Allen et al., 2000; Navar et al., 2000). Mechanistically, angiotensin II binds to specific surface receptors on mesangial cells (Pfeilschifter, 1990a), and either activates via a G-protein, a phospholipase C which hydrolyses phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and diacylglycerol, or inhibits the adenylate cyclase. By the development of specific peptidic and non-peptidic angiotensin II receptor antagonists several subtypes of angiotensin II receptors were defined, the AT_1, AT_2, AT_3 and AT_4 (Chiu et al., 1989; Whitebread et al., 1989; Smith and Timmermans, 1994; Wright et al., 1995; Unger et al., 1996). Most of the known effects of angiotensin II can be attributed to the AT_1 receptor. In rat mesangial cells the AT_1 type is well characterized (Ernsberger et al., 1992; Chansel et al., 1992; Wolf and Neilson, 1996), whereas the AT_2 type is only detectable when cell cultures are contaminated with mycoplasms (Whitebread et al., 1993). The AT_2 receptor signaling mechanisms are still largely unknown. However, it is evident that AT_2 receptors do not interact with G proteins (Bottari et al., 1991; Pucell et al., 1991) thus excluding all signaling cascades involving G proteins.

The serine–threonine-specific protein kinase B/Akt (PKB) was first identified as the human homolog of a transforming oncogene (Staal, 1987; Coffer and Woodgett, 1991), and has now gained a lot of interest because of its suggested function in cell survival signaling (Burgering

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01545-5

and Coffer, 1995; Chan et al., 1999; Scheid and Woodgett, 2001). Classical activators of PKB include growth factors, like platelet-derived growth factor or insulin-like growth factor (Franke et al., 1995; Andjelkovic et al., 1996; Alessi et al., 1996), which act through tyrosine kinase receptors and involve the small G protein $p21^{ras}$ and phosphatidylinositol 3-kinase (PI 3-kinase). The lipid products of PI 3-kinase enzymatic action like phosphatidylinositol [3,4,5]-trisphosphate (PIP_3) are important cofactors for PKB activation. Additionally, PKB requires phosphorylation on two sites for full activation, Thr^{308} in the activation loop, and Ser^{473} in a hydrophobic part at the C-terminus. The phosphoinositide-dependent kinase-1 (PDK-1) (Toker and Newton, 2000a; Casamayor et al., 1999) was identified as the responsible kinase for Thr^{308} phosphorylation whereas the kinase phosphorylating Ser^{473}, tentatively named PDK-2, has not yet been identified. Recent reports suggest that Ser^{473} is an autophosphorylation site (Toker and Newton, 2000b) or is phosphorylated by the integrin-linked kinase (Persad et al., 2001).

In this study, we report that angiotensins II and III activate the PKB pathway in rat renal mesangial cells via the AT_1 receptor and subsequent activation of the PI 3-kinase/PDK-1/PKB cascade. Furthermore, the proinflammatory cytokine interleukin-1β downregulates the angiotensin II effect on PKB by a mechanism involving induction of nitric oxide (NO) formation by the inducible NO synthase and expression of a protein phosphatase sensitive to calyculin A.

2. Materials and methods

2.1. Chemicals

Interleukin-1β and valsartan were kindly provided by Novartis Pharma (Basel, Switzerland); horseradish peroxidase-linked secondary antibodies and Hyperfilm were purchased from Amersham Pharmacia Biotech Europe (Freiburg, Germany); the PKBα/Akt1-specific antibody and the PDK-1 antibody were from Upstate Biotechnologies (Lake Placid, USA); the phospho-PKB and phospho-PDK-1 antibodies were from New England Biolabs (Frankfurt am Main, Germany); wortmannin, 2-(4-Morpholino)-8-phenyl-4*H*-1-benzopyran-4-one (LY 294002), pertussis toxin, *Clostridium difficile* toxin A, 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (U0126), ocadaic acid and calyculin A were obtained from Calbiochem-Novabiochem (Schwalbach, Germany); (*Z*)-1-[2-Aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (Deta-NO) and (*Z*)-1-[*N*-[3-aminopropyl]-*N*-[4-(3-aminopropylammonio)butyl]amino]-diazen-1-ium-1,2-diolate (spermine-NO) were from Alexis (Läufelfingen, Switzerland); (*N*-α-Nicotinoyl-Tyr-(*N*-α-CBZ-Arg)-Lys-His-Pro-Ile-OH) (CGP 42112A); *S*(+)-1-[[4-(Dimethylamino)-3-methylphenyl]-methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-6-carboxylic acid (PD 123319) and 8-bromo-cGMP were obtained from Sigma Aldrich (Deisenhofen, Germany); angiotensin II and all angiotensin II analogs were obtained from Bachem Biochemica (Heidelberg, Germany); all cell culture nutrients were from Life Technologies (Karlsruhe, Germany).

2.2. Cell culture

Rat renal mesangial cells were cultivated and characterized as previously described (Pfeilschifter, 1990b,c). In a second step, single cells were cloned by limited dilution on 96-microwell plates. Clones with apparent mesangial cell morphology were used for further processing. For the experiments in this study, passages 7–20 were used.

2.3. Cell stimulation and western blot analysis

Confluent mesangial cells in 60-mm-diameter dishes were stimulated in Dulbecco's modified Eagle medium including 0.1 mg/ml of fatty acid-free bovine serum albumin with the indicated substances. Thereafter the medium was withdrawn and the cells washed once with ice-cold phosphate-buffered saline solution. Cells were scraped into ice-

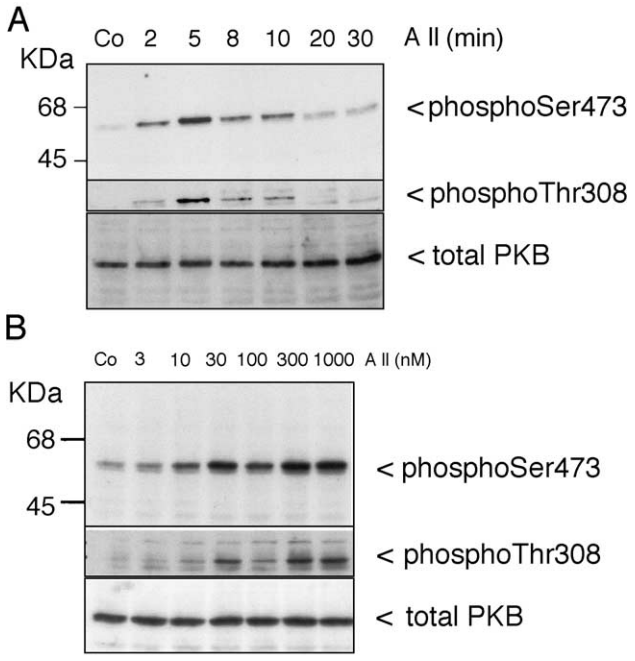

Fig. 1. Angiotensin II induces a time- and dose-dependent phosphorylation of PKB in renal mesangial cells. Quiescent mesangial cells were treated with either vehicle (Co) or angiotensin II (A II, 100 nM) for the indicated time periods (A), or for 5 min with the indicated concentrations (B). Thereafter cells were harvested and Western blot analyses were performed using specific phospho-Ser^{473}-PKB (upper panel), phospho-Thr^{308}-PKB (middle panel) and total PKBα (lower panel) antibodies at a dilution of 1:1000, 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data are representative of three independent experiments giving similar results.

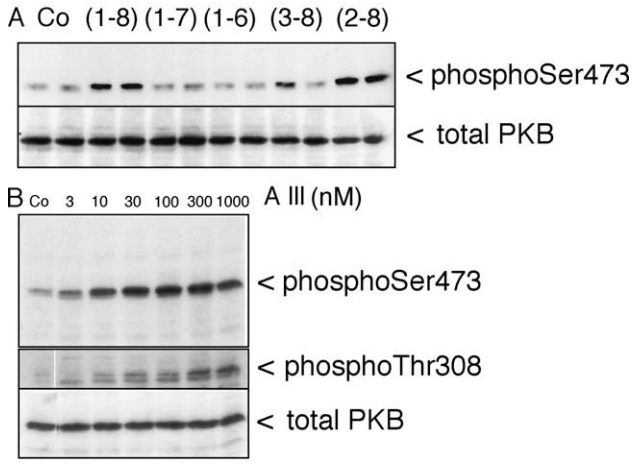

Fig. 2. Effect of angiotensin analogs on PKB phosphorylation in renal mesangial cells. (A) Quiescent mesangial cells were treated for 5 min with either vehicle (Co), angiotensin II-(1–8), angiotensin II-(1–7), angiotensin II-(1–6), angiotensin II-(3–8) or angiotensin III-(2–8) (each at 100 nM). (B) Cells were stimulated for 5 min with the indicated concentrations of angiotensin III (A III; in nM). Thereafter cells were harvested and Western blot analyses were performed using specific phospho-Ser^{473}-PKB (upper panel), phospho-Thr^{308}-PKB (middle panel) and total PKBα (lower panel) antibodies at a dilution of 1:1000, 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data show two out of three independent experiments giving similar results.

cold lysis buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X100, 2 mM EDTA, 2 mM EGTA, 40 mM β-glycerophosphate, 50 mM sodium fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 μM pepstatin A, 1 mM phenylmethyl sulphonyl fluoride) and homogenized by 10 passes through a 26-gauge needle fitted to a 1 ml syringe. Samples were centrifuged for 10 min at 14,000 × *g* and the supernatant was taken for protein determination. Cell extracts containing 70 μg of protein were subjected to sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred on to nitrocellulose paper for 1 h at 11 V using a semi-dry blotting apparatus. The blotting buffer used was 25 mM Tris, 190 mM glycine in 20% (v/v) methanol. After the transfer, immunostaining was performed as previously described in detail (Huwiler et al., 1995, 2000). Antibodies were diluted in blocking buffer as indicated in the legends of the figures. Bands were detected by the enhanced chemiluminescence method as recommended by the manufacturer.

2.4. Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA). For multiple comparisons with the same control group, the limit of significance was divided by the number of comparisons according to Bonferroni. One-way ANOVA with Bonferroni's post test was performed using GraphPad InStat version 3.00 for Windows NT, GraphPad Software (San Diego, CA, USA).

3. Results

3.1. Angiotensins II and III activate the PKB pathway via the angiotensin AT_1 type receptor

Stimulation of quiescent mesangial cells with 100 nM of angiotensin II elicits a rapid and time-dependent induction of PKB phosphorylation on Ser^{473} and Thr^{308}, which is well accepted to represent activation of the enzyme (Alessi et al., 1996). Phosphorylation is detected already after 2 min of stimulation, reaches maximal level after 5 min and thereafter declines but is still slightly elevated after 20 min (Fig. 1A).

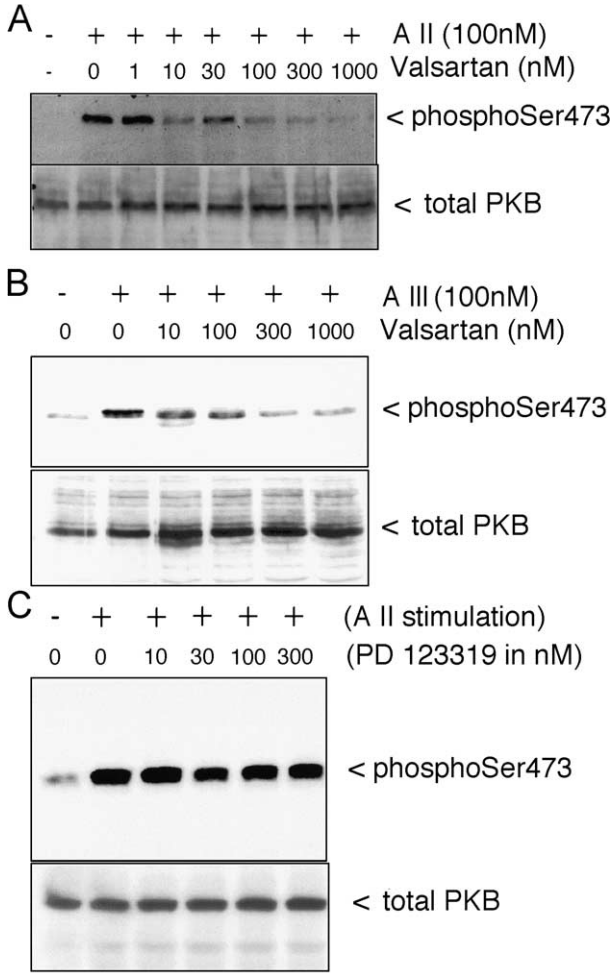

Fig. 3. Effect of the valsartan and PD 123319 on angiotensin II- and angiotensin III-induced PKB phosphorylation in renal mesangial cells. Quiescent mesangial cells were pretreated with the indicated concentrations of valsartan (in nM; A, B) or PD 123319 (in nM; C) for 20 min prior to stimulation with either vehicle (–), angiotensin II (A II; A, C) or angiotensin III (A III; B) (100 nM each) for 5 min. Thereafter, cells were harvested and Western blot analyses were performed using specific phospho-Ser^{473}-PKB (upper panel) and total PKBα (lower panel) antibodies at a dilution of 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data are representative of at least three independent experiments giving similar results.

The effect of angiotensin II occurs in a concentration-dependent manner which steadily increases up to maximal values at 300 nM after 5 min of stimulation (Fig. 1B). Furthermore, shorter fragments of the angiotensin II were tested for their ability to activate PKB. As seen in Fig. 2A neither angiotensin II-(1–7), angiotensin II-(1–6) nor angiotensin II-(3–8) (also known as angiotensin IV) have any significant stimulating effect on PKB phosphorylation, whereas angiotensin III, also know as angiotensin II-(2–8) activates PKB to a comparable extent as angiotensin II (Fig. 2A). The concentration dependency of angiotensin III-induced PKB phosphorylation is comparable to that of angiotensin II (Fig. 2B).

Pretreating cells for 20 min with increasing concentrations of valsartan, a selective AT_1 receptor antagonist (Criscione et al., 1993), blocks angiotensin II- (Fig. 3A) and also angiotensin III-induced PKB phosphorylation (Fig. 3B). In contrast, the AT_2 receptor antagonist PD 123319 has no inhibiting effect on angiotensin II- and III-induced response (Fig. 3C and data not shown). Moreover, the highly selective AT_2 receptor agonist CGP 42112A does not increase PKB phosphorylation nor does it affect the angiotensin II and III response (data not shown), thus clearly excluding the involvement of the angiotensin AT_2 receptor.

3.2. Angiotensin II stimulates PKB and PDK phosphorylation in a PI 3-kinase-dependent, but pertussis toxin- and toxin A-independent manner

To see whether angiotensin II also induces phosphorylation of the upstream kinase PDK-1, Western blot analyses were performed using a phospho-Ser^{241}-PDK-1 antibody. Ser^{241} is an autophosphorylation site in the activation loop of PDK-1 and is necessary for PDK-1 activity (Casamayor et al., 1999). As seen in Fig. 4, unstimulated quiescent mesangial cells already show a marked amount of phosphorylated PDK-1. Upon angiotensin II stimulation, a dose-dependent increase in phospho-Ser^{241}-PDK-1 is observed.

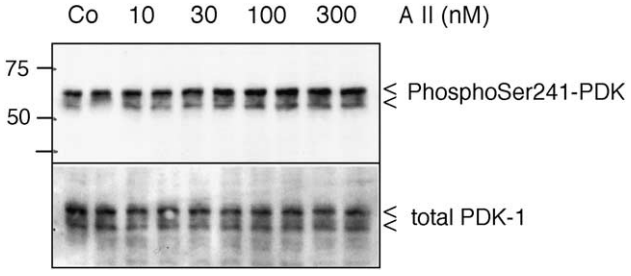

Fig. 4. Angiotensin II induces a dose-dependent phosphorylation of PDK-1 in mesangial cells. Quiescent mesangial cells were treated with either vehicle (Co) or the indicated concentrations of angiotensin II (A II; in nM) for 5 min. Thereafter cells were harvested and Western blot analyses were performed using a specific phospho-Ser^{241}-PDK-1 antibody (upper panel) or an antibody against total PDK-1 (lower panel) at a dilution of 1:1000 and 1:100, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data show two out of three independent experiments giving similar results.

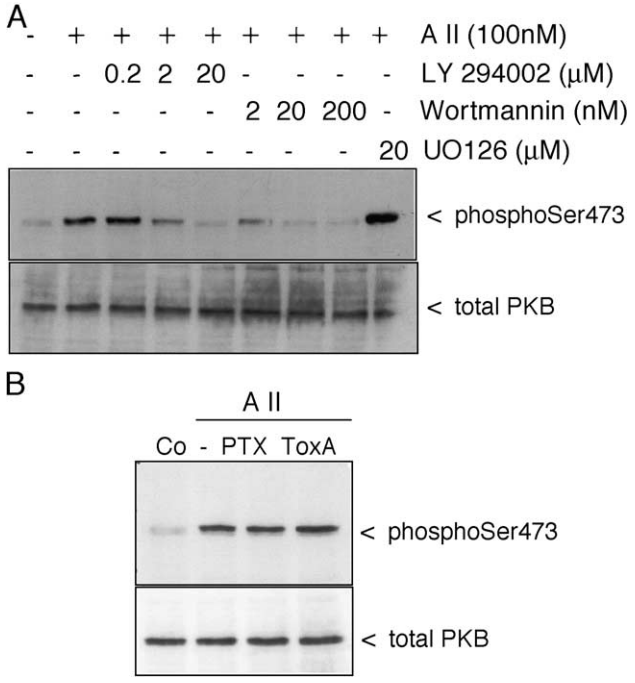

Fig. 5. Effect of PI 3-kinase inhibition and pertussis toxin on angiotensin II-induced PKB phosphorylation in renal mesangial cells. Quiescent mesangial cells were pretreated with the indicated concentrations of LY 294002 (A; in μM), wortmannin (A; in nM) and U0126 (A; 20 μM) for 20 min, or *C. difficile* toxin A (B; 20 ng/ml) and pertussis toxin (B; 100 ng/ml) for 24 h, prior to stimulation with either vehicle (co) or angiotensin II (100 nM) for 5 min. Thereafter cells were harvested and Western blot analysis was performed using specific phospho-Ser^{473}-PKB (upper panel) and total PKBα (lower panel) antibodies at a dilution of 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data are representative of at least three independent experiments giving similar results.

To trace the cascade one step further upstream, PI 3-kinase was investigated, as it is well accepted that PDK-1, but also PKB require the lipid products of PI 3-kinase, particularly PIP_3, for full activation. Evidence for the involvement of PI 3-kinase in angiotensin II-induced PKB activation is obtained by using two inhibitors of the PI 3-kinase, wortmannin (Okada et al., 1994; Powis et al., 1994) and LY 294002 (Vlahos et al., 1994), which dose-dependently block the angiotensin II-stimulated PKB activation. In contrast, the highly selective MAPK pathway inhibitor U0126 (Davies et al., 2000) has no inhibiting effect (Fig. 5A).

Furthermore, pertussis toxin, a potent inhibitor of G_i/G_o proteins (Hewlett et al., 1983), and *C. difficile* toxin A, an unselective inhibitor of the small G proteins Rho/Rac/Cdc42, were tested. However, neither pertussis toxin nor toxin *A* had any effect on angiotensin II-induced PKB activation (Fig. 5B).

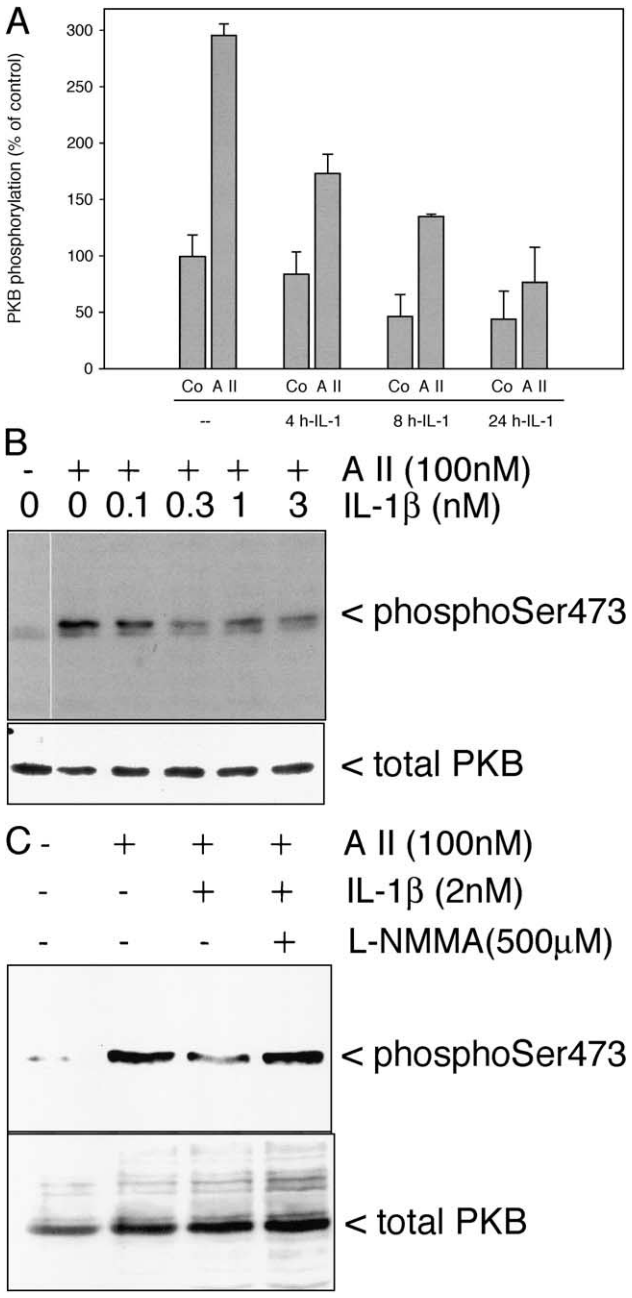

Fig. 6. Effect of interleukin-1β pretreatment on angiotensin II-induced PKB phosphorylation in mesangial cells. (A) Quiescent mesangial cells were pretreated with interleukin-1β (IL-1β, 2 nM) for the indicated time periods and then stimulated for 5 min with either vehicle (Co) or angiotensin II (A II; 100 nM). Results are expressed as % of non-pretreated vehicle-stimulated values and are means ± S.D. ($n = 3$). (B) Mesangial cells were pretreated for 24 h with the indicated concentrations of IL-1β (in nM) and then stimulated for 5 min with A II (100 nM). (C) Mesangial cells were pretreated for 24 h with IL-1β (2 nM) in the absence or presence of the NO synthase inhibitor N^G-monomethyl-arginine (L-NMMA; 500 μM) before stimulation for 5 min with A II (100 nM). Thereafter cells were harvested and Western blot analysis was performed using specific phospho-Ser^{473}-PKB and total PKB antibodies at a dilution of 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data are representative of at least three independent experiments giving similar results.

3.3. Interleukin-1 downregulates angiotensin II-induced PKB phosphorylation via induction of the inducible NO synthase

Incubation of mesangial cells for up to 24 h with the proinflammatory cytokine interleukin-1β prior to stimulation with angiotensin II leads to a time-dependent decrease of the angiotensin II-stimulated PKB phosphorylation (Fig. 6A). The phosphorylation of PKB constantly decreased between 3 and 24 h of interleukin-1β pretreatment resulting in a almost complete loss of angiotensin II-triggered phosphorylation. Fig. 6B shows a concentration-dependency of the interleukin-1β effect after 24 h of pretreatment. The inhibitory effect of interleukin-1β is completely reversed in the presence of the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA) (Fig. 6C). When exogenous NO was applied by using Deta-NO or spermine-NO, a similar reduction of angiotensin II-stimulated PKB phosphorylation is observed as obtained with interleukin-1β (Fig. 7A).

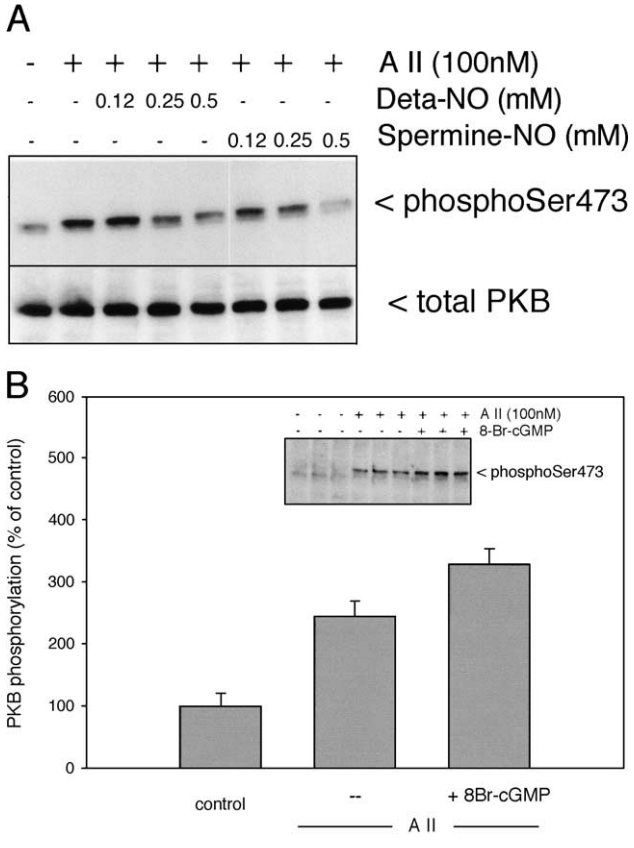

Fig. 7. Effect of nitric oxide and cGMP pretreatment on A II-induced PKB phosphorylation in mesangial cells. Quiescent mesangial cells were pretreated for 24 h with the indicated concentrations (in mM) of Deta-NO (A), spermine-NO (A) or 8-bromo-cGMP (B) and then stimulated for 5 min with A II (100 nM). Thereafter cells were harvested and Western blot analysis was performed using specific phospho-Ser^{473}-PKB (upper panel) and total PKB (lower panel) antibodies at a dilution of 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation. Data are representative of three independent experiments giving similar results.

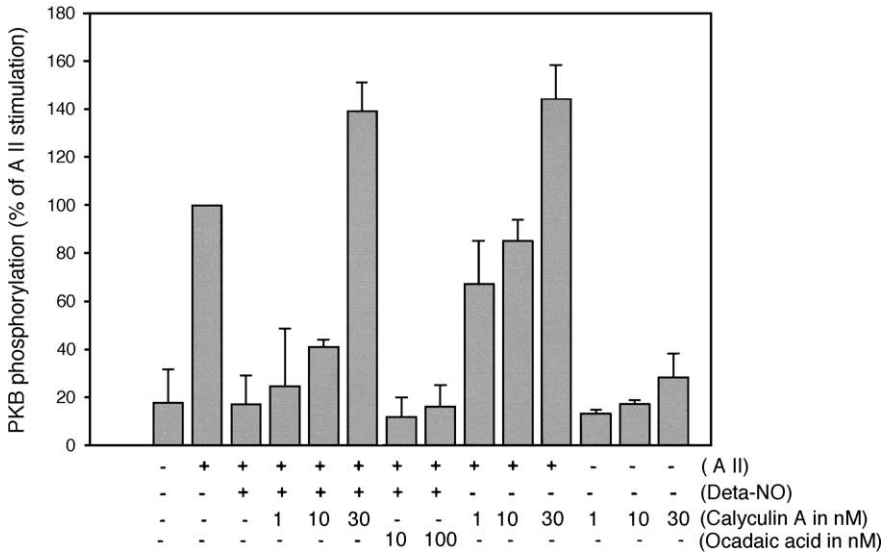

Fig. 8. Effect of calyculin A and ocadaic acid on NO-mediated inhibition of PKB phosphorylation in mesangial cells. Quiescent mesangial cells were pretreated for 24 h with Deta-NO (1 mM), then prestimulated for 20 min with the indicated concentrations of calyculin A (in nM) or ocadaic acid (in nM) before stimulation with A II (100 nM) for 5 min. Thereafter cells were harvested and Western blot analysis was performed using specific phospho-Ser^{473}-PKB and total PKB antibodies at a dilution of 1:1000 and 1:1600, respectively. Bands were detected by the ECL method according to the manufacturer's recommendation and densitometrically evaluated. Data are means ± S.D. of 3–4 independent experiments.

Again, the total protein amount of PKB does not change upon long-term NO pretreatment (Fig. 7A, lower panel). Furthermore, the mRNA level of PKBα is not affected by NO treatment (data not shown). Since NO exerts many of its effects by activating the guanylate cyclase and generating cGMP, we tested whether the NO effect is mimicked by the cell-permeable cGMP analog, 8-bromo-cGMP. As seen in Fig. 7B, pretreatment of cells for 24 h with 8-bromo-cGMP does not reduce angiotensin II-triggered PKB phosphorylation.

The inhibiting effect of NO pretreatment can be reversed in a dose-dependent manner by addition of the protein phosphatase inhibitor calyculin A (Fig. 8). However, calyculin A also increases angiotensin II-induced PKB phosphorylation, whereas in the absence of angiotensin II, calyculin A has no effect per se on PKB phosphorylation (Fig. 8). Ocadaic acid, at concentrations up to 30 nM, does not affect the NO-mediated inhibition of PKB phosphorylation.

4. Discussion

In this study we show that angiotensin II and angiotensin III, which lacks one N-terminal amino acid (angiotensin II-(2–8)), both activate the PKB cascade in mesangial cells via the angiotensin AT_1 receptor. This activation of PKB is completely PI 3-kinase-dependent, since the potent and selective PI 3-kinase inhibitors LY 294002 and wortmannin dose-dependently inhibited the angiotensin II response. At 20 μM of LY294002 and 20 nM of wortmannin a complete inhibition of PKB phosphorylation is seen (Fig. 5A). Recently, Gorin et al. (2001) reported that angiotensin II activates the PKB pathway independent of the PI 3-kinase in mesangial cells. These observations are in contrast to our present finding. The reason for this discrepancy is presently unknown. Gorin et al. used myelin basic protein phosphorylation of PKB-immunoprecipitated samples as a criterion for PKB activation, which was not inhibited by wortmannin or LY294002. In view of the fact that myelin basic protein is neither a selective nor a preferred substrate of PKB it is more likely that other kinases, which coimmunoprecipitated with PKB, are responsible for myelin basic protein phosphorylation in a PI 3-kinase inhibitor-insensitive manner.

That the activation of PKB by angiotensin II can indeed occur in a PI 3-kinase-dependent manner was also observed in smooth muscle cells (Ushio-Fukai et al., 1999; Takahashi et al., 1999). Mechanistically, it is clear that especially the γ-isoform of PI 3-kinase is activated by βγ subunits of G proteins (Stoyanov et al., 1995; Bommakanti et al., 2000; Hirsch et al., 2000), making angiotensin II a candidate activator of this PI 3-kinase isoform and of a subsequent activation of the PKB pathway. Indeed, we have shown elsewhere that rat mesangial cells do express the γ-isoform of PI 3-kinase (Huwiler et al., 2002).

Our present data further demonstrate the involvement of a pertussis toxin-insensitive pathway in angiotensin II signaling to PKB, which confirms data obtained from smooth muscle cells (Takahashi et al., 1999). Earlier studies in mesangial cells have shown that angiotensin II-induced inositol trisphosphate formation and prostaglan-

din synthesis requires a pertussis toxin-sensitive G protein (Pfeilschifter and Bauer, 1986). However, coupling of angiotensin II to Gq and G11 proteins has subsequently been documented as the main pathway leading to phospholipase C activation (Langlois et al., 1994). Furthermore, we could show that small G protein of the Rho/Rac/Cdc42 family are not involved in angiotensin II-mediated PKB activation, since toxin A from *C. difficile* has no inhibitory effect.

Most interestingly, the angiotensin II-induced PKB activation is downregulated by pretreatment with the proinflammatory cytokine interleukin-1β which involves the induction of the inducible NO synthase. This is corroborated by the following findings: (i) addition of an exogenous NO donor mimics the interleukin-1β effect on angiotensin II-induced PKB phosphorylation, and (ii) the interleukin-1β effect is completely reversed in the presence of the NO synthase inhibitor L-NMMA (Fig. 6B). Earlier studies in mesangial cells have revealed that interleukin-1β can induce the expression of the inducible NO synthase, but no other NO synthase isoform, in mesangial cells (Pfeilschifter and Schwarzenbach, 1990; Pfeilschifter et al., 1992; Kunz et al., 1994). The NO effect occurs independently of cGMP generation, since the cell permeable cGMP analog, 8-bromo-cGMP, could not mimic the inhibitory effect of NO. This is in line with many other reports which have shown that in mesangial cells most NO effects are not mediated by cGMP (for review, see: Pfeilschifter et al., 2001).

The NO-mediated inhibition of PKB most probably involves the upregulation of a phosphatase activity, since calyculin A, a potent inhibitor of protein phosphatase types 1 and 2A (IC_{50} of 0.4 and 2 nM, respectively (Ishihara et al., 1989) can reverse the inhibitory action of NO. Indeed, NO has been reported to stimulate a membrane-associated protein tyrosine phosphatase activity in T cells (Lander et al., 1993) and in rat aortic smooth muscle cells (Kaur et al., 1998). In addition, it was reported that NO can upregulate the mitogen-activated protein kinase phosphatase-1 expression in human embryonic lung fibroblasts (Marquis and Demple, 1998). However, NO has also been reported to inhibit tyrosine phosphatase activities in mesangial cells (Callsen et al., 1998, 1999). Despite these apparently discrepant findings it is obvious that protein phosphatases are targets of NO and also reactive oxygen species and may sense the cellular redox state (for review, see Pfeilschifter et al., 2001).

In summary, we have shown that angiotensin II and angiotensin III trigger activation of the PKB pathway which has important signaling functions in cell protection. Moreover, we report that exogenous NO as well as endogenous NO production after cytokine-induced inducible NO synthase expression operates in a delayed feedback mechanism to attenuate PKB signaling. Further work is required to identify the NO-induced calyculin A-sensitive phosphatase, which dephosphorylates and thus inactivates the PKB.

Acknowledgements

We thank Mrs. Simone Dorsch for her excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (HU 842/2-2, PF 361/1-1 and SFB 553), the August-Scheidel Stiftung and the VERUM Stiftung für Umwelt und Verhalten.

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