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Activation of Phospholipase Cγ_1 Protects Renal Arteriolar VSMCs from H_2O_2-Induced Cell Death

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Key Words
Apoptosis · Microcirculation · Oxidative stress · Phospholipases · Vascular smooth muscle cells

Abstract
Background: We evaluated the effect of hydrogen peroxide (H_2O_2) on viability of vascular smooth muscle cells (VSMCs) of renal resistance arterioles and determined whether responses are modulated by activation of PLCγ_1. ***Methods:*** Phospholipase C (PLC)-isozyme protein levels and activity were measured using Western blot analysis and enzymatic production of phosphoinositol 1,4,5-trisphosphate (IP_3), respectively. Stimulation of PLCγ_1 was assessed by immunoblots of tyrosine phosphorylation. ***Results:*** Cytotoxicity of H_2O_2 exposure was concentration-dependent (30% death with 250 μM; 87% with 500 μM at 8 h) and time-dependent (7% at 1 h; 30% at 8 h with 250 μM H_2O_2). Catalase abolished such relations. H_2O_2 increased PLCγ_1 expression more than that of PLCδ_1 and almost doubled total PLC enzymatic activity between 2 and 8 h, changes prevented by catalase. The PLC inhibitor U73112 (3 μM) enhanced the cytotoxic concentration and time effects of H_2O_2. In acute studies, H_2O_2 rapidly caused tyrosine phosphorylation of PLCγ_1. ***Conclusion:*** H_2O_2 increased PLCγ_1 expression and almost doubled total PLC activity, changes abolished by catalase. We conclude that H_2O_2 is cytotoxic to cultured VSMCs of renal preglomerular arterioles, a process that is attenuated by compensatory increases in PLCγ_1 protein level, tyrosine phosphorylation of PLCγ_1 and PLC enzymatic activity to generate IP_3.

Introduction

Recent evidence indicates a role for reactive oxygen species (ROS) as intracellular signaling molecules controlling vascular structure and function. The physiological activity of ROS such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) is normally controlled by the ability of superoxide dismutase (SOD) and catalase to convert potentially damaging free radicals into innocuous molecules [1–4]. Nitric oxide is another important scavenger of superoxide anion. During vascular dysfunction and remodeling associated with hypertension, diabetes, and inflammation, this balance is disrupted due to an increased production of ROS and/or reduced degradation. Such oxidative stress may be causative and play a key role in cardiovascular and renal diseases [3–6].

ROS and the intracellular redox status are involved in mechanisms of signal transduction that mediate various cell functions. For example, increased production of O_2^- interrupts nitric oxide-mediated vasorelaxation by forming $ONOO^-$, as well as having direct effects on endothe-

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lial cells and vascular smooth muscle cells (VSMCs) [2, 6]. ROS can mediate phenotypical changes in the vasculature that may be considered both physiological and pathophysiological, including growth, growth arrest, survival and apoptosis [7]. In this regard, ROS is known to affect cell proliferation of VSMCs of large diameter arteries and aorta and other cell types [1, 2, 4, 6, 8]. Differences in remodeling have been noted between the vasculature and the heart [9]. The number of viable, proliferating cells usually decreases as the number of apoptotic cells increases. Various ROS may dictate the fate of life or death. ROS may directly or indirectly modulate functions of major enzyme cascades (e.g., PLCγ_1-PKC, mitogen-activated protein (MAP) kinases), transcription factors, and caspases involved in vascular remodeling and damage [3, 6, 10, 11]. Responses are determined by their intracellular target(s) in different cell types, with mechanisms involving combinations of vasoactive agents, growth factor receptors and oxidation of signal transduction molecules.

Ang II, and perhaps H_2O_2 in some situations, favors hypertrophy/hyperplasia, in part mediated by transactivation of EGF and PDGF receptors and downstream signaling involving PLCγ_1. Such proliferative effects are antagonized by NAD(P)H oxidase inhibition, catalase and other antioxidants [4, 12]. Overexpression of catalase reduces intracellular H_2O_2 and inhibits the ability of Ang II to stimulate hypertrophy of cardiac myocytes and aortic VSMCs [4, 8]. Within the vasculature, certain arteries tend to respond more strongly with proliferation than others [13]. VSMCs from media and intima may differ with regard to phenotype and their rates of proliferation and apoptosis [5]. Interestingly, animal studies reveal a catalase effect on aortic wall thickness but not on arterial blood pressure in Ang II-induced hypertension, suggesting a major effect on large diameter conduit arteries with minimal impact on physiologically important small-diameter arteries/arterioles contributing to peripheral vascular resistance. One possible explanation for this disassociation is that H_2O_2 plays a more significant role in hypertrophy of VSMCs of aorta than in small-diameter terminal resistance arterioles. It should be appreciated that the majority of in vitro studies to date have been conducted on cultured VSMCs of aorta, a large diameter conduit vessel, significantly more than on VSMC of resistance arterioles [4, 14]. The mechanisms of vascular remodeling in hypertension appear to vary with vessel function, differing qualitatively between large conduit arteries and small resistance arterioles, in particular renal afferent arterioles [15]. Little is known about the proliferative and viability effects of oxidative stress on VSMCs of small-diameter resistance arterioles in the microcirculation.

To this end, we sought to evaluate the effect of H_2O_2 on VSMCs viability and on PLC activity linked to cytosolic Ca^{2+} metabolism in VSMCs isolated from renal resistance arteries/arterioles, vascular segments important in the regulation of glomerular capillary pressure, glomerular filtration rate, sodium excretion and renin release. We postulated that H_2O_2 activates PLCγ_1, increases enzymatic activity to increase IP_3 production, and increases the expression PLCγ_1, all of which collectively tend to promote VSMCs viability and thereby counteract injury induced by H_2O_2. Our results provide new information demonstrating that: (a) H_2O_2 is cytotoxic to preglomerular arteriolar VSMCs in a concentration- and time-dependent manner. (b) H_2O_2 stimulates tyrosine phosphorylation of PLCγ_1, increases PLC enzymatic activity, and upregulates the expression of PLCγ_1, in these VSMCs of preglomerular resistance arterioles. (c) Such H_2O_2 effects are completely blocked by catalase, an enzymatic antioxidant that scavenges H_2O_2. (d) The effects of H_2O_2 are enhanced by the inhibitor of PLC activity U73122.

Methods

Experiments were performed with 6-week-old Sprague-Dawley rats (Taconic Farm, Germantown, N.Y., USA) in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publ. No. 85-23, revised 1996). Preglomerular arteriolar VSMCs were isolated and cultured using the techniques that are standard for our laboratory [16–18].

Post-confluent monolayers of preglomerular arteriolar VSMCs (passage 3–7) were tested at 48 h after being growth-arrested in medium free of fetal calf serum. The cells were bathed in HEPES-balanced salt solution (HBSS) containing (mM) 20 HEPES, 130 NaCl, 5 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 5 $NaHCO_3$, 5.6 D(+)-glucose, pH 7.4. After a 30-min stabilization period, the cells were challenged with H_2O_2 using different concentrations (125–1,000 μM) and times of exposure (1–8 h for experiments of PLC isozyme protein expression, ≤30 min for experiments of PLCγ_1 tyrosine phosphorylation). This range of H_2O_2 concentrations was selected based on published studies' results [19, 20]. Vascular H_2O_2 concentrations in the μM range have been reported for pathological conditions such as ischemia-reperfusion injury and vascular damage associated with hypertension and atherosclerosis [2].

Protein concentration was determined with BCA Protein Assay Kit (Pierce Biotechnology, Rockford, Ill., USA) following the manufacturer's protocol. Western blot analyses were completed as described previously using the same experimental conditions and reagents including primary and secondary antibodies to test PLC isozyme protein expression [17, 18] and PLCγ_1 tyrosine

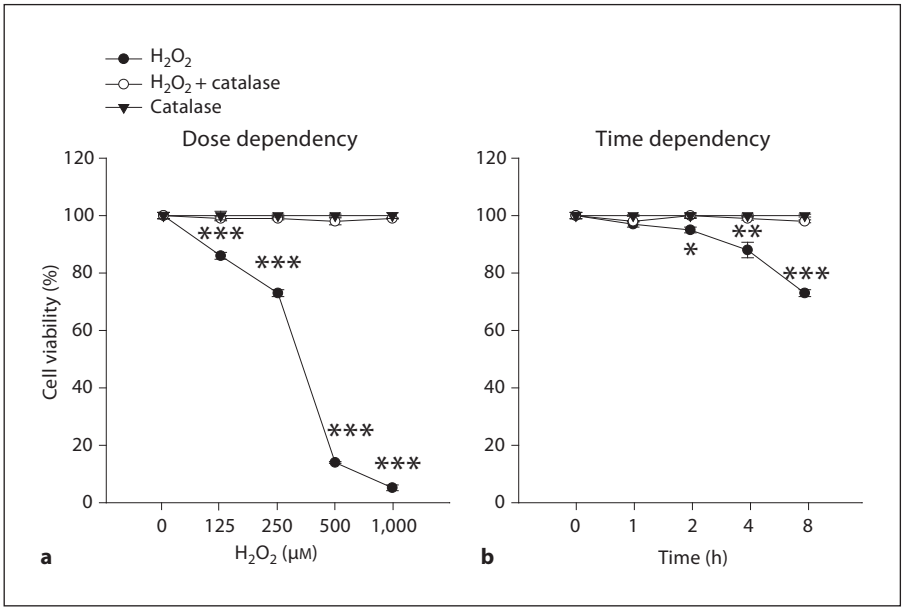

Fig. 1. Cell viability in response to H_2O_2 in the absence or presence of catalase in rat renal VSMCs. **a** Effects of different concentrations of H_2O_2 (125–1,000 μM) for 8 h. **b** Effects of different times of exposure to H_2O_2 (250 μM) for 1–8 h. Means ± SEM ($n \geq 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

phosphorylation using an antiphosphotyrosine monoclonal antibody (4G10) [20].

PLC enzymatic activity was determined after treating monolayers of VSMCs for 30 min with 250 μM H_2O_2 in the absence and presence of catalase (20 IU/ml, 10 min pretreatment). PLC activity was measured by a method based on the hydrolysis of [^{3}H]-phosphatidylinositol 4,5-bisphosphate (PIP_2) to IP_3 and DAG using whole cell lysates [21].

Cell viability was determined using the conventional trypan blue exclusion method [20]. Following treatment, cells were harvested and stained with trypan blue solution (0.4%, Sigma, St. Louis, Mo., USA) for 10 min at room temperature, and the percentage of non-stained live cells were counted using a hemocytometer and light microscopy.

Expression of a PLC isozyme is presented as the ratio of band intensity of the particular PLC isozyme to that of β-actin. Phosphorylation of PLCγ$_1$ is given as the ratio of band intensity of tyrosine phosphorylation to that of total PLCγ$_1$. Results are means ± SEM of at least three independent experiments. Data evaluation was performed by one-way ANOVA. $p < 0.05$ was considered statistically significant.

Results

Influence of H_2O_2 on Viability of Preglomerular Arteriolar VSMCs

H_2O_2 had concentration- and time-dependent effects to reduce viability of preglomerular arteriolar VSMCs (fig. 1). The cytotoxic actions of H_2O_2 at 8 h varied with concentration (30% death with 250 μM and 87% with 500 μM H_2O_2) and time, ranging from 7% at 1 h to 30% at 8 h when both groups were treated with 250 μM H_2O_2. Both the concentration- and time-dependent deleterious effects were completely blocked by catalase (20 IU, 10 min pretreatment) inactivation of H_2O_2 (fig. 1), indicating a causal relation. In the absence of H_2O_2, catalase was without effect in time control studies, suggesting the basal level of endogenous H_2O_2 in these quiescent VSMCs is low and does not affect viability. To our knowledge, these findings are novel for VSMCs of resistance arterioles.

In contrast, inhibition of PLC activity with U73122 amplified cytotoxicity of H_2O_2. Survival of renal VSMC was significantly reduced as a result of U73122 pretreatment (1 and 3 μM, 30 min) prior to H_2O_2 incubation (fig. 2). U73112 (3 μM) enhanced the deleterious action of H_2O_2 at 8 h to 82 and 100% death with 250 and 500 μM H_2O_2, respectively. A time-dependent response to U73122 enhancement of H_2O_2 (250 μM)-induced cell death was also evident: 48% death at 1 h and 82% at 8 h. By itself, neither 1 nor 3 μM U73122 affected viability of VSMCs over the 8-hour observation period during control conditions (no H_2O_2).

H_2O_2 Induces Phosphorylation of PLCγ$_1$

Western blot analyses show that stimulation with H_2O_2 induces tyrosine phosphorylation of PLCγ$_1$ in cultured VSMCs of renal resistance arterioles (fig. 3). The effects were dose-dependent. More than 50% stimulation was observed at 3.75 min, with maximum stimulation sustained between 7.5 and 30 min.

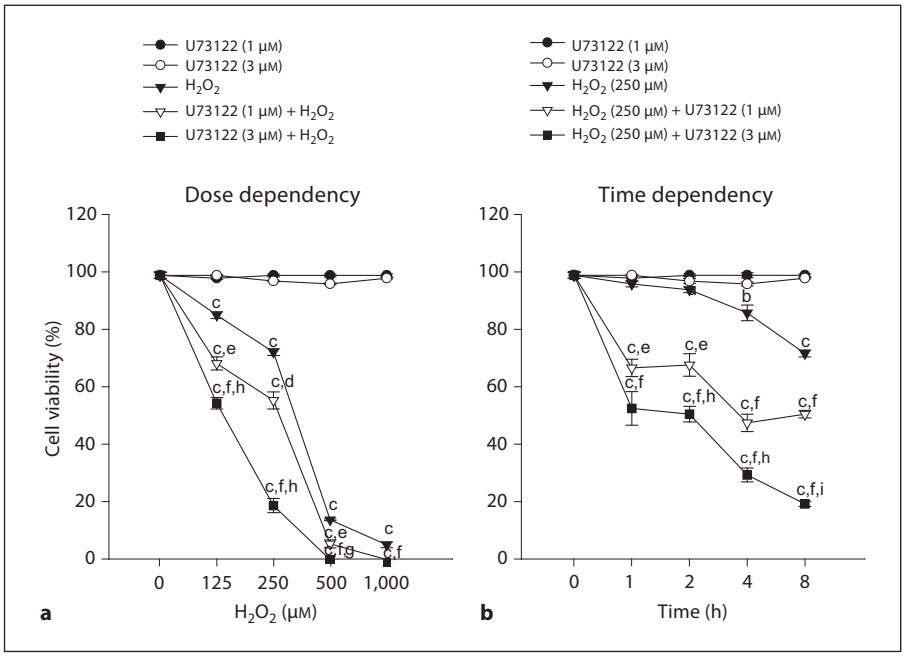

Fig. 2. Pretreatment with the inhibitor of PLC (U73122) for 30 min increases the cytotoxic effect of H_2O_2 in a concentration- and time-dependent manner. **a** Effects of different concentrations of H_2O_2 (125–1,000 μM) for 8 h. **b** Effects of different times of exposure to H_2O_2 (250 μM) for 1–8 h. Means ± SEM (n ≥3). [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs. control. [d] $p < 0.05$, [e] $p < 0.01$, [f] $p < 0.001$ vs. H_2O_2. [g] $p < 0.05$, [h] $p < 0.01$, [i] $p < 0.001$ vs. U73122 (1 μM).

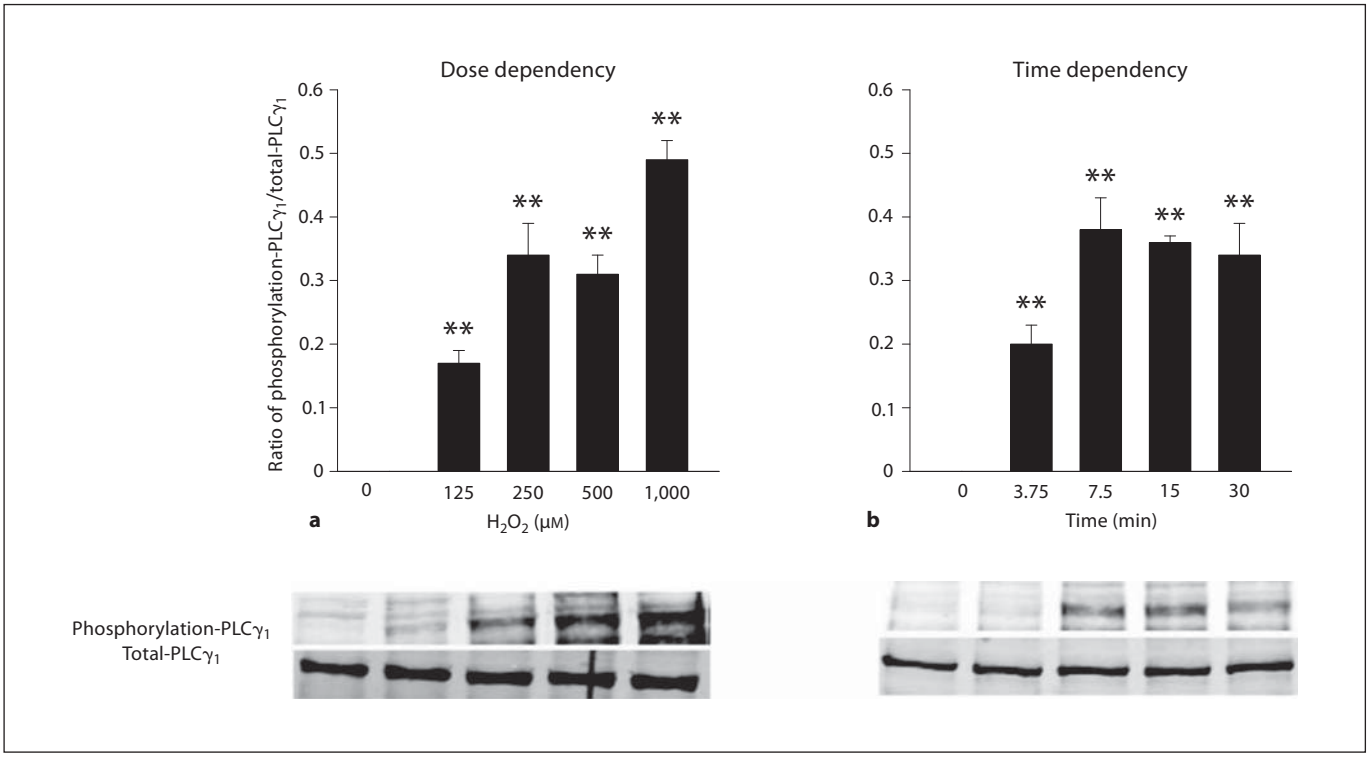

Fig. 3. Western blot analysis indicating that H_2O_2 induces tyrosine phosphorylation of PLCγ1 in cultured VSMCs of renal resistance arterioles. **a** Effect of different concentrations of H_2O_2 (125–1,000 μM) exposed for 30 min. **b** Time-dependent effect of duration of exposure to H_2O_2 (250 μM) for 3.75–30 min. Means ± SEM (n ≥3). ** $p < 0.01$ vs. control (0 μM or 0 min).

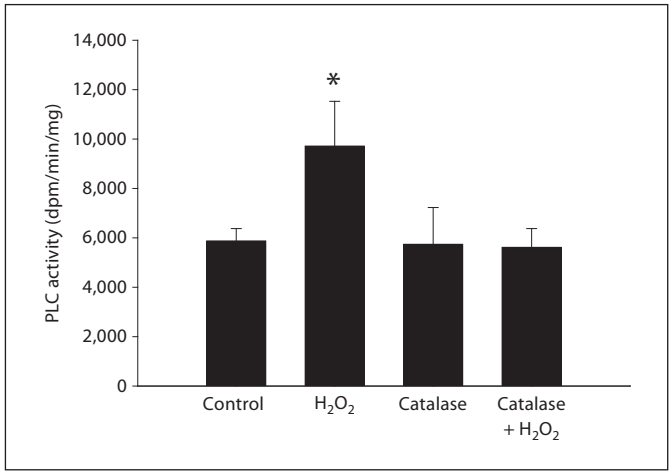

Fig. 4. Catalase (20 IU/ml) pretreatment (10 min) has no effect on PLC activity in the absence of H_2O_2, but completely or markedly inhibits H_2O_2 (250 μM, 30 min)-induced increased activity of PLC in cultured VSMCs of renal resistance arterioles. Means ± SEM ($n \geq 3$). * $p < 0.05$ vs. control.

H_2O_2 Enhances PLC Activity in Preglomerular Arteriolar VSMCs

We next assessed whether H_2O_2 affects PLC enzymatic activity in preglomerular arteriolar VSMCs. The results show that H_2O_2 treatment (250 μM for 30 min) markedly increased PLC activity measured as IP_3 production, almost twofold on the average (fig. 4). Moreover, the increase was effectively abolished by pretreatment with catalase. In the absence of H_2O_2, pretreatment with catalase had no effect on basal PLC activity.

H_2O_2 Upregulates the Expression of PLCγ_1

Western blot analyses indicate that PLCγ_1 and PLCδ_1 are present in these cultured renal arteriolar VSMCs. Moreover, the protein level of PLCγ_1 was enhanced 50–100% in response to H_2O_2 treatment of 250–500 μM for 8 h (fig. 5a) as well as time-dependent stimulation by 250 μM H_2O_2 between 2 and 8 h (fig. 5b). In contrast, similar concentrations of H_2O_2 had no significant effect on expression of PLCδ_1. Exposure to 1,000 μM H_2O_2 for 8 h decreased the protein level of PLCδ_1. It is noteworthy that PLCβ_1 was below detection levels during either control or experimental conditions (data not shown). The same antibody recognizes PLCβ_1 in freshly isolated preglomerular arterioles [17], so it seems to disappear during our culture conditions.

Other experiments examined the ability of catalase to prevent H_2O_2 stimulation of PLCγ_1. The data in figure 6 show the selective twofold stimulation of PLCγ_1 vs. PLCδ_1 protein expression in response to challenge with H_2O_2 (250 μM for 8 h). The increase was completely inhibited by catalase. Under control conditions in the absence of H_2O_2 exposure, catalase had no effect of protein levels of either PLCγ_1 or PLCδ_1, indicating specificity of action against H_2O_2 effects.

Discussion

The present study demonstrates for the first time in VSMCs of renal preglomerular arterioles that: (a) Oxidative stress induced by H_2O_2 is cytotoxic, reducing cell viability in a concentration- and time-dependent fashion. (b) H_2O_2 induces tyrosine phosphorylation of PLCγ_1, increases total PLC enzymatic activity, and upregulates expression of PLCγ_1 in the absence of changes in protein levels of PLCβ_1 or PLCδ_1. (c) Importantly, both the concentration- and time-dependent cytotoxic effects of H_2O_2 are completely blocked by catalase degradation of H_2O_2. (d) In contrast, U73122 enhances the cytotoxic effect of H_2O_2. Collectively, these data indicate that H_2O_2 activates PLCγ_1 with resultant increases in IP_3 production in combination with increased PLCγ_1 protein, changes abolished by catalase inactivation of H_2O_2. The cytotoxic effect of high concentration of H_2O_2 is attenuated by compensatory increases in PLCγ_1 protein expression and activity. An increase in enzymatic activity appears to precede increased protein expression as evidenced by rapid tyrosine phosphorylation of PLCγ_1 and its ability to generate IP_3. Thus, PLCγ_1 has a pro-survival function in the response of VSMCs of renal arterioles to acute H_2O_2-induced oxidative stress.

As pointed out in a recent review, ROS can have distinct functional effects on each cell type in the vasculature (endothelial cells, VSMCs, and adventitial fibroblasts) which include a wide spectrum of growth, apoptosis, migration, inflammatory gene expression, and matrix regulation [4]. Moreover, cellular actions may vary according to particular responsible reactive oxygen molecule, vessel type and location along the vascular tree (e.g., conduit artery vs. resistance arteriole) in addition to local concentrations and duration of insult. Few studies have addressed the functional consequences of PLCγ_1 activation during H_2O_2-induced oxidative stress and whether PLCγ_1 acts in a pro- or anti-apoptotic manner, in particular in VSMCs of resistance arterioles. To our knowledge, the present findings are novel for VSMCs of small-diameter arterioles that are physiologically impor-

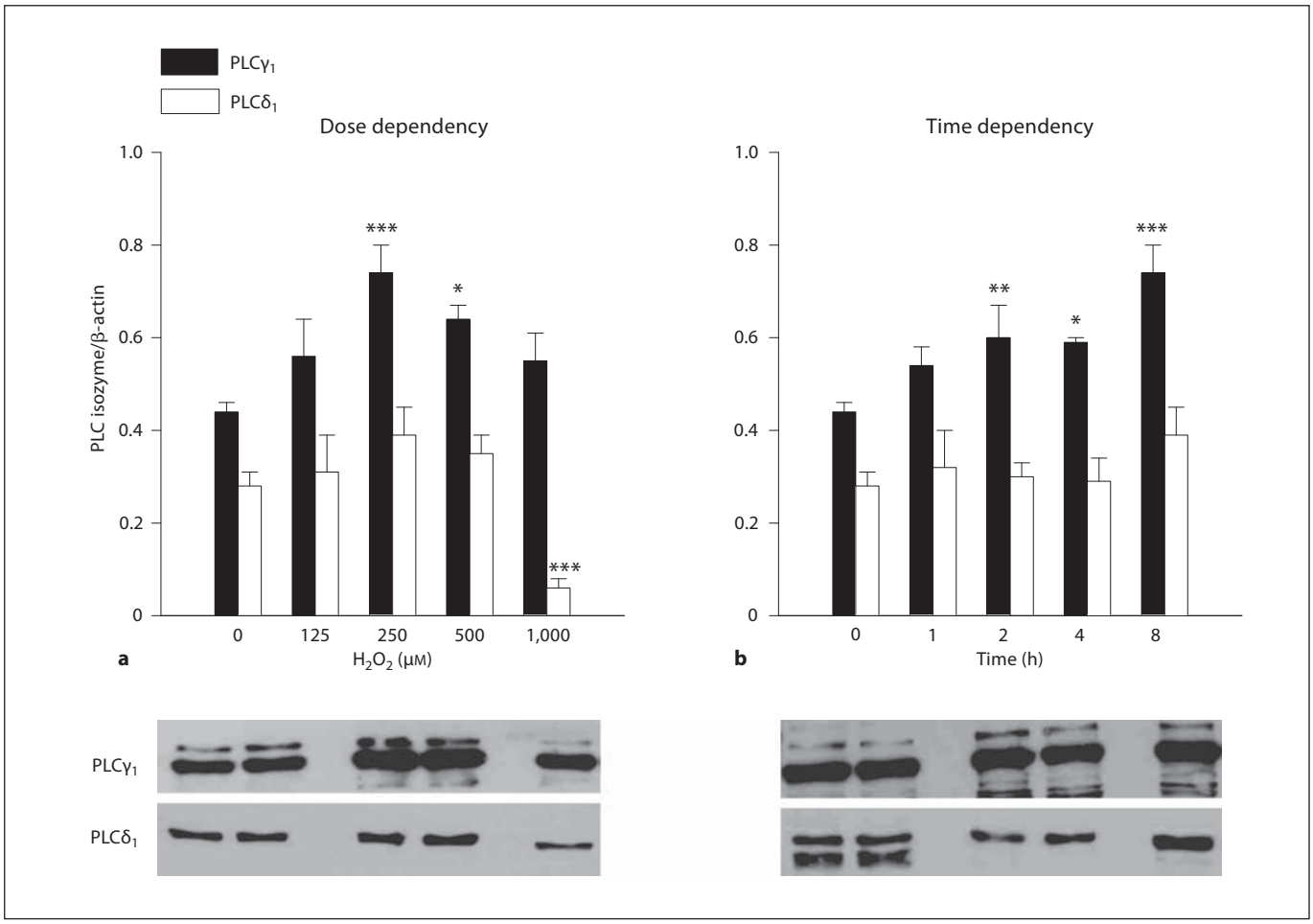

Fig. 5. Western blot analysis showing that H_2O_2 increases protein expression of PLCγ_1 in renal VSMCs of 6-week-old Sprague-Dawley rats. Protein levels of PLCδ_1 are stable until the highest concentration of H_2O_2. **a** Effect of different concentrations of H_2O_2 (125–1,000 μM) for 8 h. **b** Effect of duration of exposure to H_2O_2 (250 μM) for 1–8 h. Means ± SEM ($n \geq 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

tant regulators of vascular resistance and tissue perfusion.

ROS in general and H_2O_2 specifically can impact on multiple signaling pathways that influence cell function and survival [1–3, 6, 19]. Evidence suggests a general trend for concentration- and time-dependent effects. Typically, concentrations of H_2O_2 <100–200 μM tend to be mitogenic and promote cell proliferation and vascular hypertrophy [1, 4, 8]. On the other hand, higher concentrations often result in either temporary or permanent growth arrest [22]. In addition to endothelial cells and VSMCs, adventitial cells and macrophages release large amounts of H_2O_2. High concentrations of H_2O_2 (400–1,000 μM) induce cell death via apoptosis, whereas more moderate concentrations usually cause cell cycle arrest [19]. The mechanism(s) by which H_2O_2 reduces cell function and viability is associated with degeneration of lipids, proteins, and DNA. The precise mechanisms and critical signaling pathways mediating these polarized, dual pro- and anti-apoptotic effects await clarification.

Stimulation of second messenger systems by G-protein-coupled receptors and growth factor receptors generally favors survival of aortic VSMCs; interruption of signaling induces apoptosis mediated by caspases [23]. Contributing to complexity of the system, diverse model systems often present with variable results depending on cell type and experimental conditions. ROS appear to have variable effects on the vasculature, with O_2^- causing

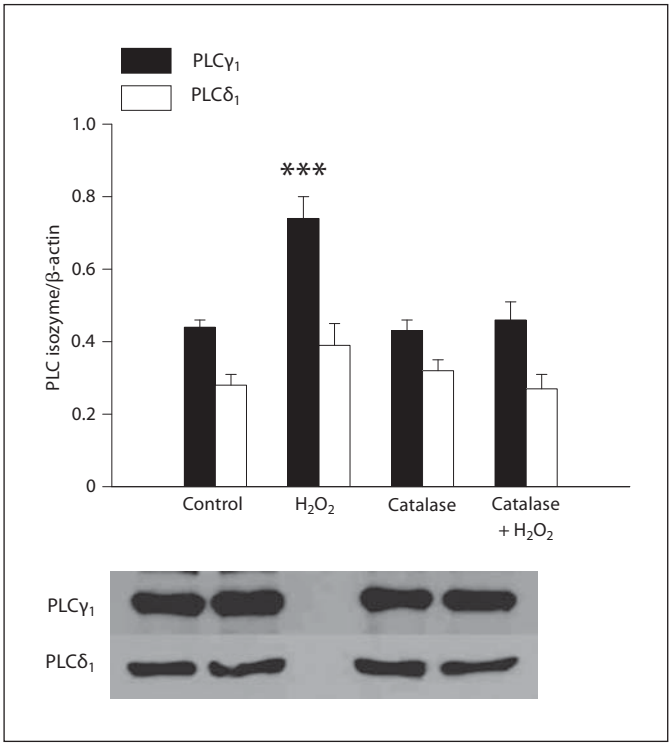

Fig. 6. Western blot analysis showing catalase inhibition of H_2O_2-induced increases in protein expression of PLCγ_1 but not PLCδ_1 in renal VSMCs of 6-week-old Sprague-Dawley rats when exposed to 250 μM H_2O_2 for 8 h. Catalase (20 IU/ml) pretreatment for 10 min has no effect on basal protein levels during control conditions without H_2O_2 treatment. Means ± SEM ($n \geq 3$). *** $p < 0.001$ vs. control.

proliferation of cardiac fibroblasts and apoptosis of cardiac myocytes [24]. H_2O_2 and O_2^- appear to activate MAP kinases differently in VSMCs [1]. Most studies indicate ERK activation as a survival factor during oxidant injury in cardiac myocytes, embryonic fibroblasts and PC12 pheochromocytoma cells [25]. In contrast, this pathway seems to act as pro-apoptotic in other cell types such as mesangial cells and macrophages [26, 27].

Within the vasculature, certain arteries tend to respond more strongly with proliferation than others [13]. VSMCs from media and intima may differ with regard to phenotype and their rates of proliferation and apoptosis [5]. It is noteworthy that renal microvascular VSMC proliferate appreciably slower than aortic VSMC [28]. Furthermore, the expression of some NAD(P)H oxidases seem to vary along the vasculature, reportedly being present in VSMCs of resistance arteries but absent in aorta [6]. Conflicting evidence for H_2O_2 suggests it can either positively or negatively modulate vascular growth and cell death [19]. It has been reported that O_2^-, but not H_2O_2, is proliferative and mitogenic to aortic VSMCs [29]. On the other hand, other evidence suggests that increased cellular H_2O_2 production impacts on proliferation mediated by epidermal- and platelet-derived growth factors (EGF and PDGF) in VSMCs, with high levels producing cell death and apoptosis [29]. Thus, sensitivity and the relative importance of signal transduction cascades appear to contrast among cell types and sites along the vasculature.

PLCγ_1 and PLCγ_2 are key components regulating cell growth/proliferation activated by oxidative stress. It has been shown recently that PLC isozymes in various cell types undergo phosphorylation and overexpression in response to treatment with H_2O_2 [6, 20, 30, 31]. H_2O_2 has been shown to tyrosine phosphorylate PLCγ_1 in fibroblasts, lymphocytes, and platelets [20, 32]. Although the key activation events are not certain, stimulation by growth factor receptor tyrosine kinases or non-receptor tyrosine kinases appear to be involved. Oxidative stress is reported to upregulate the expression of endothelin-1 and PLCγ_1 in mouse embryonic fibroblasts and PC12 cells [1, 3, 4, 20, 30, 31].

A proliferative role of PLC is suggested by the finding that cytosolic Ca^{2+} is central to activation of growth factor signaling such as ERK1/2, p38MAP kinase and Akt in A10 VSMCs [33]. On the other hand, H_2O_2-induced apoptosis has been noted in VSMCs of conduit arteries such as aorta and cerebral artery and cardiac myocytes, effects that may be related to impaired Ca^{2+} metabolism [5, 29, 34]. A recent study suggests that H_2O_2 (250–500 μM) activates PLCγ_1 in cultured human venous VSMCs through tyrosine phosphorylation in a dose- and time-dependent manner, with downstream responses related to $[Ca^{2+}]_i$ mobilization and morphological changes which are attenuated by the general tyrosine kinase inhibitor genistein or by the PLC inhibitor U73122 [35]. H_2O_2 (250 μM) causes rapid stimulation of cytosolic Ca^{2+} in cultured venous VSMCs and embryonic fibroblasts that results from tyrosine kinase activation of PLC [20, 35]. Acting via Src family and EGF receptor tyrosine kinase activities, H_2O_2 stimulates PLCγ_1 in a concentration- and time-dependent manner.

In mouse embryonic fibroblasts, PLCγ_1 activation by protein tyrosine kinases, mediated in part by Src family tyrosine kinases and EGFR tyrosine kinase, appears to play an anti-apoptotic role in oxidative stress [20]. Subsequent studies showed that PLCγ_1 phosphorylation afforded survival protection by activating PKC, phosphor-

ylating Bcl-2 and inhibiting caspase-3 activity in the same cell type in response to H_2O_2 in a dose- (50–200 μM) and time-dependent (2–5 min) manner [30]. In a similar dose- (400 μM to 2 mM) and time- (5–30 min) dependent manner, H_2O_2 treatment activates PI_3-kinase and Akt activity secondary to EGFR phosphorylation that results in pro-survival protection against oxidative stress-induced apoptosis in NIH3T3 and HeLa cells [20]. Additional supportive evidence of a protective role of PLCγ$_1$ is provided by the observation that mouse embryonic fibroblasts deficient in PLCγ$_1$ function are more sensitive to the cytotoxic actions of H_2O_2 than wild-type cells [20]. In PC12 pheochromocytoma cells, overexpression of PLCγ$_1$ attenuates UV stress-induced apoptosis [31]. Further support for this notion of PLC-mediated protection derives from studies of human intestinal epithelial Caco-2 cells, where EGF treatment promotes survival against oxidants through PLCβ$_1$ and PLCγ$_1$-dependent signaling in intestinal epithelial cells [36]. In association with reduced ventricular cardiomyocyte viability, H_2O_2 exposure for 15 min rapidly activates PLCγ$_1$ by tyrosine phosphorylation in a dose-dependent manner (20–100 μM) [37]. Such short-term exposure is reported to cause parallel increases in PLCγ$_1$ protein content and mRNA levels. PLCγ$_1$ is pro-survival related to apparent U73122-sensitive PLC-dependent PKCε activation and phosphorylation of the anti-apoptotic protein Bcl-2 [37]. Further studies are needed to address the generality of this effect to other cell types and to identify the downstream targets mediating the protective effects in VSMCs of different vessel types.

The downstream mechanism appears to involve Ca^{2+} mobilization and PKC, in some cases independent of EGF signaling [20, 38]. PKC appears to be important in mediating ROS-induced apoptosis of fibroblasts and aortic VSMCs [30]. We conducted studies on VSMC of resistance arterioles to test the generality of the protection afforded by PLCγ$_1$ against H_2O_2-induced apoptosis and present results similar to those reported previously for cultured mouse embryonic fibroblasts and fresh ventricular cardiomyocytes. However, it should be noted that our results for arteriolar VSMCs contrast with the reported detrimental effects mediated by PLC in venous VSMCs (U73122) [35]. In VSMCs from this source, an apoptotic-like response equated with detachment of cultured venous VSMCs produced by H_2O_2 (250–500 μM) exposure for 18 h was attenuated by the PLC blocker U73122.

In conclusion, vascular dysfunction and growth/remodeling associated with cardiovascular and renal disease are modulated by chronic oxidative stress. H_2O_2 is known to impact on Ca^{2+} signaling and the proliferating effect of growth factors in many cell types, although our knowledge of effects in the vasculature is largely limited to endothelial cells, VSMCs and adventitial fibroblasts of large diameter arteries and aorta. In general, low levels of H_2O_2 promote growth, with high levels producing cell death and apoptosis. Our study provides new information about the effects of H_2O_2 on viability of VSMCs derived from a major resistance arteriole in the renal microcirculation. We observe that relatively high levels of H_2O_2 have a cytotoxic effect. H_2O_2 increases tyrosine phosphorylation of PLCγ$_1$, almost doubles total PLC activity, changes that are completely inhibited by catalase inactivation of H_2O_2. Protective compensatory increases in PLCγ$_1$ function are suggested by the finding that inhibition of PLC activity with U73122 accelerates cell death. Longer-term effects of H_2O_2 in renal resistance VSMC stimulate PLCγ$_1$ but not PLCδ$_1$ (or PLCβ$_1$) protein expression. Thus, H_2O_2 may play a key role in VSMCs viability and arteriolar structure and function during inflammatory processes.

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