ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 6, 2002


# Forum Original Research Communication

# Angiotensin II Induces a Rapid and Transient Increase of Reactive Oxygen Species

M. RODRÍGUEZ-PUYOL,[1,3] M. GRIERA-MERINO,[1] G. PÉREZ-RIVERO,[1]
M.L. DÍEZ-MARQUÉS,[1] M.P. RUIZ-TORRES,[1] and D. RODRÍGUEZ-PUYOL[2,3]

## ABSTRACT

**Vascular smooth muscle cells (VSMC) exhibit a hypertrophic and contractile response after angiotensin II (Ang II) treatment, and the NADH/NADPH oxidase-dependent synthesis of hydrogen peroxide ($H_2O_2$) seems to play a central role in these responses. Present experiments were designed to analyze the mechanisms responsible for the rapid changes induced by Ang II in the intracellular $H_2O_2$ concentration in VSMC. Ang II induced a quick and transient increase of dichlorodihydrofluorescein (DCHF) fluorescence in VSMC, an effect that was completely abolished by catalase and by diethyldithiocarbamate, a cell-permeable superoxide dismutase inhibitor. Losartan and pertussis toxin prevented the stimulatory effect of Ang II. Both diphenylene iodonium (NADH/NADPH oxidase blocker) and 3-(4-octadecylbenzoyl)acrylic acid (phospholipase A2 blocker) inhibited the changes in DCHF fluorescence induced by Ang II, in a dose-dependent fashion, and the effects of both inhibitors were additive. These data demonstrate that Ang II induces a very quick and transient increase of $H_2O_2$ in VSMC. This effect depends on the receptor type 1, is linked to a G protein, and involves both NADH/NADPH oxidase and phospholipase A2 activation. The mechanism may be related to the previously proposed role of $H_2O_2$ in the genesis of the Ang II-induced cell contraction.** *Antioxid. Redox Signal.* 4, 869–875.

## INTRODUCTION

ANGIOTENSIN II (Ang II) is now considered not only an important physiological regulator of vascular tone or aldosterone secretion, but also a growth factor for some cell lines. Vascular smooth muscle cells (VSMC) exhibit a hyperplastic–hypertrophic response after Ang II treatment (5, 11, 19), and the mechanisms responsible for this effect are rather well outlined. NADH/NADPH oxidase plays a central role in this mechanism. After activation by Ang II, the enzyme synthesizes significant amounts of superoxide anion and, in the presence of superoxide dismutase (SOD), hydrogen peroxide ($H_2O_2$) (2, 7, 12, 22). Both reactive oxygen intermediates (ROI) are able to modify the activity of intracellular kinases (20, 21, 24) and/or trancription factors (9, 14), thus modifying nuclear cell activity.

The Ang II-dependent increased ROI synthesis takes place as early as 1 min after Ang II stimulation, and it is sustained for at least 4 h (20). This pattern of response suggests a modulation of NADH/NADPH oxidase activity and content by Ang II (4, 20). Some studies have proposed that the activation of this oxidase by Ang II depends on arachidonic acid metabolites (23), perhaps derived ultimately from phospholipase D-mediated phosphatidylcholine hydrolysis (18).

Recently, some authors have proposed that ROI could also mediate other Ang II effects not linked to nuclear activation, particularly cell contraction (18). Changes in intracellular calcium concentration or in the activity of different enzymes such as calcium calmodulin-dependent myosin light kinase, protein kinase C, and myosin light chain phosphatase have been proposed as the main mechanisms involved in the regulation of smooth muscle cell contraction (8). However, catalase

Departments of [1]Physiology and [2]Medicine, Alcalá University, [3]Fundación de Investigación Hospital Príncipe de Asturias, and IRSIN, Madrid, Spain.

abrogates the contractile effect of different vasoactive agonists in VSMC and mesangial cells, suggesting a role for $H_2O_2$ in the regulation of contractility (17).

In that study, Torrecillas *et al.* demonstrated that Ang II induced a very quick increase of $H_2O_2$ concentration in VSMC (17), but they did not explore the mechanisms involved in this process. The previously described NADH/NADPH oxidase system may be also responsible for this stimulation, but alternative pathways could be considered. The present experiments were designed to analyze the mechanisms involved in the genesis of the rapid changes induced by Ang II in the intracellular $H_2O_2$ concentration in VSMC.

## MATERIALS AND METHODS

### *Materials*

Collagenase type IV, Ang II, catalase, diphenylene iodonium (DPI), $H_2O_2$, diethyldithiocarbamate (DETC), indomethacin, and pertussis toxin were purchased from Sigma (St. Louis, MO, U.S.A.). 3-(4-Octadecylbenzoyl)acrylic acid (OBAA) was from Calbiochem–Novabiochem Corp. (La Jolla, CA, U.S.A.). 2′,7′-Dichlorodihydrofluorescein diacetate (DCHF-DA) was obtained from Molecular Probes (Eugene, OR, U.S.A.). Dulbecco's modified Eagle's medium (DMEM)/F-12, fetal calf serum, trypsin-EDTA (0.02%), and penicillin–streptomycin were purchased from Biowhittaker (Walkersville, MD, U.S.A.). Culture plates were from Nunc (Kamstrup, Denmark). All the other reagents were of the highest commercially available grade.

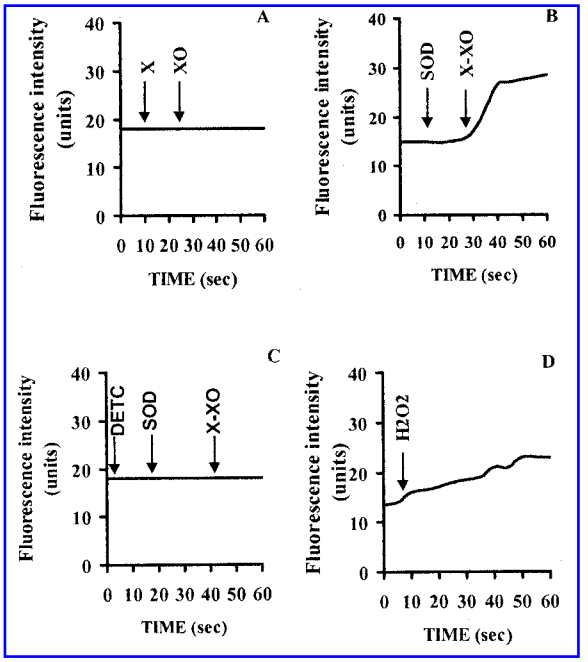


**FIG. 1. Evaluation of DCHF responses to different ROI in solutions without cells.** Different reagents were added to aqueous solutions containing the probe (20 μ*M*), and fluorescence changes were registered. (**A**) Xanthine (X; 100 μ*M*) and xanthine oxidase (XO; 1 U/L). (**B**) The same concentrations of X and XO plus SOD (100 U/ml). (**C**) The same concentrations of X and XO with DETC (1 μ*M*). (**D**) $H_2O_2$ (1 m*M*). Results shown are representative of five experiments.

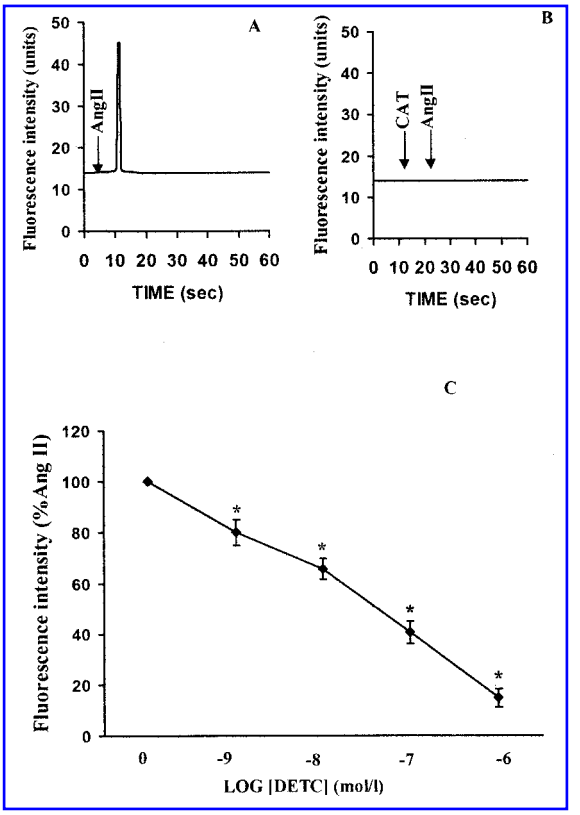


**FIG. 2. Changes in DCHF fluorescence in the presence of Ang II (1 μ*M*).** Cells were loaded with the probe, incubated with the different reagents, and fluorescence changes were registered. (**A** and **B**) Ang II alone or in the presence of catalase (CAT; 80 U/ml). Results shown are representative of five experiments. (**C**) Ang II in the presence of different concentrations of DETC. Results shown are the means ± SEM of five different experiments, and they are expressed as percentage of the signal elicited by Ang II. *$p < 0.05$ vs. cells without DETC.

### *Cell culture*

VSMC were obtained from thoracic aortas from Wistar rats by methods described previously (3, 17). In brief, Wistar rats (125–200 g) were killed and thoracic aortas were removed, cleaned of surrounding tissues, dissected into small strips, and incubated in DMEM/F-12 medium with collagenase type IV at 37°C for 45 min. The digested strips were seeded onto 100-mm-diameter dishes and maintained in 10 ml of DMEM/F-12 medium with 10% fetal calf serum, at 37ºC, in a humidified atmosphere of 5% $CO_2$. Confluent cultures were serially passaged by trypsinization (trypsin-EDTA) The cells were used between the second and fourth passages. Cells exhibited characteristics of VSMC (3, 17).

### *Measurement of the $H_2O_2$ synthesis*

$H_2O_2$ synthesis by VSMC was measured by using DCHF-DA, a fluorescent probe that has been proved useful for the

measurement of different ROI, including $H_2O_2$ (10). DCHF-DA diffuses readily to the intracellular compartment, where it is desacetylated to the non–membrane-permeable DCHF. Then, during the cellular production of $H_2O_2$, DCHF is oxidized and emits a fluorescent signal.

For the measurement, cells were plated onto 12-mm glass coverslips in a 24-well plate. When cells reached confluence, the medium in the dish was replaced, and cells were washed twice with a Krebs–Ringer–HEPES (KRH) solution (125 m*M* $CaCl_2$, 5 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* $KH_2PO_4$, 2 m*M* $CaCl_2$, 6 m*M* glucose, and 25 m*M* HEPES, pH 7.4). Then VSMC were incubated with 20 μ*M* DCHF-DA for 30 min at 37°C. Thereafter, coverslips were removed from the plate, and cells were then washed twice with KRH and placed in fresh KRH (30 min, 37°C). Fluorescence measurements were performed essentially as described by Torrecillas *et al.* (17), by placing the glass coverslips in a diagonal position at 30° angle to the incident light, inside a standard 1-cm square cuvette containing 1.5 ml of KRH. Fluorescence measurements were made with a Perkin–Elmer LB, 50B-fluorescence spectrophotometer, and excitation and emission wavelengths were 488 and 525 nm, respectively. Thereafter, the different reagents were added. Rapid mixing of the agents was achieved by continuous stirring with a magnetic bar placed at the bottom of the cuvette. The fluorescent signal was registered as a function of time.

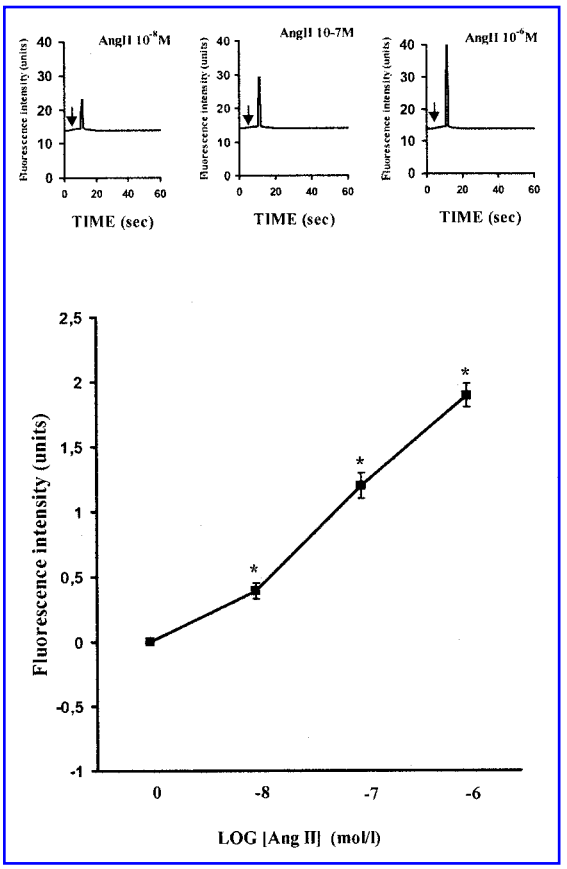


**FIG. 3. Changes in DCHF fluorescence in the presence of different Ang II concentrations.** Cells were loaded with the probe, incubated with increasing Ang II concentrations, and fluorescence changes were registered. The upper part of the figure shows a representative experiment with different Ang II concentrations. In the lower part, the means ± SEM of five different experiments are shown. *$p < 0.05$ vs. control values.

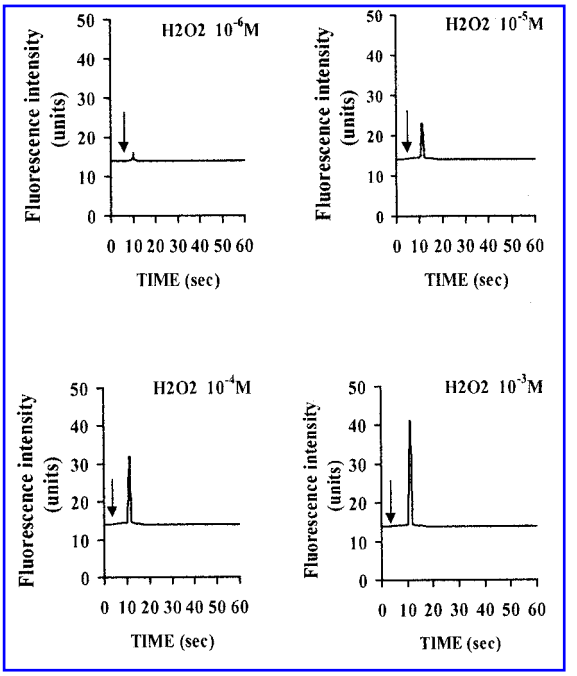


**FIG. 4. Changes in DCHF fluorescence in the presence of different $H_2O_2$ concentrations.** Cells were loaded with the probe, incubated with increasing $H_2O_2$ concentrations, and fluorescence changes were registered. The results shown are representative of five different experiments.

## *Experimental design*

Before the experiments in cells were performed, the specificity of the DCHF-dependent signals was tested in aqueous solution without cells. DCHF-DA (20 μ*M*) in KRH was chemically desacetylated and incubated with 100 μ*M* xanthine plus 1 U/L xanthine oxidase, with or without SOD (100 U/ml) and DETC (1 μ*M*), as well as with 1 m*M* $H_2O_2$.

For experiments on cells, variable concentrations of Ang II and $H_2O_2$ were used. These reagents were directly added to the cuvette containing the coverslip, as described. Catalase (80 U/ml) and losartan (1 μ*M*), a competitive antagonist of the Ang II type 1 receptor, were also added to the cuvette, immediately before Ang II (10 s). In other experiments, cells were incubated with variable concentrations of DETC, DPI, and OBAA, or with 1 μ*M* indomethacin for 30 min before including the covers in the cuvette. The incubation time was 18 h in the case of pertussis toxin (0.5 μg/ml).

## *Statistical analysis*

In all cases, the data shown are the means ± SEM of a variable number of experiments (see legends to the figures), and in some cases they are expressed as percentage of the control values. As the number of data in each distribution was never >10, nonparametric statistics, particularly Friedman's test, was selected to compare the different groups of results. A $p < 0.05$ was considered statistically significant.

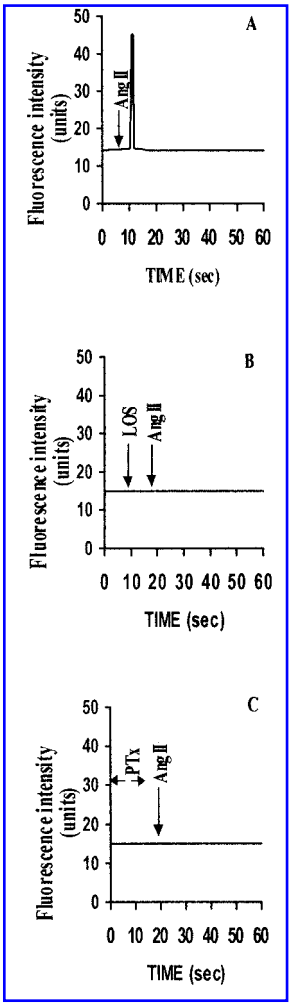


**FIG. 5. Importance of the receptor type and G proteins in the Ang II (1 μ*M*)-dependent changes in DCHF fluorescence.** Cells were loaded with the probe, incubated with the different reagents, and fluorescence changes were registered. (**A**) Ang II. (**B**) Ang II in the presence of losartan (LOS; 1 μ*M*). (**C**) Ang II in the presence of pertussis toxin (PTx; 0.5 μg/ml). Results shown are representative of five different experiments.

## RESULTS

The specificity of the DCHF fluorescent signal was tested in some preliminary experiments included in Fig. 1, performed without cells. Xanthine plus xanthine oxidase did not induce any change in fluorescent signal (Fig. 1A), but a significant and progressive fluorescence increase was observed when the reaction was performed in the presence of SOD (Fig. 1B). As expected, DETC prevented the SOD effect (Fig. 1C), and the direct addition of $H_2O_2$ induced a sustained fluorescent signal (Fig. 1D). Catalase prevented the changes in fluorescent signals in all cases.

Ang II induced a rapid, transient, and significant increase of probe fluorescence in VSMC (Fig. 2). It appeared within 2–4 s of peptide addition (Fig. 2A), remained elevated for ~1–2 s (Fig. 2A), and was prevented by previous incubation with catalase (Fig. 2B) and DETC (Fig. 2C). The pattern of response was dependent on the Ang II dose (Fig. 3). Moreover, the changes in probe fluorescence in response to Ang II were qualitatively similar to those observed after $H_2O_2$ treatment (Fig. 4). Thus, the quantitative changes in fluorescence intensity elicited by 1 μ*M* Ang II and 1 m*M* $H_2O_2$ were comparable.

In order to analyze the mechanisms involved in the rapid changes in probe fluorescence after Ang II incubation, different experiments were performed. First, losartan and pertussis toxin completely prevented the Ang II effect on cells (Fig. 5). Second, both DPI, an NADH/NADPH oxidase inhibitor, and OBAA, a phospholipase A2 blocker, inhibited the Ang II-induced changes in probe fluorescence in a dose-dependent fashion (Fig. 6). Third, the blocking effects of DPI and OBAA were additive, as shown in the Fig. 7. The presence of low doses of DPI and OBAA in the incubation media induced an inhibition of the Ang II-induced changes in DCHF fluorescence that was significantly higher than the blockade observed with any particular drug at a comparable concentration. Fourth, indomethacin, a cyclooxygenase inhibitor, also completely prevented the fluorescence changes induced by Ang II (Fig. 8). *In vitro* assays of the intrinsic antioxidant properties of the above-mentioned drugs, performed in aqueous solution containing the probe, the drug, and exogenously added $H_2O_2$, discarded the potentially intrinsic antioxidant role of these compounds, as they did not prevent the $H_2O_2$-

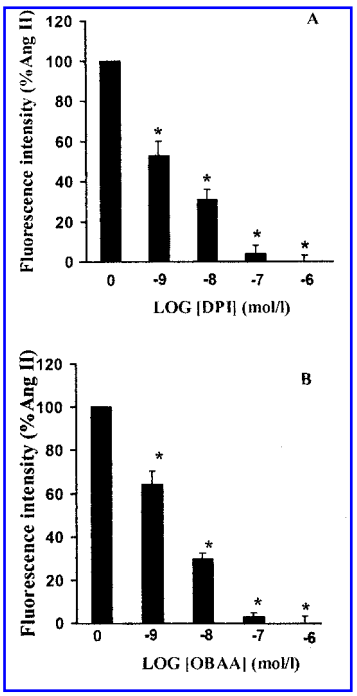


**FIG. 6. Importance of two different ROI generating systems in the Ang II (1 μ*M*)-dependent changes in DCHF fluorescence.** Cells were loaded with the probe, incubated with the different reagents, and fluorescence changes were registered. (**A**) Ang II in the presence of different concentrations of DPI. (**B**) Ang II in the presence of different concentrations of OBAA. In both panels, results shown are the means ± SEM of five different experiments, and they are expressed as percentage of the signal elicited by Ang II. *$p < 0.05$ vs. Ang II-treated cells.

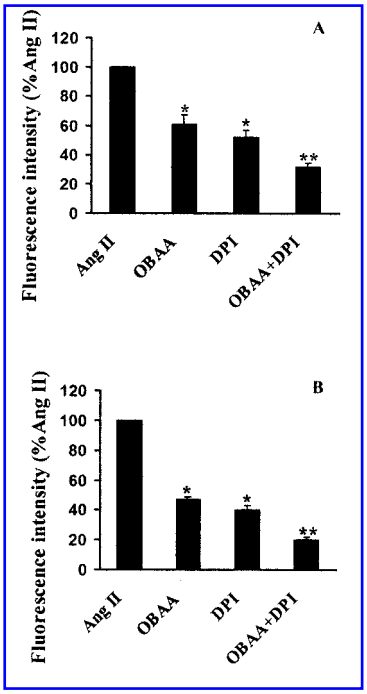


**FIG. 7. Analysis of the interactions between phospholipase A2 and NADH/NADPH oxidase in the Ang II (1 μ*M*)-dependent changes in DCHF fluorescence.** Cells were loaded with the probe, incubated with the different reagents, and fluorescence changes were registered. (**A**) Ang II in the presence of OBAA (200 p*M*), DPI (200 p*M*), or both (100 p*M* OBAA plus 100 p*M* DPI). (**B**) Ang II in the presence of OBAA (2 n*M*), DPI (2 n*M*), or both (1 n*M* OBAA plus 1 n*M* DPI). In both panels, results shown are the means ± SEM of five different experiments, and they are expressed as percentage of the signal elicited by Ang II. *$p < 0.05$ vs. Ang II-treated cells. **$p < 0.05$ vs. the other groups.

dependent increased DCHF fluorescence. Moreover, other pharmacological antagonists such as sulotroban (thromboxane A2 receptor blocker) or BN 52021 (platelet activating factor blocker) did not modify the Ang II-dependent signals (data not shown).

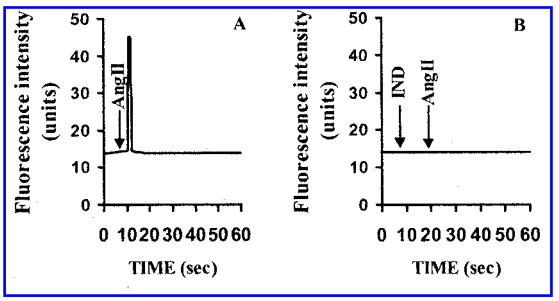


**FIG. 8. Importance of cyclooxygenase in the Ang II (1 μ*M*)-dependent changes in DCHF fluorescence.** Cells were loaded with the probe, incubated with the different reagents, and fluorescence changes were registered. (**A**) Ang II. (**B**) Ang II in the presence of indomethacin (IND; 1 μ*M*). Results shown are representative of five different experiments.

## DISCUSSION

The present results demonstrate that Ang II induces a very rapid and transient increase in DCFH fluorescence in VSMC, which probably reflects a burst of $H_2O_2$ production. Different results support this conclusion. First, in aqueous solutions, the probe fluorescence was modified only in the presence of $H_2O_2$. The coincubation of xanthine plus xanthine oxidase is a widely accepted system for superoxide anion generation and, by the action of SOD, $H_2O_2$ synthesis (1). In our experiments, xanthine plus xanthine oxidase only elicited fluorescent signals when active SOD was present in the incubation media or when $H_2O_2$ was directly added to the solution, thus supporting the lack of response of the dye to superoxide anion. Second, the increased DCHF fluorescence elicited by Ang II was completely abolished by by catalase, a specific $H_2O_2$ scavenger (1). Although catalase, which was added to the incubation media, does not readily cross cell membranes (15), $H_2O_2$ does, and it is quickly scavenged by extracellular catalase (17). Third, the pattern of cell response after Ang II incubation was qualitatively similar to the fluorescent changes observed in the presence of $H_2O_2$.

The mechanism responsible for this rapid and transient increase in the synthesis of $H_2O_2$ elicited by Ang II seems to be comparable to that previously described for later and more sustained stimulations (2, 7, 12, 16, 19, 22). The effect depended on the Ang II type 1 receptor, coupled to a G protein, because it was prevented by losartan and pertussis toxin. Moreover, the $H_2O_2$ generation pathway activated by Ang II involved the NADH/NADPH oxidase-dependent synthesis of superoxide anion, as DPI, an NADH/NADPH oxidase inhibitor, and DETC, a cell-permeable SOD blocker, abolished the changes in DCHF fluorescence induced by Ang II.

On the other hand, the pharmacological blockade of phospholipase A2 with OBAA also prevented the changes in $H_2O_2$ induced by Ang II. This inhibitory effect was quantitatively comparable to that of DPI. Two mechanisms can account for the effects of OBAA. It could be suggested that the Ang II-dependent NADH/NADPH oxidase activation depends on a previous phospholipase A2 stimulation, because it has been demonstrated that increased activities of phospholipase A2 are readily detected after Ang II treatment (13). The relationship between the rapid metabolism of membrane phospholipids and NADH/NADPH oxidase activity has been previously reported, but rather linked to the phospholipase D action (13). Recently, Gorin *et al.* stressed the importance of an arachidonic acid/redox-dependent pathway in the activation of Akt/protein kinase B by Ang II (6), suggesting a possible interaction between phospholipase A2 and NADH/NADPH oxidase. Thus, it could be proposed that the observed OBAA-dependent blockade was due indirectly to the NADH/NADPH oxidase inhibition, as phospholipase A2 could be involved in the activation of this enzyme. However, an alternative explanation may be suggested. Ang II could activate simultaneously both enzymes, generating $H_2O_2$ by at least two different mechanisms. To test this possibility, simultaneous incubations with both pharmacological antagonists were performed, and it was possible to demonstrate that both blockades were additive, suggesting the simultaneous activa-

tion of NADH/NADPH oxidase and phospholipase A2 by Ang II, with the subsequently increased synthesis of ROI.

Two characteristics of the Ang II-dependent $H_2O_2$ burst shown in these experiments must be considered. First, it disappears very quickly, probably because the newly generated ROI are readily removed by antioxidant intracellular enzymes, as was the case of the direct addition of $H_2O_2$ to cells. Second, it takes place in seconds, almost immediately after stimulus addition. This kind of response suggests a possible role of the system in the regulation of short-term cell functions, such as contraction. In this sense, previous results from our group demonstrate that catalase blocks the calcium peak and cell contraction elicited by Ang II (17), stressing the role of $H_2O_2$ as a second messenger of Ang II in the regulation of different cell functions.

In summary, the present results demonstrate that Ang II induces a very quick and transient increase of $H_2O_2$ in VSMC. This effect depends on the receptor type 1, was linked to a G protein, and involves both NADH/NADPH oxidase and phospholipase A2 activation. The mechanism may be linked to the previously proposed role of $H_2O_2$ in the genesis of the Ang II-induced cell contraction.

### *Perspectives*

The present results suggest that the metabolism of arachidonic acid by cyclooxygenase, following the activation of phospholipase A2, is also a relevant mechanism of Ang II-induced $H_2O_2$ synthesis, but more extensive experiments are needed to carefully explore this pathway.

## ACKNOWLEDGMENTS

The present studies were supported by the FIS (01/0434) and BFI-2001/1036. M. Griera was funded by the IRSIN (Instituto Reina Sofía de Investigación en Nefrología), and G. Pérez is a fellow from Ministerio de Ciencia y Technologia of Spain.

## ABBREVIATIONS

Ang II, angiotensin II; DCHF-DA, 2´,7´-dichlorodihydrofluorescein diacetate; DETC, diethyldithiocarbamate; DMEM, Dulbecco's modified Eagle's medium; DPI, diphenylene iodonium; $H_2O_2$, hydrogen peroxide; KRH, Krebs–Ringer–HEPES; OBAA, 3-(4-octadecylbenzoyl)acrylic acid; ROI, reactive oxygen intermediate; SOD, superoxide dismutase; VSMC, vascular smooth muscle cells.

## REFERENCES

1. Baud L and Ardaillou R. Reactive oxygen species production and role in the kidney. *Am J Physiol* 251: F765–F776, 1986.
2. Bech-Laursen J, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, and Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 95: 588–593, 1997.
3. Chamley-Campbell J, Campbell GR, and Ross R. The smooth muscle cell in culture. *Physiol Rev* 59: 1–61, 1979.
4. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Q 4th, Taylor WR, Harrison DG, de Leon H, Wilcox JN, and Griendling KK. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertension rat. *Circ Res* 80: 45–51, 1997.
5. Gibbons GH, Pratt RE, and Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. *J Clin Invest* 90: 456–461, 1992.
6. Gorin Y, Kim NH, Feliers D, Bhandari B, Ghosh CG, and Abboud HE. Angiotensin II activates Akt/protein kinase B by an arachidonic acid/redox-dependent pathway and independent of phosphoinositide 3-kinase. *FASEB J* 15: 1909–1920, 2001.
7. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
8. Horowitz A, Menice CB, Laporte R, and Morgan KG. Mechanisms of smooth muscle contraction. *Physiol Rev* 76: 967–1003, 1996.
9. Jornot L, Petersen H, and Junod AF. Modulation of the DNA binding activity of transcription factors CREP, NFkappaB and HSF by $H_2O_2$ and TNF alpha. Differences between in vivo and in vitro effects. *FEBS Lett* 416: 381–386, 1997.
10. Lopez-Ongil S, Hernandez-Perera O, Navarro-Antolin J, Perez de Lema G, Rodriguez-Puyol M, Lamas S, and Rodriguez-Puyol D. Role of reactive oxygen species in signaling cascade of cyclosporine A-mediated up-regulation of eNOS in vascular endothelial cells. *Br J Pharmacol* 124: 447–454, 1998.
11. Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE, and Dzau VJ. The angiotensin II type 2 (AT 2) receptor antagonizes the growth effects of the AT1 receptor: gain-of-function study using gene transfer. *Proc Natl Acad Sci U S A* 92: 10663–10667, 1995.
12. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, and Harrison DG. Angiotensin II mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 97: 1916–1923, 1996.
13. Schlondorff D, De Candido S, and Satriano JA. Angiotensin II stimulates phospholipases C and A2 in cultured rat mesangial cells. *Am J Physiol* 253: C113–C120, 1987.
14. Simon AR, Rai U, Fanburg BL, and Cochran BH. Activation of the JAK-STAT pathway by reactive oxygen species. *Am J Physiol* 275: C1640–C1652, 1998.
15. Sundaresan M, Yu Z, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of $H_2O_2$ for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
16. Takahashi T, Taniguchi T, Okuda M, Takahashi A, Kawasaki S, Domoto K, Taguchi M, Ishikawa Y, and

Yokoyama M. Participation of reactive oxygen intermediates in the angiotensin II-activated signaling pathways in vascular smooth muscle cells. *Ann N Y Acad Sci* 902: 283–287, 2000.
17. Torrecillas G, Boyano-Adanez MC, Medina J, Griera M, López-Ongil S, Arilla E, Rodriguez-Puyol M, and Rodriguez-Puyol D. The role of hydrogen peroxide in the contractile response to angiotensin II. *Mol Pharmacol* 59: 104–112, 2001.
18. Touryz RM and Schiffrin EL. Ang II-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells. *Hypertension* 34: 976–982, 1999.
19. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, and Griendling KK. p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J Biol Chem* 271: 23317–23321, 1996.
20. Ushio-Fukai M, Alexander RW, Akers M, and Griendling KK. p38 MAP kinase is a critical component of the redox-sensitive signaling pathways by angiotensin II: role in vascular smooth muscle cells hypertrophy. *J Biol Chem* 273: 15022–15029, 1998.
21. Yoshizumi M, Abel J, Haendeler J, Huang Q, and Berk BC. Ser and Cas mediate JNK activation but not ERK1/2 and p38 kinases by reactive oxygen species. *J Biol Chem* 275: 11706–11712, 2000.
22. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, and Griendling K. Role of NADH/NADPH oxidase-derived $H_2O_2$ in angiotensin II-induced vascular hypertrophy. *Hypertension* 32: 488–495, 1998.
23. Zafari AM, Ushio-Fukai M, Minieri CA, Akers M, Lasségue B, and Griendling KK. Arachidonic acid metabolites mediate angiotensin II-induced NADH/ NADPH oxidase activity and hypertrophy in vascular smooth muscle cells. *Antioxid Redox Signal* 1: 167–179, 1999.
24. Zhang J, Jin N, Liu Y, and Rhoades RA. Hydrogen peroxide stimulates extracellular signal-regulated protein kinases in pulmonary arterial smooth muscle cells. *Am J Respir Cell Mol Biol* 19: 324–332, 1998.

Address reprint requests to:
*Manuel Rodríguez-Puyol*
*Departamento de Fisiología*
*Facultad de Medicina*
*Universidad de Alcalá*
*Campus Universitario s/n*
*Alcalá de Henares*
*28805 Madrid*
*Spain*

*E-mail:* manuel.rodriguez@uah.es

Received for publication December 14, 2001; accepted June 21, 2002.

**This article has been cited by:**


1. Senthilkumar B. Rajamohan , Gayatri Raghuraman , Nanduri R. Prabhakar , Ganesh K. Kumar . 2012. NADPH Oxidase-Derived H2O2 Contributes to Angiotensin II-Induced Aldosterone Synthesis in Human and Rat Adrenal Cortical Cells. *Antioxidants & Redox Signaling* **17**:3, 445-459. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
2. Abel Martin Garrido, Kathy K. Griendling. 2009. NADPH oxidases and angiotensin II receptor signaling. *Molecular and Cellular Endocrinology* **302**:2, 148-158. [CrossRef]
3. M. Paola Zago , Gerardo G. Mackenzie , Ana M. Adamo , Carl L. Keen , Patricia I. Oteiza . 2005. Differential Modulation of MAP Kinases by Zinc Deficiency in IMR-32 Cells: Role of H2O2. *Antioxidants & Redox Signaling* **7**:11-12, 1773-1782. [Abstract] [Full Text PDF] [Full Text PDF with Links]
4. Y HAN, Y ZHANG, H WANG, X GAO, W WANG, G ZHU. 2005. Reactive oxygen species in paraventricular nucleus modulates cardiac sympathetic afferent reflex in rats. *Brain Research* **1058**:1-2, 82-90. [CrossRef]
5. Alberto Lazaro , Julio Gallego-Delgado , Pilar Justo , Vanesa Esteban , Julio Osende , Sergio Mezzano , Alberto Ortiz , Jesus Egido . 2005. Long-Term Blood Pressure Control Prevents Oxidative Renal Injury. *Antioxidants & Redox Signaling* **7**:9-10, 1285-1293. [Abstract] [Full Text PDF] [Full Text PDF with Links]
6. B ZUCKERBRAUN, J BARBATO, A HAMILTON, S SEBTI, E TZENG. 2005. Inhibition of geranylgeranyltransferase I decreases generation of vascular reactive oxygen species and increases vascular nitric oxide production. *Journal of Surgical Research* **124**:2, 256-263. [CrossRef]
7. Susana López-Ongil, M.-Luisa Díez-Marqués, Mercedes Griera, Manuel Rodríguez-Puyol, Diego Rodríguez-Puyol. 2005. Crosstalk Between Mesangial and Endothelial Cells: Angiotensin II Down-Regulates Endothelin-Converting Enzyme 1. *Cellular Physiology and Biochemistry* **15**:1-4, 135-144. [CrossRef]
8. Jose M. López-Novoa . 2002. Role of Reactive Oxygen Species in Renal Function and Diseases. *Antioxidants & Redox Signaling* **4**:6, 867-868. [Citation] [Full Text PDF] [Full Text PDF with Links]