A possible role for platelet-activating factor in the hydrogen peroxide-induced TXB_2 and PGE_2 glomerular synthesis

I. Arribas-Gómez,* I. Duque-Marín, G. Pérez de Lema, M. L. Díez-Marqués, J. Lucio-Cazaña, M. Rodríguez-Puyol, and D. Rodríguez-Puyol[1,†]

Departments of Physiology and Pharmacology and Medicine, University of Alcalá de Henares, and Clinical Biochemistry Department* and Nephrology Section,† University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain

Abstract Hydrogen peroxide stimulates both prostanoid and platelet-activating factor (PAF) biosynthesis in cultured rat mesangial cells and isolated rat glomeruli. The present experiments were designed to try to establish some relationship between prostanoid and PAF synthesis in these renal structures, in the presence of hydrogen peroxide. Cells and glomeruli were incubated with hydrogen peroxide under different experimental conditions, and thromboxane B_2 (TXB_2), the stable metabolite of thromboxane A_2 (TXA_2), and prostaglandin E_2 (PGE_2) concentrations were measured in the supernatants of the cells or glomeruli. Moreover, H_2O_2-dependent PAF synthesis was measured by high performance liquid chromatography (HPLC) ([^{3}H]acetate incorporation) and radioimmunoassay. H_2O_2 induced increased TXB_2 and PGE_2 production in cultured rat mesangial cells and isolated rat glomeruli. This effect was blocked by incubation in the presence of a PAF-receptor antagonist, BN-52021. This antagonist has no intrinsic effect either in basal prostanoid synthesis or in arachidonic acid-stimulated glomerular TXB_2 synthesis. Alprazolam, another PAF antagonist, nonchemically related to BN-52021, also completely blocked the H_2O_2-induced production of TXB_2 by isolated rat glomeruli. Moreover, H_2O_2 was also able to induce an increased [^{3}H]acetate incorporation into a fraction comigrating with a PAF standard in HPLC in isolated glomeruli, and this effect was dependent on the H_2O_2 concentration tested. Moreover, H_2O_2 was also able to induce an increased [^{3}H]acetate incorporation and increased synthesis of radioimmunoassayable PAF in cultured mesangial cells. These results suggest that the increased synthesis of PGE_2 and TXB_2 induced by H_2O_2 could be dependent on platelet-activating factor production.—**Arribas-Gómez, I., I. Duque-Marín, G. Pérez de Lema, M. L. Díez-Marqués, J. Lucio-Cazaña, M. Rodríguez-Puyol, and D. Rodríguez-Puyol.** A possible role for platelet-activating factor in the hydrogen peroxide-induced TXB_2 and PGE_2 glomerular synthesis. *J. Lipid Res.* 1995. **36:** 260–265.

Supplementary key words glomeruli • mesangial cells

Reactive oxygen species (ROS) have been considered as mediators of renal injury in some pathophysiologic conditions such as glomerulonephritis, renal ischemia, and some toxic-induced nephropathies (1–3). ROS, particularly hydrogen peroxide (H_2O_2), may modify the synthesis of some prostanoids by both isolated glomeruli and cultured rat mesangial cells (4, 5). These mediators, with well-known vasoactive properties, could contribute, at least partially, to the genesis of the changes in renal blood flow (RBF) and glomerular filtration rate (GFR), which characterize these pathological conditions. Moreover, it has also been published that a platelet-activating factor (PAF) antagonist, BN-52021, could inhibit the H_2O_2-induced changes in the planar cell surface area of cultured mesangial cells, thus suggesting a role for PAF in the effects of ROS on these cells (6). On the other hand, Lewis et al. (7) and Duque et al. (6) have demonstrated that H_2O_2 induces an increased synthesis of PAF by endothelial and mesangial cells, respectively. PAF, a lipid with well-defined hemodynamic properties (8), could be also involved in the genesis of the renal dysfunction in those situations characterized by an increased ROS synthesis.

The synthesis of both prostanoid and PAF shares common metabolic pathways, but the exact relationships between them have not been extensively defined. Some authors (9, 10) have suggested that thromboxane A_2 (TXA_2) could be the mediator of the PAF effects in some pathophysiologic conditions. Moreover, PAF seems to induce an increased prostaglandin E_2 (PGE_2) synthesis in cultured rat mesangial cells (11). Thus, experiments were designed to analyze the interactions between prostanoids

Abbreviations: H_2O_2, hydrogen peroxide; PGE_2, prostaglandin E_2; TXB_2, thromboxane B_2; PAF, platelet-activating factor; RBF, renal blood flow; GFR, glomerular filtration rate; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species; BN, BN-52021, a PAF-receptor antagonist; HPLC, high performance liquid chromatography.

[1]To whom correspondence should be addressed.

and PAF in isolated glomeruli and cultured mesangial cells incubated with H_2O_2, in an attempt to assess whether the effect of H_2O_2 on the synthesis of these eicosanoids can be mediated by PAF, in order to further define the pathophysiologic mechanisms that characterize those renal diseases in which ROS seem to be involved.

MATERIALS AND METHODS

Materials

Collagenase type I A, L-glutamine, phenylmethylsulfonyl fluoride (PMSF), nonlabeled PAF (1-*O*-alkyl-2 acetyl-*sn*-glycero-3-phosphocholine), TXB_2 and PGE_2 standards, and arachidonic acid were purchased from Sigma (St. Louis, MO). RPMI 1640, Hanks balanced salt solution, and fetal calf serum were obtained from Flow Laboratories (Woodcock Hill, UK). [^{3}H]acetate and labeled PAF (1-*O*-[^{3}H]alkyl-2 acetyl-*sn*-glycero-3-phosphocholine, 90 Ci/mmol), tritiated TXB_2 (120 Ci/mmol), tritiated PGE_2 (150 Ci/mmol), and PAF RIA kit were from DuPont (Boston, MA). BN-52021 was kindly provided by Dr. P. Braquet, Institut Henri Beaufour (Le Plessis-Robinson, France). TXB_2 and PGE_2 rabbit antisera were from Advanced Magnetics, Inc. (Cambridge, UK). Alprazolam was a gift of Upjohn Farmoquímica (Madrid, Spain). Bond-Elut C-18 extraction columns of 200 mg were from Analytichem International (Harbor City, CA). A 300 mm × 4 mm stainless-steel μPorasil column was from Waters (Milford, MA).

Glomerular isolation and mesangial cell culture

Renal glomeruli were isolated from Wistar rats, weighing 150–200 g, by successive mechanical sieving (105 and 75 μm) of kidney cortex, after renal perfusion with heparinized saline solution, as previously described (12). Buffer A (Tris 20 mM, NaCl 130 mM, KCl 10 mM, sodium acetate 10 mM, glucose 5 mM, pH 7.45) was used in all the steps of the isolation procedure. Isolated glomeruli (from rats weighing 100–150 g) obtained by a similar mechanical sieving procedure (150 and 50 μm), but under sterile conditions, were treated with collagenase, plated in plastic culture flasks, and incubated as previously described (6, 13) in order to obtain mesangial cells. Studies were performed on days 20–22, and the identity of the cells was confirmed by morphological and functional criteria (6, 13).

Prostanoid synthesis

Isolated glomeruli were centrifuged (120 *g*, 3 min) and resuspended in buffer A with 2.5 mM $CaCl_2$, and incubated in control conditions (buffer A) or in the presence of 10^{-4} M H_2O_2, with or without BN-52021 (5×10^{-5} M, final concentration of BN). The incubations were performed in a final volume of 1 ml at 37°C and the incubation time was 40 min in every case. BN was always added at time 0, but H_2O_2 was added after 10 min. Incubations were stopped by centrifugation at 300 *g* for 3 min at 4°C. Supernatants were collected and frozen (−40°C) until prostaglandin radioimmunoassays were performed. Storing time was never over 15 days. Cultured cells grown in standard cultured flasks were washed twice with buffer A and incubated under the same experimental conditions described for glomeruli. After finishing the incubations, the supernatants were removed and stored at −40°C until analyzed. TXB_2, the stable hydrolysis product of TXA_2, and PGE_2 were measured by radioimmunoassay in glomerular and mesangial cell supernatants, as previously described (12). In every case, results were corrected for the protein content (14). Cell viability was assessed, under the different experimental conditions mentioned above, by measuring the trypan blue dye exclusion and the LDH activity in the supernatants (6).

In another set of experiments directed towards the analysis of specificity of the effects observed, isolated glomeruli were incubated for 30 min at 37°C with different concentrations of H_2O_2 (1 μM–1000 μM) or with a fixed amount of H_2O_2 (100 μM) for variable times (0–30 min). Samples were processed as described above, and TXB_2 was measured in the supernatants. Moreover, the H_2O_2-dependent glomerular synthesis of TXB_2 was also tested after preincubation of glomeruli for 10 min with different BN-52021 concentrations (0.05 μM–50 μM), and with alprazolam (10 μM), another PAF antagonist. The experimental design and the sample management were completely comparable to that previously described for the BN preincubation, and the H_2O_2 concentration selected was 100 μM. Finally, the effect of BN on the arachidonic acid-stimulated glomerular production of TXB_2 was analyzed. For that purpose, isolated glomeruli were preincubated for 10 min with BN-52021 (50 μM) and then incubated for 30 min with or without arachidonic acid. Incubations were performed at 37°C and samples were processed as in the other experiments.

PAF synthesis

Specific [^{3}H]acetate incorporation into PAF was measured in isolated glomeruli and cultured mesangial cells, as previously described (6, 15). Buffer for glomeruli isolation or culture medium of cells was replaced with 1 ml of Krebs buffer (pH 7.4) containing 25 μCi [^{3}H]acetate and 10^{-4} M PMSF. After 15 min at room temperature, H_2O_2 (1–1000 μM in the case of glomeruli and 100 μM in the case of cells) or buffer A was added to the samples, and an additional 30-min incubation at 37°C was performed. A time-course study (0–30 min) of the [^{3}H]acetate glomerular incorporation in presence of 100 μM H_2O_2 was also performed. Reactions were stopped by the addition of 1 ml 20% acetic acid. Suspensions of lysed glomeruli or cells were centrifuged at 500 *g* for 15 min; the

supernatants were passed through Bond-Elut C-18 columns washed with methanol, water, and acetic acid (10%). The samples were applied to the columns, followed by 2 ml 10% acetic acid, 6 ml ethyl acetate, and 6 ml methanol. The eluate was extracted by the method of Bligh and Dyer (16). The chloroform phase was recovered, evaporated to dryness under nitrogen, and stored at −40°C. Extracts were resuspended in 100 μl chloroform, nonlabeled PAF (5×10^{-3} M) was added, and HPLC was performed (6, 17). A normal-phase isocratic HPLC system was used. The mobile phase was acetonitrile–methanol–85% phosphoric acid 130:5:1.5 (by volume) at a flow rate of 1 ml/min; a UV detector (wavelength 203 nm) was used. Calculation of [^{3}H]acetate incorporated as [^{3}H]acetyl-PAF was done by measuring the radioactivity in the fraction eluting with unlabeled PAF standard corrected for recovery (see below).

Finally, immunoassayable PAF produced by mesangial cells was measured by a commercial RIA kit (6). The lipid extraction procedure was comparable to that mentioned above. The recovery was monitored by the addition of 20,000 cpm of ^{3}H-labeled PAF to each sample (mean recovery: 41 ± 5%). The sensitivity of the RIA was 35 pg/tube, and the intraassay and interassay variability were 6.8% and 10.4%, respectively.

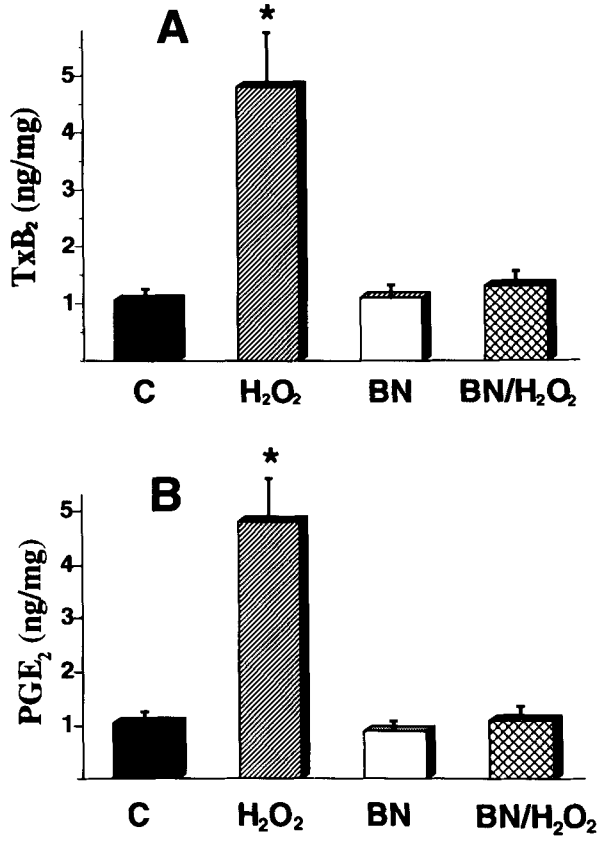

Fig. 1. (A) Production of thromboxane B_2 (TXB_2) and (B) prostaglandin E_2 (PGE_2) by isolated rat glomeruli. C, isolated glomeruli incubated in control conditions with buffer only; H_2O_2, glomeruli incubated with 100 μM H_2O_2; BN, glomeruli incubated with 50 μM BN-52021; BN/H_2O_2, glomeruli incubated with 50 μM BN-52021 and then with added 100 μM H_2O_2. See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 10 experiments. *$P < 0.05$ vs. C, BN and BN/H_2O_2.

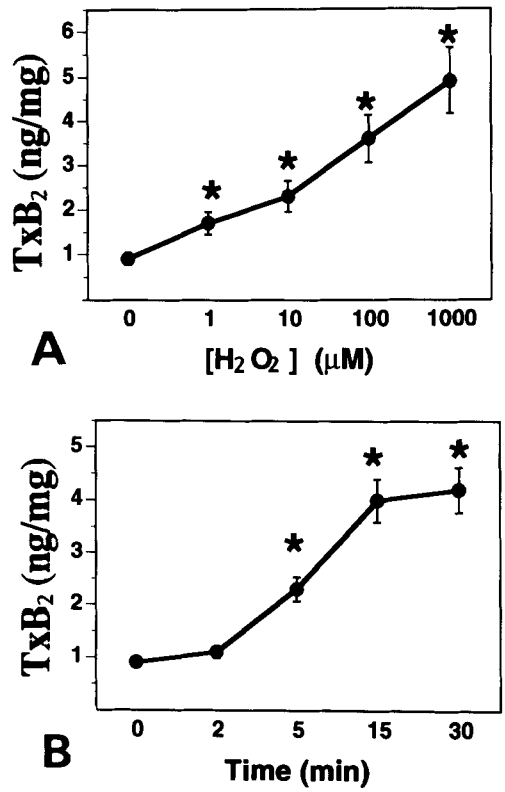

Fig. 2. (A) Effect of different H_2O_2 concentrations on TXB_2 production by isolated rat glomeruli and (B) time-course study of the TXB_2 production by isolated rat glomeruli in presence of H_2O_2 (100 μM). See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 5 experiments. *$P < 0.05$ vs. 0.

Statistical methods

Results are expressed as mean ± SEM and the number of experiments is shown in every case. Comparisons were performed by the unpaired Student's *t*-test, one-way analysis of variance, and Scheffe's multiple comparison test as needed. A $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the effects of H_2O_2 on the glomerular synthesis of TXB_2 (panel A) and PGE_2 (panel B). H_2O_2 increased the synthesis of both prostanoids by about 5-fold, and this effect was completely blocked by preincubation with BN-52021 (Fig. 1, both panels). The H_2O_2-induced TXB_2 glomerular synthesis showed a concentration-dependent pattern (**Fig. 2A**). It appeared in a

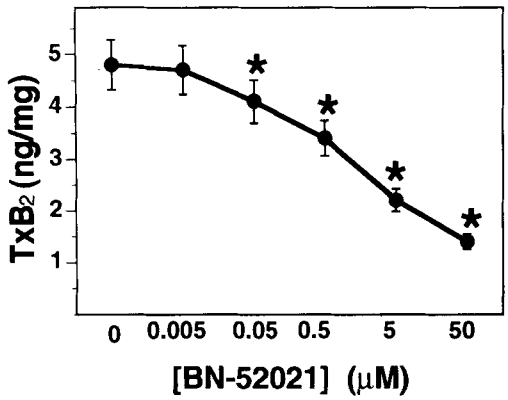

Fig. 3. Effect of different BN-52021 concentrations on TXB_2 synthesis by isolated rat glomeruli. See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 6 experiments. $^*P < 0.05$ vs. 0.

relatively short time, reaching a maximum after 30 min of incubation (Fig. 2B). Moreover, the inhibitory effect observed with BN-52021 on this H_2O_2-dependent TXB_2 glomerular synthesis was also dependent on the PAF antagonist concentration, with a threshold for the BN-52021 inhibitory action of 50 nM and a complete inhibition at 50 μM (**Fig. 3**).

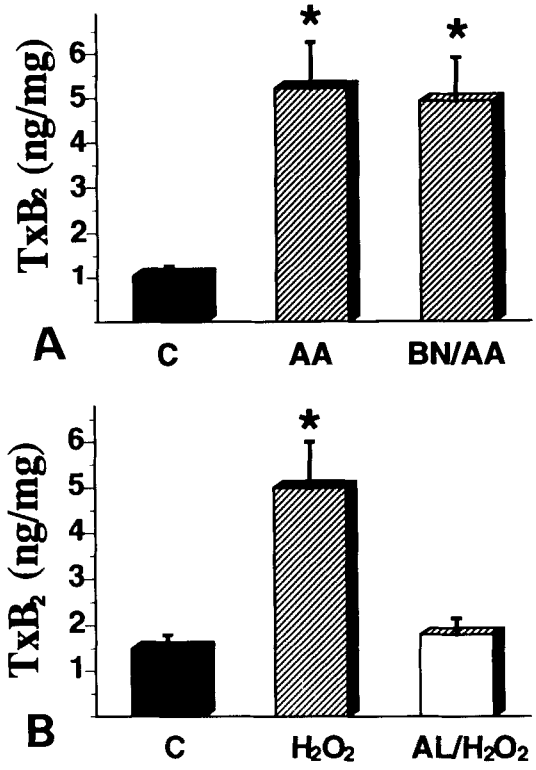

Fig. 4. Production of thromboxane B_2 (TXB_2) in presence of (A) arachidonic acid (AA) and (B) alprazolam (AL) by isolated rat glomeruli. C, isolated glomeruli incubated under control conditions with buffer only; AA, isolated glomeruli incubated with AA 1 μM; BN/AA, isolated glomeruli incubated with 50 μM BN-52021 and then with added 1 μM AA; AL/H_2O_2, isolated glomeruli incubated with 10 μM AL and then with added 100 μM H_2O_2. See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 6 experiments. $^*P < 0.05$ vs. C and AL/H_2O_2.

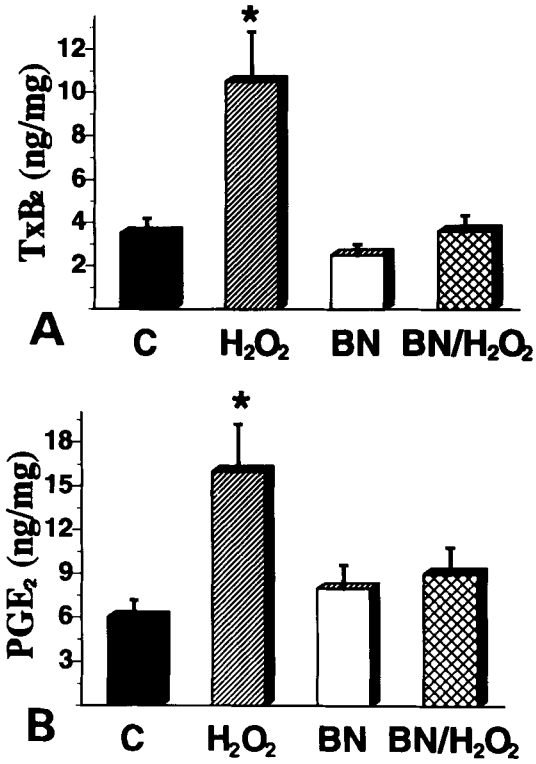

Fig. 5. (A) Production of thromboxane B_2 (TXB_2) and (B) prostaglandin E_2 (PGE_2) by cultured rat mesangial cells. C, cells incubated under control conditions with buffer only; H_2O_2, cells incubated with 100 μM H_2O_2; BN, cells incubated with 50 μM BN-52021; BN/H_2O_2, cells incubated with 50 μM BN-52021 and then with added 100 μM H_2O_2. See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 10 experiments. $^*P < 0.05$ vs. C and BN/H_2O_2.

Figure 4 includes two different kinds of results. Panel A shows the effect of preincubation with BN-52021 on the arachidonic acid-induced TXB_2 glomerular synthesis. As shown in the figure, arachidonic acid induced a significant increase in the TXB_2 production by isolated glomeruli, and this effect was not blocked by the PAF antagonist. Panel B analyzes the effect of alprazolam, another PAF antagonist not related chemically to BN-52021, on the synthesis of TXB_2 by isolated glomeruli. As in the case of BN-52021, alprazolam completely blocked the effect of H_2O_2 on the synthesis of this prostanoid.

The experiments included in **Fig. 5** are comparable to those depicted in Fig. 1, but performed in cultured mesangial cells. The basal TXB_2 (panel A) and PGE_2 (panel B) synthesis by cells was significantly higher than in the case of isolated glomeruli. H_2O_2 induced a significant increase in the cellular production of these prostanoids, an effect that was again completely blocked by BN-52021 (Fig. 5, both panels).

In isolated glomeruli, H_2O_2 induced a dose- and time-dependent increase in the [^{3}H]acetate incorporation in a lipid fraction that eluted with unlabeled PAF in an HPLC system (**Fig. 6**). As these parameters can be considered as a reliable index of PAF synthesis, it can be proposed that

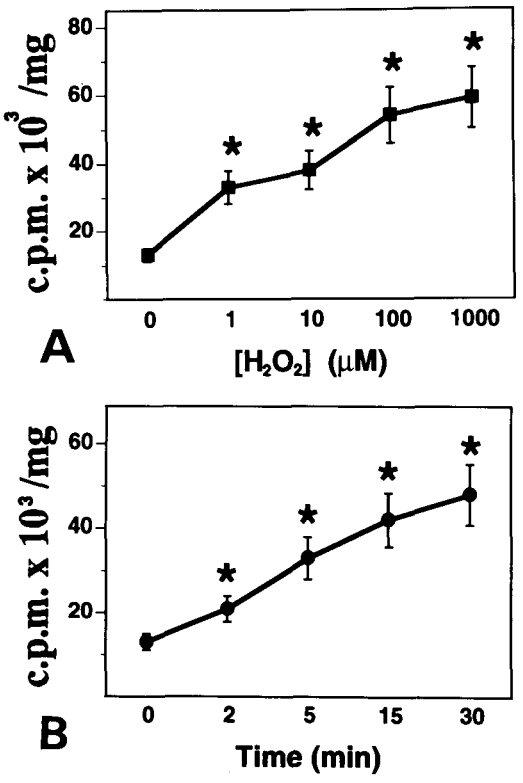

Fig. 6. Effect of different H_2O_2 concentrations on [^{3}H]acetate incorporation by isolated rat glomeruli and (B) time-course study of the [^{3}H]acetate incorporation by isolated rat glomeruli in the presence of H_2O_2 (100 μM). See Methods for further details about incubations. Data are expressed as mean ± SEM and are the mean of 5 experiments. *$P < 0.05$ vs 0.

H_2O_2 stimulates the synthesis of PAF by isolated rat glomeruli in a concentration- and time-dependent manner (Fig. 6). This H_2O_2-dependent PAF stimulation was also observed in cultured rat mesangial cells, and it was possible to demonstrate this action, not only by measuring the [^{3}H]acetate incorporation (**Fig. 7**, panel A), but also by a direct RIA measurement (Fig. 7, panel B).

The LDH release by isolated glomeruli incubated for 30 min with 1 mM H_2O_2 did not differ from that observed in control glomeruli (control: 78 ± 8, H_2O_2: 81 ± 7 mU/mg of glomerular protein). Cells incubated for 30 min with 100 μM H_2O_2 excluded the trypan blue dye in a fashion similar to control cells (96 ± 3 and 97 ± 2%, respectively) and the LDH release was comparable in both cases (control: 270 ± 15, H_2O_2: 278 ± 18 mU/mg of mesangial cell protein).

DISCUSSION

The present results clearly demonstrate that H_2O_2 induced an increased synthesis of prostanoids and PAF in isolated glomeruli and cultured rat mesangial cells. Although the TXA_2/PGE_2 hydroperoxide-induced production was previously described in these structures (4), the H_2O_2-dependent PAF synthesis has been only recently reported (6). Two possible explanations could account for this simultaneous stimulation. First, H_2O_2 might activate phospholipase A_2, thus providing specific substrates for both prostanoid and PAF synthesis. However, the PAF antagonist results do not support this hypothesis, as the glomerular and mesangial effects of H_2O_2 on prostanoid synthesis were completely blocked by BN and alprazolam preincubation. Second, as the main pharmacological effect of BN-52021 seems to be a blockade of the PAF receptors in the cell membrane (18), a better explanation for the results obtained would be that PAF synthesis would be the first step in the prostanoid release. In other words, hydrogen peroxide would induce an increased PAF synthesis in cultured rat mesangial cells and isolated glomeruli and this PAF, secondarily, would stimulate prostanoid synthesis, particularly that of PGE_2 and TXA_2. Different experimental evidence supports this hypothesis. First, the present results clearly show an in-

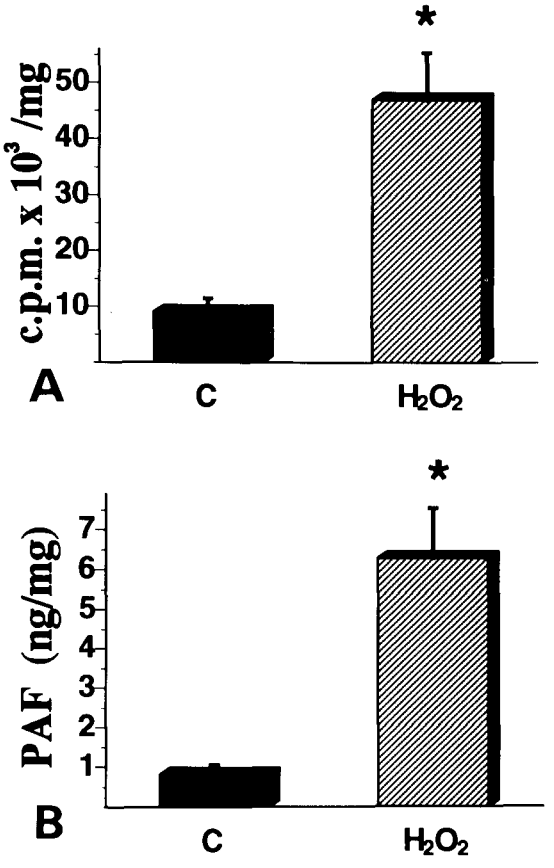

Fig. 7. (A) [^{3}H]acetate incorporation by cultured rat mesangial cells. (B) Immunoreactive PAF production by cultured mesangial cells. C, cells incubated under control conditions with buffer only; H_2O_2, cells incubated with 100 μM H_2O_2. See Methods for further details about incubation. Data are expressed as mean ± SEM and are the mean of 10 experiments. *$P < 0.05$ vs. C.

creased PAF production after H_2O_2 treatment, confirming previous reports of our own group (6); in the particular case of cells, the HPLC [^{3}H]acetate incorporation analysis and the immunoreactive PAF measurement yielded similar results, showing a 6- to 10-fold increase in PAF production after H_2O_2 stimulation. Similar results were published by Lewis et al. (7) in cultured endothelial cells. Second, the time course of H_2O_2-induced TXB_2 glomerular synthesis paralleled that of PAF synthesis, although PAF stimulation appeared just after 2 min of incubation. Third, it is a well-known fact that PAF can stimulate PGE_2 (8) and TXA_2 (9, 10) synthesis under different experimental situations. Finally, when PAF action was prevented by using a PAF receptor antagonist, H_2O_2 was unable to induce an increased synthesis of the prostanoids analyzed.

The possibility that BN itself could have some nonspecific effect on prostanoid synthesis in these studies can be reasonably ruled out. BN alone did not modify TXA_2 and PGE_2 production at all in cultured cells and isolated glomeruli under basal conditions. Moreover, BN did not modify TXA_2 production by isolated glomeruli stimulated with arachidonic acid, the natural substrate of eicosanoids. In addition, alprazolam, another PAF-receptor antagonist, also inhibited the H_2O_2-induced production of glomerular TXA_2.

In conclusion, our results demonstrate the simultaneous production of PGE_2, TXA_2, and PAF in rat mesangial cells or rat glomeruli treated with H_2O_2. In addition, they suggest an intermediate role of PAF in the secondary production of prostanoids. The increased mesangial and glomerular presence of these metabolites as a consequence of ROS stimulation could contribute to an explanation of the pathophysiology of those renal diseases characterized by increased ROS production.

This work was supported by a CICYT Grant (SAF 93/0713).

Manuscript received 15 January 1993, in revised form 26 July 1993, and in re-revised form 19 July 1994.

REFERENCES

1. Couser, W. G. 1985. Mechanisms of glomerular injury in immunocomplex disease. *Kidney Int.* **28:** 569–583.
2. Paller, M. S., J. R. Hoidal, and T. F. Ferris. 1984. Oxygen free radicals in ischemic acute renal failure in the rat. *J. Clin. Invest.* **74:** 1156–1164.
3. Diamond, J. R., J. V. Bonventre, and M. J. Karnovsky. 1986. A role for oxygen free radicals in aminonucleoside nephrosis. *Kidney Int.* **29:** 478–483.
4. Baud, L., M. P. Nivez, D. Chansel, and R. Ardaillou. 1981. Stimulation by oxygen radicals of prostaglandin production by rat renal glomeruli. *Kidney Int.* **20:** 332–339.
5. Schlondorff, D., and R. Ardaillou. 1986. Prostaglandins and other arachidonic acid metabolites in the kidney. *Kidney Int.* **29:** 108–119.
6. Duque, I., C. García-Escribano, M. Rodríguez-Puyol, M. L. Díez-Marqués, J. M. López-Novoa, I. Arribas, I. Hernando, and D. Rodríguez-Puyol. 1992. Effects of reactive oxygen species on cultured rat mesangial cells and isolated rat glomeruli. *Am. J. Physiol.* **263:** F466–F473.
7. Lewis, M. S., R. E. Whatley, P. Cain, T. M. McIntire, S. M. Prescott, and G. A. Zimmerman. 1988. Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. *J. Clin. Invest.* **82:** 2045–2055.
8. Schlondorff, D., and R. Neuwirth. 1986. Platelet-activating factor and the kidney. *Am. J. Physiol.* **251:** F1–F11.
9. Badr, K. F., D. K. DeBoer, K. Takahashi, R. C. Harris, A. Fogó, and H. R. Jacobson. 1989. Glomerular responses to platelet-activating factor in the rat: role of thromboxane A_2. *Am. J. Physiol.* **256:** F35–F43.
10. Macconi, D., A. Benigni, M. Morigi, A. Ubiali, S. Orisio, M. Livio, N. Perico, T. Bertani, G. Remuzzi, and C. Patrono. 1989. Enhanced glomerular thromboxane A_2 mediates some pathophysiologic effect of platelet-activating factor in rabbit nephrotoxic nephritis: evidence from biochemical measurements and inhibitor trials. *J. Lab. Clin. Med.* **113:** 549–560.
11. Schlondorff, D., J. A. Satriano, J. Hagege, J. Perez, and L. Baud. 1984. Effect of platelet-activating factor and serum-treated zymosan on prostaglandin E_2 synthesis, arachidonic acid release and contraction of cultured rat mesangial cells. *J. Clin. Invest.* **73:** 1227–1231.
12. Rodríguez-Puyol, D., G. Arriba, A. Blanchart, J. C. Santos, C. Caramelo, A. Fernandez-Cruz, L. Hernando, and J. M. López-Novoa. 1986. Lack of a direct regulatory effect of atrial natriuretic factor on prostaglandin and renin release by isolated rat glomeruli. *Biochem. Biophys. Res. Commun.* **138:** 496–501.
13. Rodríguez-Puyol, D., S. Lamas, A. Olivera, A. López-Farre, G. Ortega, L. Hernando, and J. M. Lopez-Novoa. 1989. Actions of Cyclosporin A on cultured rat mesangial cells. *Kidney Int.* **35:** 632–637.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1952. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **73:** 265–268.
15. López-Farré, A., D. Gómez-Garre, F. Bernabeu, I. Montañés, I. Millás, and J. M. López-Novoa. 1991. Renal effects and mesangial cell contraction induced by endothelin are mediated by PAF. *Kidney Int.* **39:** 624–630.
16. Bligh, E. G., and W. J. Dyer. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
17. Wardlow, M. L. 1985. Rapid isocratic procedure for separation of platelet-activating factor from phospholipids in human saliva by high performance liquid chromatography. *J. Chromatogr.* **342:** 380–384.
18. Braquet, P., T. Y. Shen, L. Toqui, and B. Vargaftig. 1987. Perspectives in platelet-activating factor research. *Pharmacol. Rev.* **39:** 97–145.