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Nitric Oxide Donors Induce Stress Signaling via Ceramide Formation in Rat Renal Mesangial Cells*

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Exogenous NO is able to trigger apoptosis of renal mesangial cells, and thus may contribute to acute lytic phases as well as to resolution of glomerulonephritis. However, the mechanism involved in these events is still unclear. We report here that chronic exposure of renal mesangial cells for 24 h to compounds releasing NO, including spermine-NO, (*Z*)-1-{*N*-methyl-*N*-[6-(*N*-methylammoniohexyl)-amino]}diazen-1-ium-1,2-diolate (MAHMA-NO), *S*-nitrosoglutathione (GS-NO), and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) results in a potent and dose-dependent increase in the lipid signaling molecule ceramide. Time courses reveal that significant effects occur after 2–4 h of stimulation with NO donors and reach maximal levels after 24 h of stimulation. No acute (within minutes) ceramide production can be detected. When cells were stimulated with NO donors in the presence of phorbol ester, a direct activator of protein kinase C, both ceramide production and DNA fragmentation are completely abolished. Furthermore, addition of exogenous ceramide partially reversed the inhibitory effect of phorbol ester on apoptosis, thus suggesting a negative regulation of protein kinase C on ceramide formation and apoptosis. In contrast to exogenous NO, tumor necrosis factor (TNF)-α stimulates a very rapid and transient increase in ceramide levels within minutes but fails to induce the late-phase ceramide formation. Moreover, TNF fails to induce apoptosis in mesangial cells.

Interestingly, NO and TNFα cause a chronic activation of acidic and neutral sphingomyelinases, the ceramide-generating enzymes, whereas acidic and neutral ceramidases, the ceramide-metabolizing enzymes, are inhibited by NO, but potently stimulated by TNFα. Furthermore, in the presence of an acidic ceramidase inhibitor, *N*-oleoylethanolamine, TNFα leads to a sustained accumulation of ceramide and in parallel induces DNA fragmentation.

In summary, our data demonstrate that exogenous NO causes a chronic up-regulation of ceramide levels in mesangial cells by activating sphingomyelinases and concomitantly inhibiting ceramidases, and that particularly the late-phase of ceramide generation may be responsible for the further processing of a proapoptotic signal.

In recent years, nitric oxide (NO),[1] a gas previously considered a potentially toxic chemical, has become established as a diffusible universal messenger mediating cell-cell communication throughout the body. NO is a well known mediator of blood vessel relaxation that helps to maintain blood pressure. In the central nervous system NO acts as a nonconventional type of neurotransmitter contributing to long term potentiation. In addition NO is responsible for parts of the cytotoxic activity of macrophages that helps to defeat microbes and tumor cells. Excessive and uncontrolled production of NO is associated with severe diseases like septic shock, stroke, neurodegeneration, diabetes mellitus, arthritis, and other forms of acute and chronic inflammation (1–4). In mammals, the synthesis of NO is catalyzed by nitric-oxide synthase (NOS), which exists in several isoforms. NOS catalyzes the oxidation of L-arginine to citrulline and NO.

Renal mesangial cells exposed to proinflammatory cytokines like interleukin-1 or tumor necrosis factor-α express an inducible NOS and produce large amounts of NO (5, 6), which may contribute to certain forms of glomerulonephritis (4, 7, 8). Glomerular mesangial and endothelial cells are not only production sites of NO but are also themselves targets for NO and undergo apoptotic cell death upon exposure to high concentrations of NO (9, 10). Apoptosis is a controlled biological strategy to remove damaged or unwanted cells from a given tissue and thus is involved in important physiological and pathophysiological processes (11, 12).

NO-induced apoptosis has been described for a variety of cell types including macrophages (13, 14), neurons (15, 16), thymocytes (17, 18), and glomerular mesangial, endothelial, and epithelial cells (9, 10). By contrast, under certain circumstances NO can inhibit apoptosis for example in endothelial cells where NO inhibits interleukin 1β-converting enzyme-like proteases (19).

In this context, another molecule, the sphingolipid ceramide, has attracted attention as a candidate regulator of apoptosis. Ceramide is generated by sphingomyelinase-catalyzed sphingolipid turnover and has been characterized as an important intracellular mediator of stress signaling, which under certain conditions can cause apoptosis (20, 21).

In this study we present evidence that exogenously applied NO induces a chronic up-regulation of intracellular ceramide levels and that this delayed increase in ceramide is paralleled by the onset of apoptosis.

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[1] The abbreviations used are: NO, nitric oxide; NOS, nitric-oxide synthase; TNFα, tumor necrosis factor α; DMEM, Dulbecco's modified Eagle's medium; MAHMA-NO, (*Z*)-1-{*N*-methyl-*N*-[6-(*N*-methylammoniohexyl)amino]}diazen-1-ium-1,2-diolate; spermine-NO, (*Z*)-1-{*N*-[3-aminopropyl]-*N*-[4-(3-aminopropylammonio)butyl]amino}-diazen-1-ium-1,2-diolate; GS-NO, *S*-nitrosoglutathione; SNAP, *S*-nitroso-*N*-acetyl-D,L-penicillamine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C.

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EXPERIMENTAL PROCEDURES

Chemicals—(*Z*)-1-{*N*-Methyl-*N*-[6-(*N*-methylammoniohexyl)amino]}-diazen-1-ium-1,2-diolate (MAHMA-NO), (*Z*)-1-{*N*-[3-aminopropyl]-*N*-[4-(3-aminopropylammonio)butyl]amino}-diazen-1-ium-1,2-diolate (spermine-NO), *S*-nitrosoglutathione (GS-NO), and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) were from Alexis Corp., Läufelfingen, Switzerland; [^{14}C]serine (specific activity, 53 Ci/mol) and [^{14}C]sphingomyelin (specific activity, 55 Ci/mol) were from Amersham Pharmacia Biotech Buckinghamshire, UK; [^{14}C]ceramide (specific activity 55 Ci/mol) was from ICN Pharmaceuticals, Inc., Irvine, CA; 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was purchased from Calbiochem, Lucerne, Switzerland; dibutyryl cyclic GMP and *N*-oleoylethanolamine were from Sigma; all cell culture nutrients were from Life Technologies, Inc., Breda, the Netherlands; TNFα was a gift of Knoll AG, Ludwigshafen, Germany.

Cell Culture—Rat renal mesangial cells were cultivated and characterized as described previously (22, 23). In a second step, single cells were cloned by limited dilution on 96-well plates. Clones with apparent mesangial cell morphology were characterized by positive staining for the intermediate filaments desmin and vimentin, which is considered to be specific for myogenic cells, positive staining for Thy 1.1 antigen, and negative staining for Factor VIII-related antigen and cytokeratin, excluding endothelial and epithelial contaminations, respectively. For the experiments in this study passages 10–23 were used.

Lipid Extraction and Ceramide Quantitation—Confluent mesangial cells in 30-mm diameter dishes were labeled for 24 h with [^{14}C]serine (0.2 μCi/ml) and stimulated as indicated. The reaction was stopped by extraction of lipids (24), and ceramide was resolved by sequential one-dimensional TLC using chloroform/methanol/ammonia (65:35:7.5; v/v) followed by chloroform/methanol/acetic acid (9:1:1; v/v). Spots corresponding to ceramide were analyzed and quantitated using a Berthold (Nashua, NH) LB 2842 automatic TLC scanner.

Apoptosis Assay—Confluent mesangial cells in 60-mm diameter dishes were incubated with the indicated stimuli in DMEM containing 0.1 mg/ml fatty acid-free bovine serum albumin for the indicated time periods. Thereafter, oligonucleosomal DNA fragmentation, a characteristic biochemical feature of apoptotic cell death, was measured using a nucleosome DNA-enzyme-linked immunosorbent assay (Boehringer), which quantitatively records histone-associated DNA fragments.

Acidic and Neutral Sphingomyelinase Assay—Confluent mesangial cells in 60-mm diameter dishes were incubated with the indicated stimuli in DMEM containing 0.1 mg/ml fatty acid-free bovine serum albumin for the indicated time periods. Thereafter cells were homogenized in lysis buffer containing 50 mM sodium acetate, pH 4.5, 0.5% Triton X-100, 5 mM $MgCl_2$, 1 mM EDTA for the acidic sphingomyelinase, and 50 mM Tris, pH 7.4, 0.5% Triton X-100, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol for the neutral sphingomyelinase. Cell homogenates were centrifuged for 10 min at 14,000 × *g*, and the supernatant was taken for an *in vitro* assay. 100 μg of protein in a total volume of 100 μl was incubated for 30 min at 37 °C with 20 nCi of [^{14}C]sphingomyelin. Thereafter, the reaction was stopped by the addition of 200 μl of water and 2 ml of chloroform/methanol (2:1; v/v). After phase separation, the radioactivity in the upper phase containing the phosphocholine was counted in a β-counter.

Acidic and Neutral Ceramidase Assay—Confluent mesangial cells were stimulated as described above and homogenized in lysis buffer containing 50 mM sodium acetate, pH 4.5, 0.5% Triton X-100, 5 mM $MgCl_2$, 1 mM EDTA, and 5 mM D-galactonic acid-γ-lactone for the acidic ceramidase, and 50 mM Tris, pH 8.0, 0.5% Triton X-100, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM D-galactonic acid-γ-lactone for the neutral ceramidase. Cell homogenates were centrifuged for 10 min at 14,000 × *g*, and the supernatant was taken for an *in vitro* assay. 100 μg of protein in a total volume of 100 μl was incubated for 20 h at 37 °C with 20 nCi of [^{14}C]ceramide. Thereafter, the reaction was stopped by the addition of 200 μl of water, and lipid extraction was performed by addition of 2 ml of chloroform/methanol (2:1; v/v). The lower phase was concentrated and lipids were resolved by TLC using chloroform/methanol/ammonia (90:20:0.5; v/v) as a solvent. Spots corresponding to ceramide and sphingosine were analyzed and quantitated using a Berthold (Nashua, NH) LB 2842 automatic TLC scanner.

RESULTS

It is well documented from our previous studies that glomerular mesangial cells and endothelial cells are able to undergo apoptosis upon stimulation with exogenous NO donors (9, 10). However the exact mechanism by which the short-lived gas NO mediates its apogenic effect is still poorly understood.

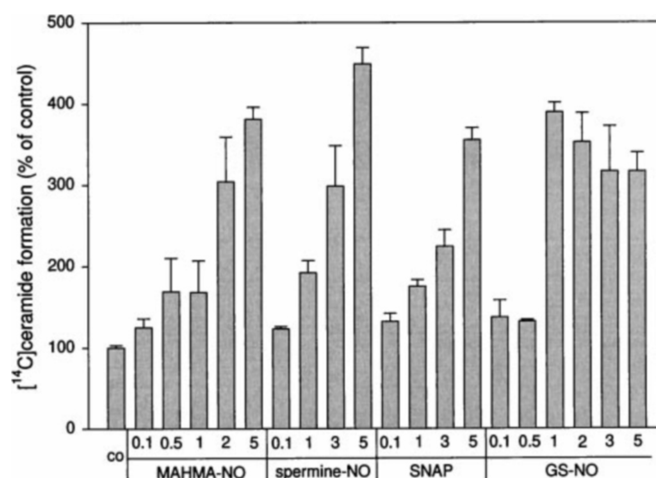

FIG. 1. **Concentration dependence of long term ceramide formation by different NO donors in mesangial cells.** [^{14}C]Serine-labeled mesangial cells were stimulated for 24 h with the indicated concentrations (in mM) of MAHMA-NO, spermine-NO, SNAP, and GS-NO. Thereafter lipids were extracted, separated by TLC as described under "Experimental Procedures," and the spots corresponding to ceramide were quantitated on a TLC scanner. Data are expressed as percentage of control values and are means ± S.D., *n* varies between 3 and 6.

In this study we evaluated the effect of exogenously delivered NO on the stress-signaling molecule ceramide in mesangial cells. Fig. 1 shows that long term treatment of mesangial cells for 24 h with different NO donors like MAHMA-NO, spermine-NO, SNAP, and GS-NO stimulates a dose-dependent increase in ceramide levels. Time course studies reveal no acute increase of ceramide within the first 60 min of NO stimulation (Fig. 2*A*). However, significant increases of ceramide levels occur after 2–4 h of stimulation and reach maximal levels (3–4-fold increase) after 24 h of stimulation with GS-NO (Fig. 2*B*). In contrast, TNFα causes a very rapid and transient increase in ceramide formation (Fig. 2*A*) but fails to induce a late-phase increase of ceramide as it is seen for NO (Fig. 2*B*).

The NO-induced ceramide production is independent of cGMP formation as the membrane-permeant cGMP analog dibutyryl cGMP (10 μM–1 mM) does not cause an increased ceramide generation (data not shown). To further elucidate the mechanism of NO-induced ceramide production we treated cells with a phorbol ester, a direct activator of protein kinase C. Phorbol esters have been reported to block the proapoptotic activity of NO in several cell types (25, 26). Fig. 3*A* shows that the phorbol ester TPA causes a dose-dependent inhibition of NO-induced ceramide formation. In parallel, NO-induced DNA fragmentation is also blocked by increasing concentrations of TPA (Fig. 3*B*), thus suggesting a negative regulatory role for PKC on NO-stimulated ceramide formation and apoptosis. To examine whether ceramide elevations are required for NO to trigger apoptosis we added exogenous C_6-ceramide to the cells exposed to spermine-NO in the presence of TPA. Indeed, as shown in Fig. 3*C* exogenous ceramide at least partially bypassed the suppression of NO-induced apoptosis by TPA.

To elucidate the differential effects of NO and TNFα on ceramide formation, we analyzed the activities of the ceramide-generating and metabolizing enzymes, *i.e.* the acidic and neutral sphingomyelinases and the acidic and neutral ceramidases, respectively. Treatment of mesangial cells with GS-NO reveals chronic activation of the acidic sphingomyelinase (Fig. 4*A*) as well as of the neutral sphingomyelinase (Fig. 4*B*), although with distinctly different time patterns. The acidic sphingomyelinase was activated more rapidly, reaching maximal activity 2 h after addition of GS-NO and thereafter slowly

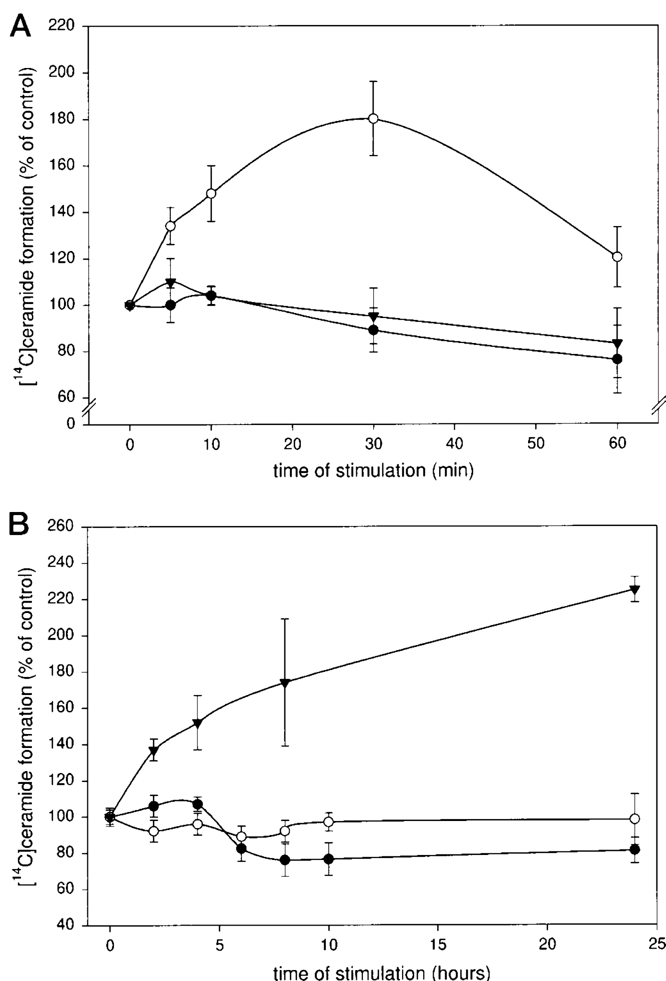

FIG. 2. **Time course of NO- and TNFα-induced ceramide formation in mesangial cells.** [^{14}C]Serine-labeled mesangial cells were stimulated with either vehicle (●), GS-NO (2 mM; ▼), or TNFα (1 nM; ○) for short term (*A*) or long term (*B*) periods as indicated. Thereafter lipids were extracted, separated by TLC as described under "Experimental Procedures," and the spots corresponding to ceramide were quantitated on a TLC scanner. Data are expressed as percentage of control values and are means ± S.D., n = 3–4.

declining over the next several hours but still remaining significantly elevated after 24 h of stimulation (Fig. 4*A*). By contrast, the neutral sphingomyelinase activity shows a lag period of 4 h before steadily increasing over the next 20 h of GS-NO stimulation. After a very rapid and transient (10–20 min) increase in neutral sphingomyelinase activity (data not shown) TNFα also triggers a delayed activation of the acidic and neutral sphingomyelinases. A lag period of 4–8 h after TNFα stimulation is required before significant chronic increases of acidic (Fig. 4*A*) and neutral (Fig. 4*B*) sphingomyelinase activities are detected which subsequently persisted for 24 h.

Strikingly, we observed that TNFα stimulation causes a chronic up-regulation of acidic and neutral ceramidase activities in mesangial cells (Fig. 5, *A* and *B*), whereas GS-NO leads to a time-dependent inhibition of acidic and neutral ceramidase activities (Fig. 5, *A* and *B*). This opposite behavior may explain why GS-NO causes a sustained increase in ceramide levels whereas TNFα-induced changes in sphingomyelinase and ceramidase activities counterbalance each other leaving ceramide levels unchanged (Fig. 2*B*).

To test whether the inhibitory effect of NO on acidic and neutral ceramidase activities is caused by a direct modulation of the enzymes, increasing concentrations of the rapidly decomposing NO donor MAHMA-NO ($t_{1/2}$ = 1–2 min) were incubated *in vitro* with the cell extracts containing active acidic and neutral ceramidases. However, up to concentrations of 1 mM MAHMA-NO, no significant inhibition of either ceramidase activity is detected thus excluding a direct inhibitory modulation of the enzymes by NO (data not shown).

We speculated that the lack of sustained ceramide generation by TNFα might be responsible for the failure of cytokines like TNFα or interleukin 1β to induce mesangial cell apoptosis, despite inducible NOS (iNOS) up-regulation and high levels of endogenously produced NO (10, 27). To evaluate this hypothesis, we used an acidic ceramidase inhibitor *N*-oleoylethanolamine (28) to deplete acidic ceramidase activity in mesangial cells. Inhibition of ceramide metabolism will lead to increased ceramide levels and given the case that ceramide is responsible for initiating apoptosis, we expected to get an increased apoptotic response. Indeed, as shown in Fig. 6, in the presence of *N*-oleoylethanolamine, mesangial cells display an enhanced rate of apoptosis when stimulated with TNFα. *N*-Oleoylethanolamine alone had no effect on apoptosis *per se*.

DISCUSSION

The majority of physiological functions of NO appears to be mediated by activation of its main intracellular receptor guanylate cyclase (29, 30). By nitrosation of the heme moiety NO increases guanylate cyclase activity by 2 orders of magnitude (31, 32). The generation of cyclic GMP and the subsequent regulation of cyclic GMP-dependent protein kinases, cyclic GMP-regulated cyclic nucleotide phosphodiesterases, and ion channels is responsible for functional cell responses triggered by NO (29, 30). However, under pathological conditions, when large quantities of NO are generated, other reactions become more prominent and predominate. Among those are interactions of NO with iron-sulfur centers, protein thiols, lipids, and zinc fingers in various important cellular molecules (33). Another important reaction partner of NO is superoxide, and the subsequent generation of peroxynitrite, which, in the presence of a metal catalyst (like Fe^{3+}), can be converted to nitronium ion that readily nitrates tyrosine residues in proteins, and thus may block critical phosphorylation reactions in signal transduction cascades (34). These latter reactions may be responsible for the pathophysiological roles played by large amounts of NO produced by the inducible isoform of NOS that contributes to inflammatory and autoimmune diseases and to host defense mechanisms required to successfully cope with microbial infections. The number of newly discovered targets of NO is steadily increasing, and NO was shown to affect crucial intracellular signaling pathways. In Jurkat T cells, human umbilical vein endothelial cells, and in mesangial cells NO was found to activate distinct subgroups of mitogen-activated protein kinases including the stress-activated protein kinases and p38 kinase (9, 35).

In this study we demonstrate for the first time that NO triggers ceramide formation in glomerular mesangial cells in a cyclic GMP-independent manner. This observation provides an unexpected link between two highly versatile signaling molecules. The sphingomyelin pathway is a ubiquitous signaling pathway that is used by an increasing number of cell surface receptors and environmental stress factors and includes the generation of ceramide by acidic or neutral sphingomyelinases (20, 21). Depending on the cell type ceramide was shown either to trigger cell differentiation or proliferation or to initiate programmed cell death (apoptosis) (20, 21). Moreover, stress-induced apoptosis was reported to require ceramide-induced signaling via the stress-activated protein kinase cascade (36). Our data now provide evidence for cross-communication between the NO signaling system and the sphingolipid signaling pathway. Strikingly, we found that NO increases ceramide levels in

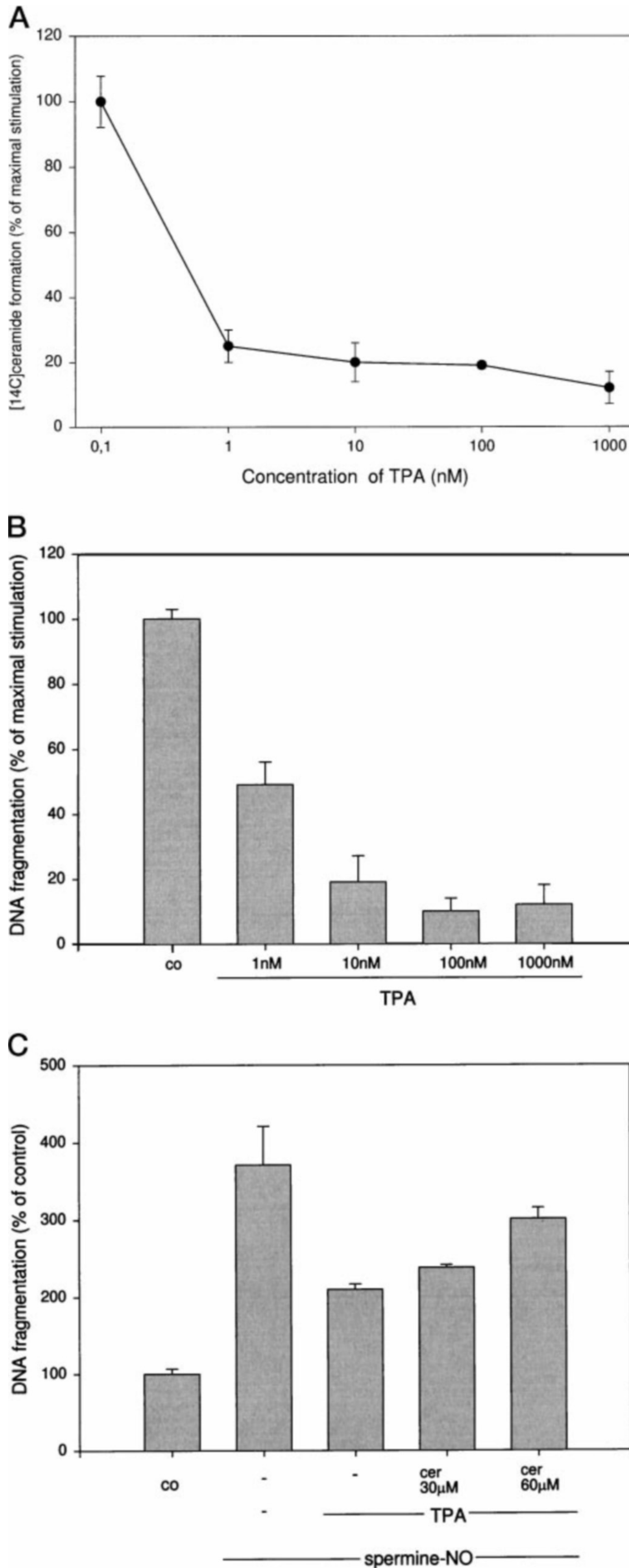

FIG. 3. **Effect of PKC on NO-induced ceramide formation (*A*) and DNA fragmentation (*B*) in mesangial cells.** *A*, [^{14}C]serine-labeled mesangial cells were stimulated for 24 h with either vehicle or 2 mM spermine-NO in the presence of the indicated concentrations of the phorbol ester TPA. Thereafter lipids were extracted and separated by TLC as described under "Experimental Procedures," and the spots corresponding to ceramide were quantitated on a TLC scanner. Data are expressed as percentage of control values and are means ± S.D., n = 2. *B*, confluent mesangial cells were stimulated for 24 h with either vehicle (*co*) or 2 mM spermine-NO in the presence of the indicated concentrations of TPA. Thereafter DNA fragmentation was measured as described under "Experimental Procedures." Data are expressed as percentage of control values and are the means ± S.D., n = 3. *C*, confluent mesangial cells were stimulated for 24 h with either vehicle (*co*) or 2 mM spermine-NO in the presence of 100 nM TPA and the indicated concentrations of C_6-ceramide. Thereafter DNA fragmentation was measured as described under "Experimental Procedures." Data are expressed as percentage of control values and are means ± S.D., n = 3.

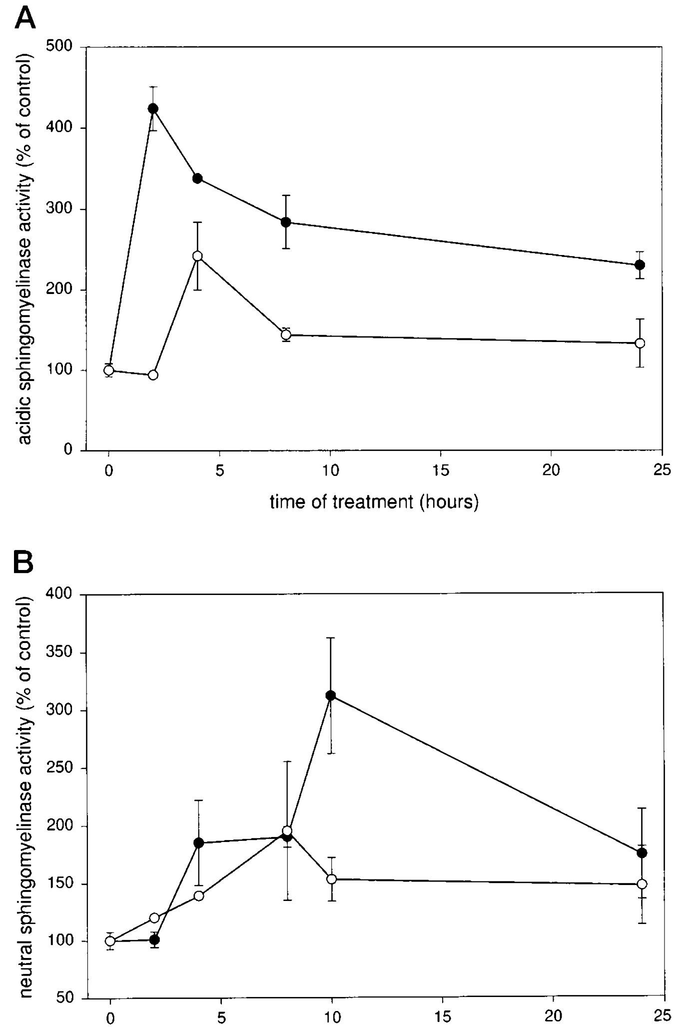

FIG. 4. **Effect of NO and TNFα on acidic and neutral sphingomyelinase activities in mesangial cells.** Confluent mesangial cells were stimulated for the indicated time periods with either GS-NO (2 mM; ●) or TNFα (2 nM; ○). Thereafter cell extracts were prepared as described under "Experimental Procedures" and analyzed for acidic (*A*) and neutral (*B*) sphingomyelinase activities. Results are expressed as percentage of control values and are means ± S.D., n = 3.

the cell by a dual mechanism; on the one hand it stimulates the ceramide-producing enzymes acidic and neutral sphingomyelinases, thus leading to an increased ceramide formation, and on the other hand NO inhibits the ceramide-metabolizing enzymes, *i.e.* acidic and neutral ceramidases, and thereby prevents degradation of ceramide resulting in an amplified increase in ceramide steady-state levels. By contrast, TNFα, besides activating the sphingomyelinases like NO does, causes a potent activation of the ceramidases, which fully compensates for the increased generation of ceramide, and no net change in chronic ceramide production is observed. This may also explain why mesangial cells undergo apoptosis when NO is delivered by exogenous sources but are resistant to endog-

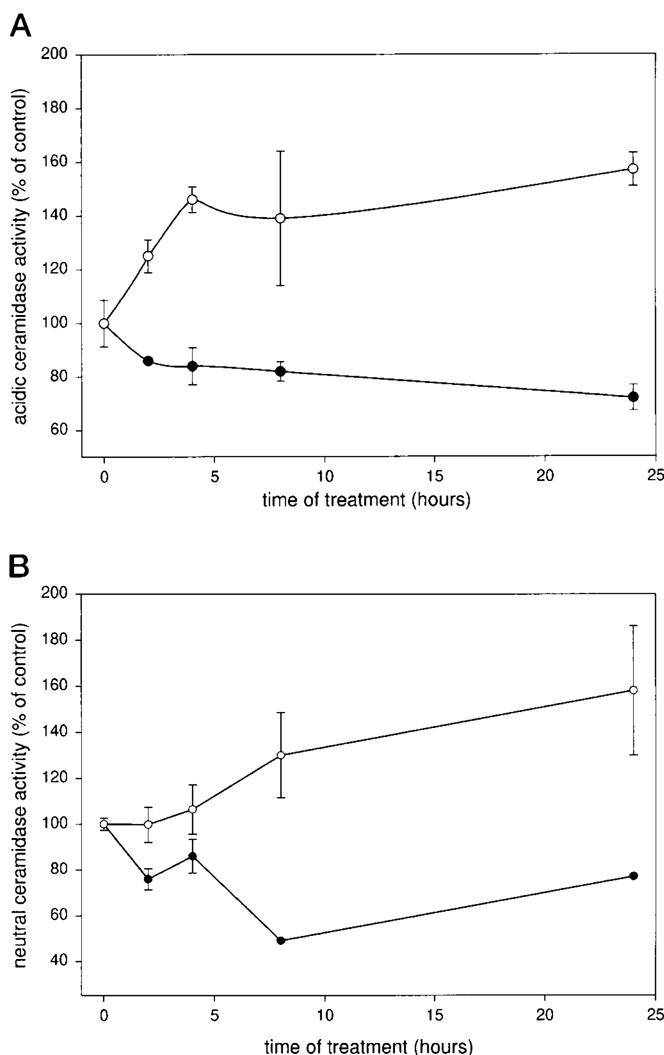

FIG. 5. **Effect of NO and TNFα on acidic and neutral ceramidase activities in mesangial cells.** Confluent mesangial cells were stimulated for the indicated time periods with either GS-NO (2 mM; ●) or TNFα (2 nM; ○). Thereafter cell extracts were prepared as described under "Experimental Procedures" and analyzed for acidic (*A*) and neutral (*B*) ceramidase activities. Results are expressed as percentage of control values and are means ± S.D., n = 3.

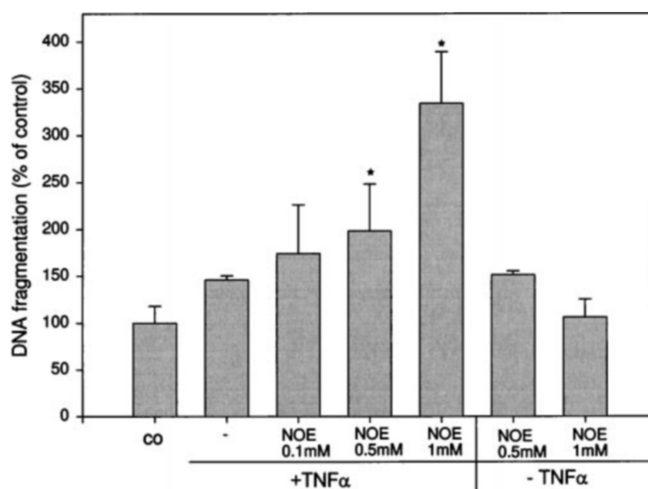

FIG. 6. **Effect of *N*-oleoylethanolamine on TNFα-induced DNA fragmentation in mesangial cells.** Confluent mesangial cells were stimulated for 24 h with TNFα (2 nM) in the absence or presence of the indicated concentrations of *N*-oleoylethanolamine (*NOE*). Thereafter DNA fragmentation was measured as described under "Experimental Procedures." Data are expressed as percentage of control values and are means ± S.D., n = 3. *, $p < 0.05$, statistically significant difference compared with TNFα-stimulated control.

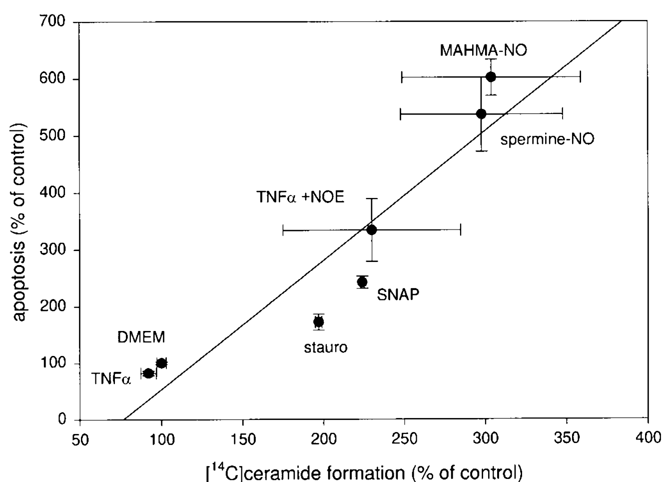

FIG. 7. **Correlation between ceramide formation and induction of apoptosis in mesangial cells.** Mesangial cells were stimulated for 24 h with either DMEM (control), TNFα (2 nM), TNFα plus NOE (1 mM), MAHMA-NO (2 mM), spermine-NO (2 mM), SNAP (1 mM), and staurosporine (*stauro*, 100 nM). Thereafter ceramide formation and cell apoptosis were measured as described under "Experimental Procedures." Data are means ± S.D. (n = 3–6). A linear regression was performed and revealed a correlation coefficient between ceramide formation and apoptosis of 0.88.

enously produced NO after cytokine-induced inducible NOS expression (10, 27). Activation of both acidic and neutral ceramidase activities has also been reported to occur in interleukin 1β-exposed rat hepatocytes (37).

It is tempting to speculate that NO either directly or indirectly affects one of the ceramide-producing or -metabolizing enzymes, *i.e.* acidic or neutral sphingomyelinases or ceramidases. Work is presently in progress to elucidate possible direct effects of NO on enzymes in the ceramide signaling cascade. Whether the NO-induced formation of ceramide is causally related to apoptosis of mesangial cells seen after exposure to NO donors (9, 10) remains to be elucidated. It is however noteworthy that it is not the formation of ceramide *per se* but rather the prolonged and sustained increase of ceramide levels that correlates with programmed cell death. This becomes obvious after inhibition of acidic ceramidase by *N*-oleoylethanolamine (28), which enables mesangial cells to undergo apoptosis after TNFα stimulation (Fig. 6). However, it should be noted that this inhibitor has only been characterized in a few systems, and data on the selectivity of the compound in mesangial cells are presently not available. Inhibition of ceramidase now unveils the stimulatory action of TNFα on acidic and neutral sphingomyelinases and gives rise to sustained increases in ceramide levels in mesangial cells, which may be required to trigger apoptosis.

In Fig. 7 we have compiled data on ceramide production in and apoptosis of mesangial cells in response to a number of different stimuli. There is a strong correlation between chronic ceramide production and apoptosis of the cells. However, further work is required to formally establish a causal link between these two phenomenons.

Another interesting aspect of this study is the negative regulatory role of PKC in NO-induced ceramide formation. Previously, we have shown that mesangial cells express four PKC isoenzymes, PKC-α, -δ, -ϵ, and –ζ (38, 39). Moreover, we demonstrated that ceramide is able to directly interact with PKC-α and –δ, but not with PKC-ϵ and -ζ isoenzymes (40, 41). These

data together with the results presented in this study raise the intriguing possibility of PKC-α and/or PKC-δ acting as negative regulators of the sphingomyelin cycle, in a way similar to the PKC-α-mediated feedback regulation of hormone-stimulated phosphoinositide turnover (38, 42–44). Interestingly, C_2-ceramide-induced internucleosomal DNA fragmentation in U937 cells could be blocked by treatment of cells with phorbol ester (45), thus providing further evidence for a link between NO-induced ceramide production and subsequent apoptosis. The direct target of PKC mediating this negative feedback regulation is not yet known. It will be important though to see whether the NO-induced ceramide signal is further propagated along the different signaling pathways thought to be targeted by this lipid and to be crucially involved in a cell's decision to live or to die.

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