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# Nitric oxide donor-induced apoptosis in smooth muscle cells is modulated by protein kinase C and protein kinase A

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**Abstract**

We have demonstrated previously that exogenously applied nitric oxide (NO) redox species induced apoptosis in smooth muscle cells. The present studies were undertaken to characterize further the potential role of protein kinase C and protein kinase A in the regulation of *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis in smooth muscle cells. *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis was prevented by the protein kinase C inhibitors, calphostin C and H-7, and was potentiated by protein kinase C activator, phorbol 12-myristate 13-acetate (PMA). Furthermore, *S*-nitroso-*N*-acetylpenicillamine prolonged membrane translocation of protein kinase C-$\varepsilon$. The membrane permeable analogue, dibutyryl–cAMP (Db–cAMP), potentiated *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis, whereas the selective protein kinase A competitive inhibitor, Rp–cAMP, prevented *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis. These results indicate that both protein kinase C and protein kinase A are involved in *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis in smooth muscle cells.


*Keywords:* Nitric oxide; Apoptosis; Smooth muscle cell; Protein kinase C; Protein kinase A

## 1. Introduction

Nitric oxide (NO) is a diffusible messenger that mediates a number of physiological processes in a range of cell and tissue types (Schmidt and Walter, 1994). Among the most thoroughly investigated actions of NO is induction of vascular relaxation. NO exerts its effects on the vasculature by increasing guanosine 3′,5′-cyclic monophosphate (cGMP) in smooth muscle cells (Ignarro et al., 1987), causing protein phosphorylate events downstream (Stamler, 1994). NO is synthesized from molecular oxygen and the guanidino group of L-arginine by several isoforms of nitric oxide synthase (Nathan, 1992). The production of large quantities of NO is coupled to the activation of the smooth muscle cells by cytokines such as interleukin-1. Under these conditions, NO redox species contribute to the cytostatic and cytotoxic activities of these cells. To date, the exact mechanisms by which NO exerts its cytostatic/cytotoxic properties are not known clearly. Nitrosylation (Stamler et al., 1992), and mono- and poly-ADP ribosylation induced by NO (Dimmeler et al., 1992), or interactions of NO with metal containing proteins leading to the inhibition of enzymes required for cellular metabolism and DNA synthesis (Henry et al., 1993) might be involved.

Recently, a link between NO formation and apoptosis was proposed (Sarih et al., 1993; Nishio et al., 1996a). The apoptotic death process is characterized by morphological change, i.e., nuclear and cytoplasmatic condensation, nuclear fragmentation, and apoptotic body formation, as well as biochemical markers, i.e., internucleosomal DNA fragmentation, known as ‘DNA laddering’. It has been reported that NO-induced apoptotic signaling causes an early accumulation of the tumor suppressor p53 prior to DNA fragmentation (Messmer and Brune, 1996a). Also, it has been reported that Bcl-2 protects macrophage from NO-induced apoptosis (Messmer et al., 1996b). However, mechanistically, the apoptogenic action of NO is still poorly understood.

It is known that smooth muscle cells can be killed by NO through apoptosis, but the mechanisms by which this occurs remain undefined. A further well-characterized effect of NO is the activation of soluble guanylyl cyclase, leading to increased levels of intracellular cGMP and subsequent activation of cGMP-dependent protein kinase. However, our previous work indicated that modulation of

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cGMP levels is not involved in the induction of apoptosis (Nishio et al., 1996a). Alternatively, NO may act through completely different mechanisms and apoptosis may be regulated by the activity of the signal transduction systems involving protein kinase C and protein kinase A. For example, activation of protein kinase C can increase NO-induced apoptosis in HL-60 cells (Chang-Duck et al., 1997).

In this study, we employed pharmacological agents to examine the importance of several signal transduction pathways in regulating NO-induced apoptosis. We demonstrated that modulation of the activity of protein kinase C and protein kinase A can ultimately influence NO-induced apoptosis in smooth muscle cells.

## 2. Materials and methods

### 2.1. Materials

*S*-nitroso-*N*-acetyl penicillamine and 3-morpholinosydnomine hydrochloride (SIN-1) were obtained from Biomol. Dibutyryl–cAMP, calphostin C, phorbol 12-myristate 13-acetate (PMA) and H-7 were from Sigma, and all cell culture materials were from Life Technologies. Rp–adenosine 3′,5′-cyclic monophosphothioate triethylamine (Rp–cAMP) was from RBI. Rabbit polyclonal antibodies to protein kinase C-$\alpha$, -$\beta$, -$\gamma$, -$\delta$, -$\varepsilon$ and -$\zeta$ were from Life Technologies.

### 2.2. Cell culture

Aortic smooth muscle cells were obtained from the thoracic aorta of Japanese white rabbits by the method described by Travo et al. (1980). The cells ($1 \times 10^5$) were seeded into 35-mm diameter dishes and maintained in 2 ml of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. The cells were used between the third and fifth passage. After subconfluence, the medium was exchanged for 2 ml of serum-free DMEM. The cells were used for experiments after 24 h.

### 2.3. Analysis of DNA fragmentation in agarose gels

Smooth muscle cells ($1 \times 10^5$) were lysed at 37°C for 60 min in a buffer containing 0.5% sodium *N*-lauroyl–sarcosinate, 50 mM Tris buffer (pH 7.8) and 10 mM EDTA. The lysate was then incubated in RNAse (100 $\mu$g/ml) for 0.5 h at 37°C. After proteinase K (100 $\mu$g/ml) treatment for 1 h at 50°C, DNA was extracted with an equal volume of phenol/chloroform, 1:1 and precipitated with 1/10th volume 3 M sodium acetate and 2.5 volume ice-cold ethanol at room temperature for 1 h. DNA samples were loaded onto 2% agarose gels and run at 100 V for $\sim$3 h (Wyllie et al., 1980).

### 2.4. [$^3$H] thymidine assay

Subconfluent plates of smooth muscle cells were labeled with [$^3$H] thymidine (1 mCi/ml) for 36 h. Then after the cells were incubated with the indicated media, DNA fragmentation was quantitated as follows: 0.5 ml of lysis buffer was added to each culture well and mixed by pipetting, and the suspension was transferred to an Eppendorf tube, incubated on melting ice for 10 min, and centrifuged at 8,000 *g* for 5 min at 4°C. Subsequently, fragmented radiolabeled DNA was counted in the supernatant by liquid scintillation counting. The radioactivity of cells treated with lysis buffer and ultrasound homogenator was used as total activity. Results are expressed as fragmented DNA as a percentage of total DNA (Baumgartner-Parzer et al., 1995).

### 2.5. Cell fractionation and protein kinase C immunoblot analysis

Cells were washed with phosphate-buffered saline (PBS) and scraped from dishes with a rubber policeman. The cell suspension was centrifuged at 110*g* for 5 min to remove PBS, and the cell pellet was lysed in a buffer containing 50 mM Tris/HCl (pH 7.5), 5 mM EDTA, 10 mM EGTA, 0.3% (w/v) 2-mercaptoethanol, 20 $\mu$g/ml leupeptin, 50 $\mu$g/ml phenylmethylsulfonyl fluoride, and 10 mM benzamidine hydrochloride (lysis buffer). The suspension was sonicated for 30 s, centrifuged at 100,000*g* for 1 h, and the resultant supernatant was used as the soluble fraction. To obtain the membrane preparation, the 100,000*g* pellet was again sonicated in lysis buffer and centrifuged at 100,000*g* for 1 h to remove contaminating soluble proteins. The pellet was then sonicated in the lysis buffer containing 1% (v/v) Triton X-100 and recentrifuged at 100,000*g* for 1 h. The supernatant (detergent-solubilized pellet) was used as the particulate fraction. When necessary, the samples were concentrated by ultrafiltration. The protein concentration was determined by the protein assay kit (the Pierce BCA protein assay). All the procedures were performed at 4°C. Cytosolic and membrane aliquots were electrophoresed on 12% polyacrylamide–sodium dodecyl sulfate (SDS) gels and electrophoretically transferred to polyvinylidene difluoride membranes as described (Nishio et al., 1996b). Enhanced chemiluminescence immunoblotting (ECL Western blotting protocol; Amersham) with affinity-purified, protein kinase C-$\alpha$ and -$\varepsilon$ specific anti-peptide polyclonal antibodies was performed using horseradish peroxidase-linked donkey anti-rabbit immunoglobulin as the secondary antibody. The immunoblots of protein kinase C-$\alpha$ and -$\varepsilon$ were quantitated by densitometry, and the membrane translocation were expressed as a percentage of cytosolic fraction plus membrane fraction.

### 2.6. Statistical analysis

Results are expressed as mean $\pm$ S.D. The data were first analyzed using two-way analysis of variance

(ANOVA), followed by the Scheffe test when $F$ ratios were significant ($P < 0.05$).

## 3. Results

### 3.1. Modulation of S-nitroso-N-acetylpenicillamine-induced apoptosis in smooth muscle cells by protein kinase C

The modulation of apoptosis by protein kinase C was studied in the following experiments. PMA (100 nM), a commonly used protein kinase C-activating agent, moderately potentiated DNA fragmentation induced by *S*-nitroso-*N*-acetylpenicillamine (100 $\mu$M) (Figs. 1 and 2). We also examined the effect of the concentration of PMA on the *S*-nitroso-*N*-acetylpenicillamine-induced DNA fragmentation (Fig. 3). PMA potentiated *S*-nitroso-*N*-acetylpenicillamine-induced DNA fragmentation in a dose dependent manner (0.1–100 nM) (Fig. 3). In line with these experiments, smooth muscle cells were treated with two pharmacological inhibitors of protein kinase C, calphostin C and H-7 to determine their effects on NO-induced apoptosis. Calphostin C (500 nM) and H-7 (50 $\mu$M) decreased NO-induced DNA fragmentation of smooth muscle cells. These results suggest that protein kinase C is involved in *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis of smooth muscle cells. Furthermore, calphostin C alone or H-7 alone induced DNA fragmentation of smooth muscle cells. This discrepancy may reflect that the appropriate protein kinase C activity or activity of a specific subtype of protein kinase C is required to regulate apoptotic activity.

### 3.2. S-nitroso-N-acetylpenicillamine-induced membrane translocation of protein kinase C subtypes in smooth muscle cells

Further, to identify the protein kinase C isoform responsible for the apoptosis induction by *S*-nitroso-*N*-acetylpenicillamine, we studied the time course analysis of the effect of *S*-nitroso-*N*-acetylpenicillamine on the translocation of protein kinase C isoforms. In the total cell lysate, protein kinase C-$\alpha$ (76 kDa), -$\varepsilon$ (91 kDa) and -$\zeta$ (70 kDa and 80 kDa) were detected by immunoblotting and the addition of 100 $\mu$M *S*-nitroso-*N*-acetylpenicillamine for 8 h to smooth muscle cell culture did not change the total amount of protein kinase C-$\alpha$, -$\varepsilon$ and -$\zeta$ (data not shown). We investigated the effect of *S*-nitroso-*N*-acetylpenicillamine on the translocation of protein kinase C-$\alpha$ and -$\varepsilon$, because protein kinase C-$\alpha$ and -$\varepsilon$ are translocated to

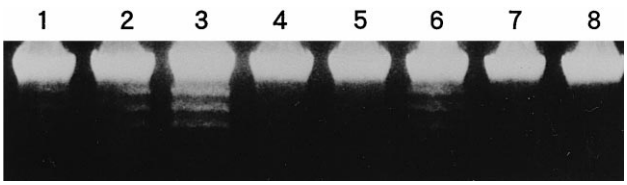


Fig. 1. Modulation of SNAP-induced apoptosis in SMCs by PKC and PKA. (a) DNA, isolated from SNAP-treated SMCs for 8 h in the absence or presence of PMA, calphostin C, dibutyryl–cAMP or Rp–cAMP, was subjected to electrophoresis in 2% agarose and visualized with ethidium bromide. The gels are representative of three individual experiments. Lanes 1–8 are: PMA (100 nM), SNAP (100 $\mu$M), SNAP + PMA (100 nM), SNAP + calphostin C (500 nM), dibutyryl–cAMP (100 $\mu$M), SNAP + dibutyryl–cAMP (100 $\mu$M), SNAP + Rp–cAMP (50 $\mu$M), control respectively.

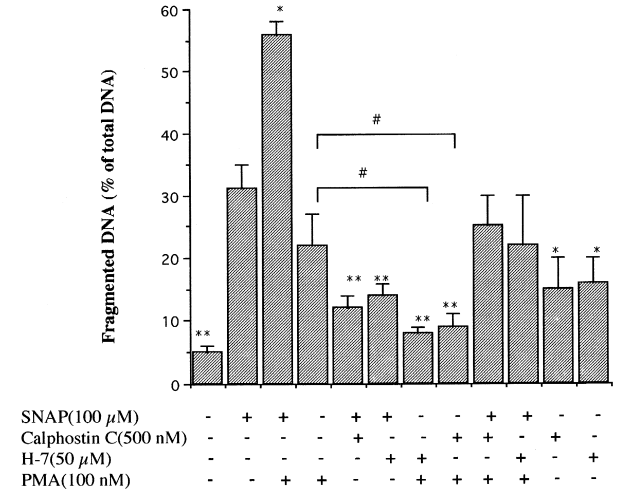


Fig. 2. Prevention by PKC inhibitor of SNAP-induced apoptosis in SMCs. Serum-deprived SMCs were incubated for 8 h with SNAP (100 $\mu$M), PMA, calphostin C (500 nM), or H-7 (50 $\mu$M) at the indicated combination. PMA, calphostin C and H-7 were added 30 min before SNAP. Controls were without any addition. DNA fragmentation is expressed as a percentage of the total DNA, by [$^3$H] thymidine incorporation as described in Section 2 and represents means ± S.D. of four independent experiments done in duplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with SNAP only. $^{\#}P < 0.01$ compared with PMA only.

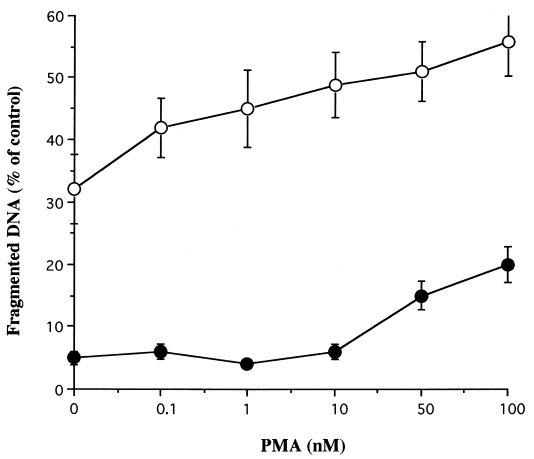


Fig. 3. Effects of PMA on SNAP-induced DNA fragmentation. SMCs were incubated for 8 h in the presence of PMA at concentrations indicated in the figure with (○) or without (●) SNAP (100 $\mu$M). DNA fragmentation is expressed as a percentage of the total DNA, as determined [$^3$H] thymidine incorporation as described in Section 2 and represents means ± S.D. of four independent experiments done in duplicate.

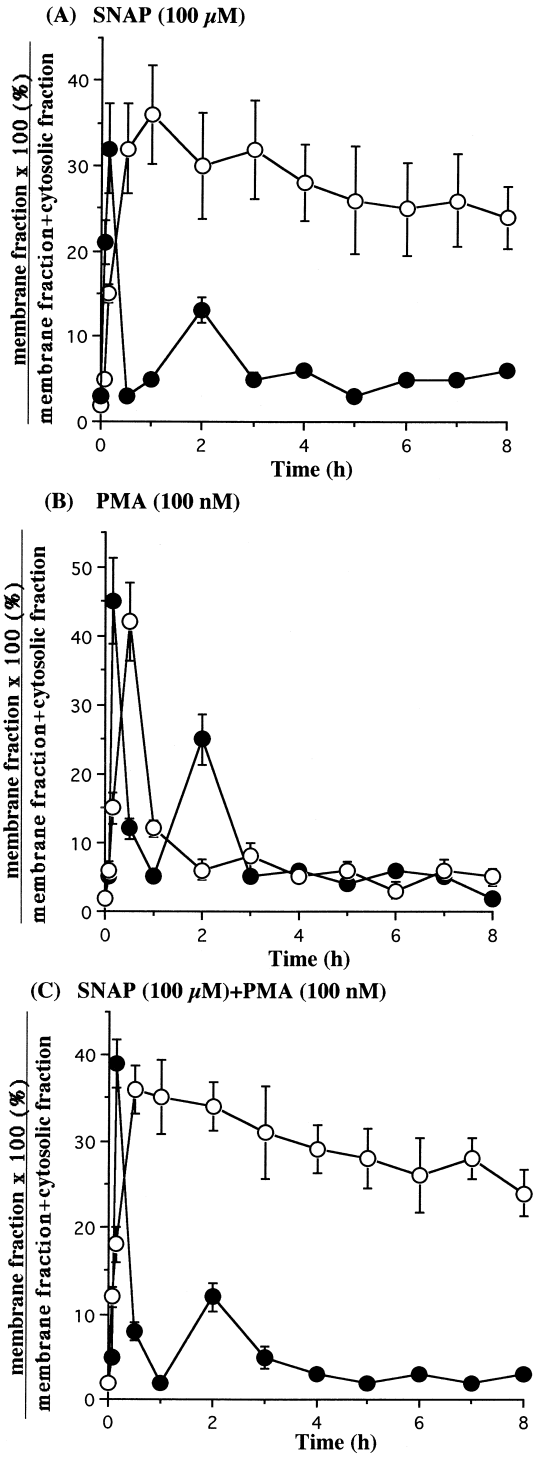


Fig. 4. Effects of SNAP on membrane translocation of PKC-$\alpha$ and PKC-$\varepsilon$ in SMCs. Serum-deprived SMCs were cultured for several hours, as indicated in figures, in the presence of 100 $\mu$M SNAP (A), 100 nM PMA (B) or SNAP and PMA in combination (C). Cells were lysed at indicated times after SNAP or PMA addition and the immunoblots of membrane and cytosolic fractions were performed as described in Section 2. The membrane translocation of PKC-$\alpha$ (●) and PKC-$\varepsilon$ (○) is expressed as a percentage of cytosolic fraction plus membrane fraction and represents means ± S.D. of four independent experiments in duplicate.

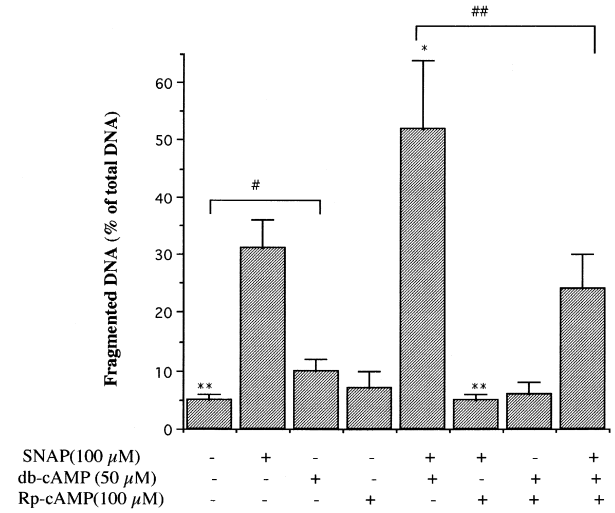


Fig. 5. Prevention by PKA inhibitor of SNAP-induced apoptosis in SMCs. Serum-deprived SMCs were incubated for 8 h with 100 $\mu$M SNAP in the absence or presence of Db–cAMP or Rp–cAMP at the indicated combinations. Db–cAMP and Rp–cAMP were 30 min before SNAP. Controls were without any addition. DNA fragmentation are expressed as a percentage of the total DNA by [$^3$H] thymidine assay as described in Section 2 and represents means ± S.D. of four independent experiments done in duplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with SNAP only. $^{\#}P < 0.05$, compared with control. $^{\#\#}P < 0.05$ compared with SNAP and Db–cAMP in combination.

membrane by PMA stimulation, but protein kinase C-$\zeta$ is not. We detected a small amount of protein kinase C-$\alpha$ and -$\varepsilon$ in the membrane fraction under basal conditions and did not find any change of protein kinase C-$\alpha$ and protein kinase C-$\varepsilon$ in the membrane fraction of smooth muscle cell treated with vehicle for 8 h (data not shown). In contrast, as demonstrated in Fig. 4A, *S*-nitroso-*N*-acetylpenicillamine (100 $\mu$M) protein gradually increased kinase C-$\varepsilon$ in the membrane fraction up to 1 h, whereafter the amount stayed at the same level for up to 8 h. Protein kinase C-$\alpha$ in *S*-nitroso-*N*-acetylpenicillamine (100 $\mu$M) treated smooth muscle cells was transiently translocated to membrane at 10 min, but this translocation to the membrane was reversed to the control level at 30 min. PMA caused only transient translocation of protein kinase C-$\alpha$ to the membrane at 10 min and protein kinase C-$\varepsilon$ to membrane at 20 min, but this translocation was reversed to the control level at 1 and 2 h respectively (Fig. 4B). The combination of PMA and *S*-nitroso-*N*-acetylpenicillamine induced the prolonged translocation of protein kinase C-$\varepsilon$ to the membrane, in contrast to the transient translocation of protein kinase C-$\alpha$ (Fig. 4C). These results suggest that the *S*-nitroso-*N*-acetylpenicillamine-induced prolonged translocation of PKC-$\varepsilon$ to the membrane is involved in *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis of smooth muscle cells. However, it is not clear whether the translocation of protein kinase C to the membrane is the cause or effect of *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis of smooth muscle cells.

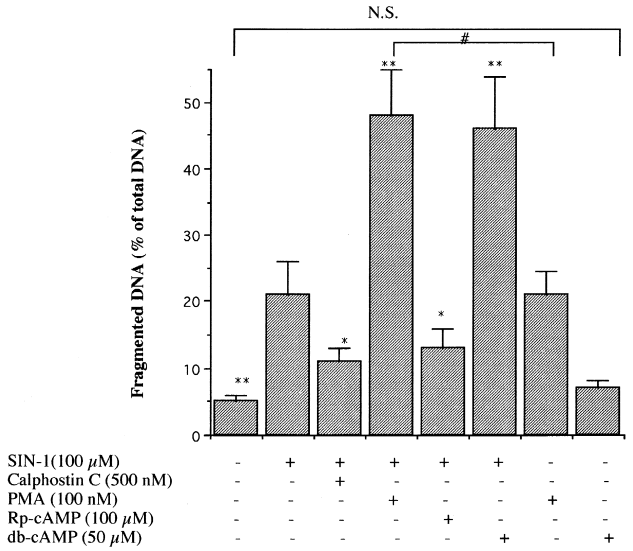


Fig. 6. Modulation of SIN-1-induced apoptosis in SMCs by PKC and PKA. Serum-deprived SMCs were incubated for 8 h with SIN-1 (100 $\mu$M), calphostin C (500 nM), Db–cAMP (50 $\mu$M), and Rp–cAMP (100 $\mu$M) at the indicated combinations. PMA, calphostin C, Db–cAMP, and Rp–cAMP were added 30 min before SIN-1. Controls were without any addition. DNA fragmentation are expressed as a percentage of the total DNA, as determined by [$^3$H] thymidine assay as described in Section 2 and represents means $\pm$ S.D. of four independent experiments done in duplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, respectively, compared with SIN-1 only. $^{\#}P < 0.01$ compared with SIN-1 and PMA in combination. N.S. indicates 'not significant' compared with no addition.

### *3.3. Modulation of S-nitroso-N-acetylpenicillamine-induced apoptosis in smooth muscle cells by protein kinase A*

Further, we focused on cAMP as a potential modulator of NO-mediated signal pathways. We addressed the question of whether a lipophilic cAMP analogue interferes with *S*-nitroso-*N*-acetylpenicillamine-induced DNA fragmentation. A membrane-permeable cAMP analogue, dibutyryl cAMP (50 $\mu$M), increased *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis (Fig. 5). Additionally, as demonstrated in Figs. 1 and 5, Rp–cAMP (100 $\mu$M), a cAMP protein kinase inhibitor, prevented the *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis in smooth muscle cells. These results suggest that protein kinase A is involved in *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis of smooth muscle cells.

### *3.4. Modulation of SIN-1-induced apoptosis in smooth muscle cells by protein kinase C and protein kinase A*

We reported previously that SIN-1, an alternative nitric oxide donor, induced apoptosis (Nishio et al., 1996a). Therefore, we investigated whether SIN-1-induced apoptosis was modulated by protein kinase C and protein kinase A. Fig. 6 shows that both protein kinase C and protein kinase A activator potentiate DNA fragmentation induced by SIN-1. Inversely, Fig. 6 shows that both the protein kinase C and protein kinase A inhibitor prevent DNA fragmentation by SIN-1. PMA (100 nM) alone induced apoptosis as much as SIN-1 (100 $\mu$M) alone. Db–cAMP (50 $\mu$M) alone did not induced apoptosis as compared with control. These results indicate that apoptosis induced by an alternative NO donor was modulated by protein kinase C and protein kinase A.

## 4. Discussion

### *4.1. Role of protein kinase C*

The role of protein kinase C in the induction of apoptosis has been complicated by conflicting reports. For instance, the observation that activation of protein kinase C by exposure to PMA induces apoptosis in promyelocytic leukemic cells (Macfarlane and Manzel, 1994) and that protein kinase C inhibitors prevent radiation-induced apoptosis of mouse thymocytes (Shaposhnikova et al., 1994) suggest that activation of protein kinase C promotes apoptosis. However, the ability of PMA to oppose steroid-induced apoptosis in thymic lymphocytes (McConkey et al., 1990) and to prevent the death of plasmacytomas deprived of interleukin-6 (Romanova et al., 1996), radiation-induced apoptosis in vitro (Tomei et al., 1988) and serum deprivation-induced apoptosis of mature lymphocytes (Lucas et al., 1991) support an inhibitory role of protein kinase C in the apoptotic process. Furthermore, pharmacological inhibition of protein kinase C induces apoptosis in human myeloid leukemia cells (Freemerman et al., 1996). These data support the idea that protein kinase C activation blocks apoptosis. It is conceivable that conflicting observations regarding the apparent role of protein kinase C in the regulation of apoptosis reflect cell type-specific responses to triggering agents (Jarvis et al., 1994; Lucas and Sanchez-Margalet, 1995).

We have taken a pharmacological approach to determine how activation or inhibition of protein kinase C in the specific signal transduction pathways can modulate the apoptotic effects of NO. Inhibition of protein kinase C activity, by a series of compounds known to inhibit protein kinase C, resulted in decreasing the NO-inducible apoptotic activity in smooth muscle cells. It is important to note, however, that calphostin C and H-7 are not totally specific for protein kinase C. Therefore, to assess the specificity of these compounds, we investigated the effect of these inhibitors on smooth muscle cells treated with *S*-nitroso-*N*-acetylpenicillamine and/or PMA. The prevention of *S*-nitroso-*N*-acetylpenicillamine-induced apoptosis by calphostin C and H-7 was inhibited by PMA (Fig. 2). These observations suggest that protein kinase C activation can contribute to NO-inducible apoptotic activity in smooth muscle cells, and it remains possible that protein kinase C

is a mediator of NO-induced apoptosis in smooth muscle cells.

It is unknown whether NO can directly modulate the activity of protein kinase C. As mentioned in text, protein kinase C-$\varepsilon$ in vehicle-treated smooth muscle cells was predominantly found in the cytoplasm with low amounts in the membranes. The addition of 100 nM *S*-nitroso-*N*-acetylpenicillamine to smooth muscle cell cultures resulted in prolonged translocation of protein kinase C-$\varepsilon$ from the cytoplasm to membrane (Fig. 4A). Because membrane translocation is essential for the enzyme's activation, it was suggested that the addition of NO caused the activation of protein kinase C-$\varepsilon$ in the cells. When smooth muscle cells were treated with PMA and *S*-nitroso-*N*-acetylpenicillamine in combination, PMA did not have a significant effect on protein kinase C-$\varepsilon$. This result suggests that *S*-nitroso-*N*-acetylpenicillamine activates protein kinase C-$\varepsilon$ and that the modulatory effect of PMA on *S*-nitroso-*N*-acetylpenicillamine induced apoptosis is due to another protein kinase C subtype. Alternatively, there may be another mechanism of activation of protein kinase C-$\varepsilon$ other than membrane translocation. Some protein kinase C-$\alpha$ was detected in the membrane fraction after 8 h. This would not necessarily mean that protein kinase C-$\alpha$ is not activated, because this isoform might be localized in or translocated to nuclei, as reported previously (James and Olson, 1992). Also, we should not underestimate the role of protein kinase C-$\alpha$ in apoptosis on the basis of the transient translocation to the membrane, because other observations indicate that a short effect may be the crucial one, e.g. short activation of ras by EGF results in proliferation while the prolonged activation of ras by NGF results in differentiation of PC12 cells (Muroya et al., 1992).

NO activates a number of enzymes in addition to guanyl cyclase, although activation of protein kinase C by NO has not been described previously. In fact, both the purified protein kinase C and protein kinase C within the macrophage cell line IC-21 are inactivated by NO in a dose-dependent fashion, mediated by *S*-nitrosylation of thiol residues on the kinase (Gopalakrishna et al., 1993). Disulfide bonds play a role in the modulation of the function of many proteins. However, various reducing agents in smooth muscle cells may create a redox state for NO that differs from that which occurs in macrophages. In fact, in mouse macrophages, protein kinase C activators such as TPA/PMA are reported to suppress NO-induced apoptotic cell death (Mebmer et al., 1995). The intracellular redox milieu determines the biochemistry of NO and thus its ultimate molecular targets (Stamler, 1994). Further study will be needed to examine how exogenous NO activates protein kinase C.

### *4.2. Role of protein kinase A*

Previous results have linked cAMP to programmed cell death during palatal development (Pratt and Martin, 1975) and in some plants (Basile et al., 1973). Our results, also, showed that Db–cAMP alone induced apoptosis and that Db–cAMP-induced apoptosis is prevented by Rp–cAMP (Figs. 5 and 6). We questioned whether protein kinase A might also regulate NO-induced apoptosis in smooth muscle cells. A membrane-permeable cAMP analogue, dibutyryl cAMP (50 $\mu$M), increased NO-induced DNA cleavage. Inversely, as presented in Fig. 2, Rp–cAMP (100 $\mu$M), protein kinase A inhibitor, prevented NO-induced apoptosis in smooth muscle cells. Furthermore, it is reported that NO activates protein kinase A (Assender et al., 1992). These results suggest that protein kinase A is involved in NO-induced apoptosis in smooth muscle cells.

However, it is not clear how protein kinase A regulate NO-induced apoptosis. Previously, it was reported that cGMP-dependent activation of protein kinase A may be responsible, at least in part, for the NO-dependent inhibition of proliferation of smooth muscle cells (Assender et al., 1992). We reported that cGMP alone did not induce apoptosis (Nishio et al., 1996a). Therefore, there may be another mechanism for the modulation by protein kinase A in NO-induced apoptosis.

Thus, the results indicate that both protein kinase C and protein kinase A are involved in smooth muscle cells apoptosis induced by NO donors. Further investigations will be needed to define the molecular mechanism by which protein kinase C and protein kinase A modulate NO-induced apoptosis of smooth muscle cells.

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