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# Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart

(nitric oxide/coronary vasodilatation/vascular injury)

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**ABSTRACT** **The effects of the oxidant species peroxynitrite ($ONOO^-$) on coronary perfusion pressure and vasodilatation elicited by acetylcholine, isoproterenol, and *S*-nitroso-*N*-acetyl-DL-penicillamine were investigated in the isolated perfused rat heart. $ONOO^-$ (0.3–1000 μM) caused a concentration-dependent vasodilatation of the coronary vasculature. This dilator response was inhibited by oxyhemoglobin, indicating that it was due to the generation of nitric oxide. Tachyphylaxis to $ONOO^-$ developed rapidly, so that the response disappeared after three or four applications of this compound. $ONOO^-$ not only induced tachyphylaxis but also inhibited the vasodilatation induced by the three vasodilators studied. This latter effect of $ONOO^-$ was critically dependent on its concentration, since it occurred at 3 μM, which was subthreshold as a dilator, and at 1000 μM, which was supramaximal, but not at 30 and 100 μM. These latter concentrations inhibited the responses to vasodilators only in the presence of oxyhemoglobin. Thus, a wide range of concentrations of $ONOO^-$ induce a vascular dysfunction, as evidenced by the tachyphylaxis to its own vasodilator actions and the long-lasting impairment of the responses to other vasodilators. However, at the same time $ONOO^-$ generates nitric oxide, which at certain concentrations of $ONOO^-$ is sufficient to counteract its deleterious action. Coinfusion of *S*-nitroso-*N*-acetyl-DL-penicillamine or prostacyclin at low concentrations that did not produce vasodilatation also protected against $ONOO^-$-induced vascular dysfunction: these compounds may be protective through a common mechanism, as yet undefined.**

Nitric oxide (NO) released by the vascular endothelium is a powerful vasodilator that plays a role in the physiological regulation of blood flow and blood pressure. In addition, NO is generated in large quantities by the vascular endothelium and smooth muscle cells following activation by bacterial endo- and exotoxins and by some cytokines. NO thus released is responsible for the vasodilatation and the hyporeactivity to vasoconstrictors characteristic of septic shock and might also be involved in tissue damage (1).

NO may induce tissue damage as a result of a direct action on iron/sulfur-centered enzymes of the respiratory cycle (2) or enzymes involved in the synthesis of DNA (3). In addition, NO may interact with oxygen-derived radicals such as superoxide anion ($O_2^-$) to generate molecules, including peroxynitrite ($ONOO^-$), that are potentially more injurious to tissues (4–6). $ONOO^-$, when protonated at physiological pH, may rapidly decompose to another oxidant with hydroxyl radical-like activity, as well as to nitrogen dioxide ($NO_2$) (6, 7). These properties make $ONOO^-$ a potent tissue-damaging species that can exert its cytotoxicity by interacting with various molecular targets such as thiols (8), lipids (9), and proteins containing aromatic amino acids (10). Recently, we have found that $ONOO^-$ causes aggregation of washed human platelets (11). However, this aggregatory action of $ONOO^-$ was abolished in the presence of thiols as a result of the generation of *S*-nitrosothiols and/or NO, which inhibited aggregation. This led us to suggest that the fate and the actions of $ONOO^-$ are critically dependent on the microenvironment in which this oxidant is generated (11).

The present experiments were carried out to investigate the actions of $ONOO^-$ in the coronary circulation, where NO is produced physiologically (12–15) and where $O_2^-$ (16) and possibly $ONOO^-$ (17) may be generated in pathological conditions.

## METHODS

**Isolated Perfused Heart Preparation.** Male Wistar rats (200–250 g) were anesthetized with isoflurane (2%), injected with heparin (200 units) and decapitated. The isolated perfused hearts were prepared according to a modified method of Langendorff. In brief, the hearts were isolated and a proximal pulmonary arteriotomy was performed for drainage of coronary effluent. They were then placed in a jacketed chamber (37°C) and perfused through the aorta by means of a roller pump (Minipuls2, Gilson) with Krebs–Henseleit solution (118 mM NaCl/2.8 mM KCl/1.2 mM $KH_2PO_4$/2.5 mM $CaCl_2$/1.2 mM $MgSO_4$/25 mM $NaHCO_3$/5.5 mM glucose, pH 7.4) at 37°C that had been bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. The perfusion rate was maintained at 9 ml/min, resulting in an initial coronary perfusion pressure (CPP) of 50–60 mmHg (1 mmHg = 133 Pa). Hearts with a CPP outside this range were discarded. The CPP was monitored with an Elcomatic EM750 transducer connected to a Gould TA-4000 monitoring system. The hearts were electrically paced at a frequency of 320 beats/min with an S44 Grass stimulator. They were allowed to stabilize for 10 min, after which time the CPP was increased to 120–140 mmHg by using the thromboxane $A_2$ mimetic U-46619 ($EC_{80} \approx 6$ nM; $n = 6$). Each experiment was completed within 2 hr of isolation of the preparation.

**Experimental Design.** $ONOO^-$ (0.3–1000 μM) or the products of its decomposition (dec $ONOO^-$, see below) were infused directly into the aortic cannula (<1-sec delay before reaching the heart) for 30 sec at 100 μl/min. The pH of the perfusate was not affected by the addition of $ONOO^-$ at these concentrations. Unless otherwise stated, 5 min was allowed to elapse between recovery from each infusion and the subsequent treatment.

$ONOO^-$ (100 μM) was infused into the hearts and the CPP response was allowed to recover to control levels (5–10 min).

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Abbreviations: ACh, acetylcholine; CPP, coronary perfusion pressure; dec $ONOO^-$, decomposed peroxynitrite; ISO, isoproterenol; SNAP, *S*-nitroso-*N*-acetyl-DL-penicillamine.

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This procedure was repeated three to four times ($n$ = 3). Other hearts were infused with a single concentration of $ONOO^-$ (0.3–1000 $\mu$M; $n$ = 3–6 for each concentration) in the presence or absence of oxyhemoglobin (6 $\mu$M, 100 $\mu$l/min for 10 min, starting 3 min before the infusion of $ONOO^-$).

In other experiments, hearts were infused (50 $\mu$l/min for 30 sec) in a random order with isoproterenol (ISO), acetylcholine (ACh), and *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), each at a concentration which resulted in approximately half-maximal dilatation: 12.5–25 nM, 30–300 nM, and 5–10 nM, respectively. A single concentration per heart of $ONOO^-$ or dec $ONOO^-$ (0.3–1000 $\mu$M) was then infused, followed by a second administration of ISO, ACh, and SNAP ($n$ = 3). In some of these experiments the administration of $ONOO^-$ (30 $\mu$M or 100 $\mu$M) was preceded by an infusion of oxyhemoglobin (6 $\mu$M, 100 $\mu$l/min for 10 min, starting 3 min before the infusion of $ONOO^-$; $n$ = 3–6). A period of 20 min, determined in a preliminary set of experiments, was allowed for washout of oxyhemoglobin before the second exposure to the vasodilators. In further experiments, the infusion of $ONOO^-$ (3 $\mu$M) was preceded by an infusion of SNAP (2 nM, 100 $\mu$l/min for 10 min, starting 3 min before $ONOO^-$) or of prostacyclin (2 nM, 100 $\mu$l/min for 15 min, starting 5 min before $ONOO^-$) ($n$ = 3 for each). ISO, ACh, and SNAP were then administered again.

In a separate series of experiments, ISO (25 nM) was infused 5 min before and 5, 15, 25, 35, 55, and 75 min after the administration of $ONOO^-$ (3 $\mu$M; $n$ = 5).

**Synthesis of $ONOO^-$.** $ONOO^-$ was synthesized by the reaction of acidified $NaNO_2$ with $H_2O_2$ in a quenched flow reactor (18). The $ONOO^-$ formed was stabilized by rapid mixing (1–2 sec) with NaOH. To prepare dec $ONOO^-$, the addition of NaOH to the $H_2O_2/NaNO_2$ mixture was delayed for 3 min, after which no $ONOO^-$ was present. Alternatively, $ONOO^-$ was diluted in glucose-free Krebs buffer and then neutralized with 1 M HCl. The same results were observed for either method of preparation of dec $ONOO^-$. Both $ONOO^-$ and dec $ONOO^-$ were diluted in Milli-Q water (Millipore water purification system) immediately prior to use, shielded from light, and kept on ice. Under these conditions the diluted $ONOO^-$ stock solutions were stable for 1–2 hr.

The vasodilatation of coronary vasculature (reduction in CPP) was measured from experimental tracings as the area under the curve ($cm^2$) using the SIGMA SCAN computer program (Sigma) with a Jande table. The data (mean ± SEM, $n$ = 3–6) were compared by means of the GraphPad INSTAT (version 2.04) computer program (Sigma); $P < 0.05$ was considered as significant.

**Reagents.** 9,11-Dideoxy-9$\alpha$,11$\alpha$-methanoepoxyprostaglandin $F_{2\alpha}$ (U-46619, Calbiochem), ACh bromide and ISO hydrochloride (Sigma), and prostacyclin sodium salt (Wellcome Foundation, Beckenham) were obtained as indicated. SNAP was synthesized by H. Hodson (Wellcome) and oxyhemoglobin was prepared by the method of Paterson *et al.* (19). All compounds were dissolved and diluted in physiologic saline.

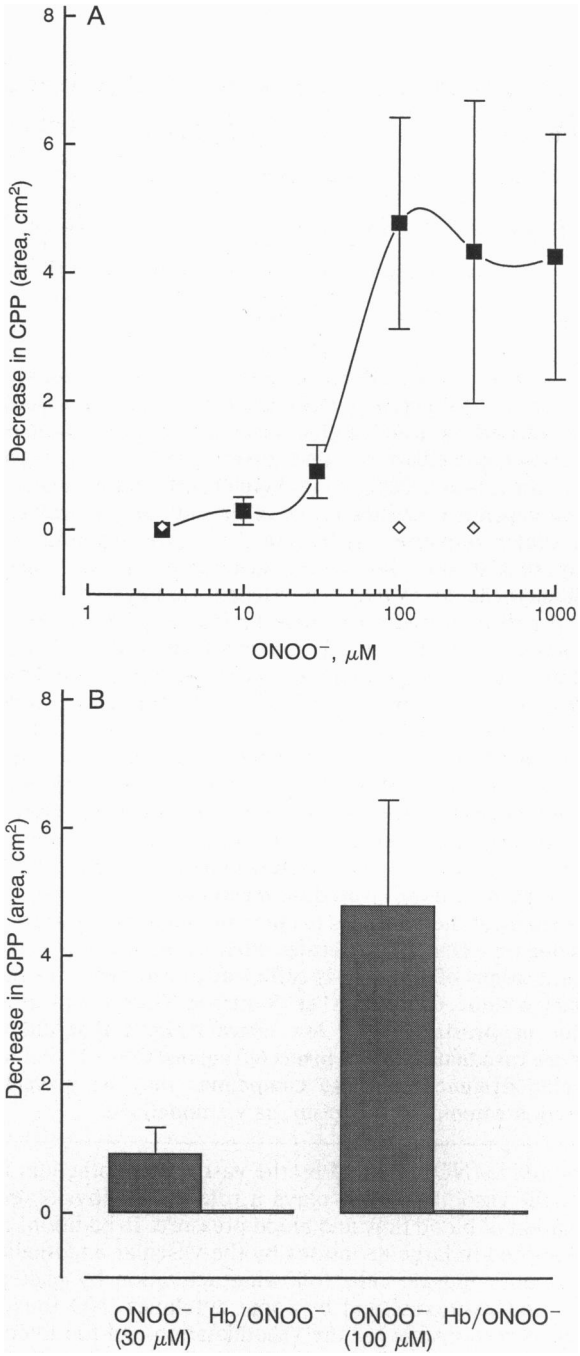


FIG. 2. Effect of $ONOO^-$ on CPP. $ONOO^-$ (■), but not dec $ONOO^-$ (◇), induced a concentration-dependent vasodilatation (*A*). These effects of $ONOO^-$ (30 and 100 $\mu$M) were abolished by oxyhemoglobin (6 $\mu$M; $Hb/ONOO^-$) (*B*). Results are means ± SEM, $n$ = 3–6.

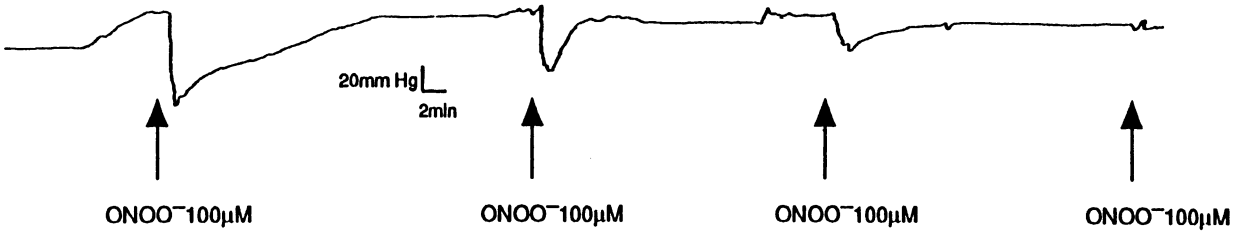


FIG. 1. Tachyphylaxis of CPP to repeated infusions of $ONOO^-$ (100 $\mu$M) in isolated perfused rat hearts. Trace is typical of four separate observations.

## RESULTS

**Effect of $ONOO^-$ on CPP.** Infusion of $ONOO^-$ (30 and 100 $\mu$M) to the heart induced a vasodilatation which decreased rapidly and progressively on its repeated administration (Fig. 1). Because of this tachyphylaxis, dose–response curves (0.3–1000 $\mu$M) were constructed for individual hearts, each exposed to a single, randomly allocated dose of $ONOO^-$ (Fig. 2*A*). $ONOO^-$-induced vasodilatation occurred after a delay of 20 sec and lasted from 5 to 10 min. The maximum effective concentration was 100 $\mu$M. The responses to this and to higher concentrations of $ONOO^-$ were variable (Fig. 2*A*). The vasodilator effect of $ONOO^-$ was abolished by oxyhemoglobin (6 $\mu$M; Fig. 2*B*). dec $ONOO^-$ (3–300 $\mu$M, $n = 3$) did not induce vasodilatation (Fig. 2*A*).

**Effect of $ONOO^-$ on the Vasodilatation Elicited by ISO, ACh, and SNAP.** Infusions of $ONOO^-$ not only induced tachyphylaxis but also reduced significantly the responses to other vasodilators (ISO, ACh, and SNAP) which otherwise induced dose-dependent reproducible dilatations (data not shown). However, this effect was dependent on the concentration of $ONOO^-$. At 3 and 1000 $\mu$M, $ONOO^-$ caused a significant reduction in the vasodilator effects of these compounds (Fig. 3 *A* and *B*). In contrast, $ONOO^-$ at 10, 30, and 100 $\mu$M did not affect significantly the responses to these vasodilators (Fig. 3*C* shows the responses for 30 $\mu$M $ONOO^-$). However, in the presence of oxyhemoglobin (6 $\mu$M), 30 and 100 $\mu$M $ONOO^-$ did cause significant reduction in the vasodilatation induced by ISO, ACh, and SNAP (Fig. 3*D* shows the response for 30 $\mu$M $ONOO^-$). Infusion of oxyhemoglobin alone, in the absence of $ONOO^-$, followed by washout (20 min) did not affect the responses to these vasodilators ($n = 3$; data not shown). dec $ONOO^-$ (3 $\mu$M) had no significant effect on the vasodilatation elicited by ACh (0.45 ± 0.16 $cm^2$ before and 0.58 ± 0.23 $cm^2$ after), ISO (0.67 ± 0.03 $cm^2$ before and 0.45 ± 0.09 $cm^2$ after) and SNAP (1.15 ± 0.33 $cm^2$ before and 1.44 ± 0.66 $cm^2$ after) ($n = 3$ for each).

To determine whether these effects of $ONOO^-$ were persistent, infusions of ISO were administered at intervals for up to 75 min after exposure to $ONOO^-$ (3 $\mu$M). When given before $ONOO^-$, isoproterenol (25 nM) caused a vasodilatation of 1.51 ± 0.22 $cm^2$ ($n = 5$). $ONOO^-$ (3 $\mu$M) induced a significant decrease in the effect of ISO (0.26 ± 0.09 $cm^2$, $n = 5$, $P < 0.01$) measured 5 min after administration of $ONOO^-$. The vasodilatation elicited by ISO had not recovered 75 min after $ONOO^-$ (0.35 ± 0.06 $cm^2$, $n = 5$, $P < 0.01$).

The effect of $ONOO^-$ (3 $\mu$M) on the response to vasodilators was prevented by coinfusion of SNAP (2 nM). Prostacyclin (2 nM) also inhibited the effect of $ONOO^-$ (Fig. 4 and Table 1). At the concentrations used, neither SNAP nor prostacyclin induced vasodilatation ($n = 3$; data not shown).

## DISCUSSION

We have shown that $ONOO^-$ induces a concentration-dependent vasodilatation of the rat coronary vasculature. This effect was abolished by oxyhemoglobin, suggesting that it was mediated via generation of NO and/or NO donor(s). A vasodilator effect of $ONOO^-$ associated with the release of NO has been reported in isolated vessels such as calf pulmonary artery, dog artery rings, and human coronary arteries

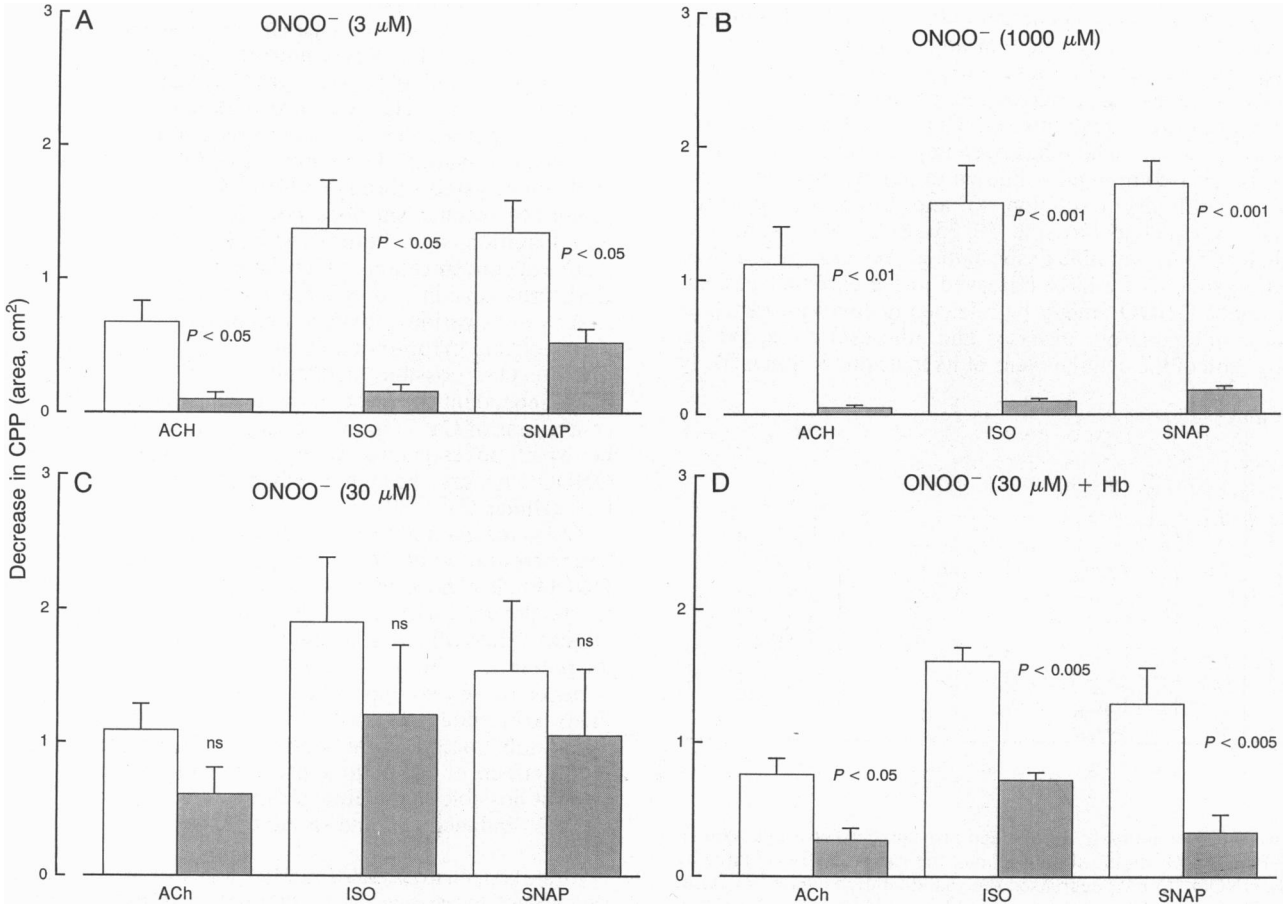


FIG. 3. Effect of $ONOO^-$ on vasodilatation elicited by ACh, ISO, and SNAP. At 3 $\mu$M and 1000 $\mu$M (*A* and *B*), but not at 30 $\mu$M (*C*), $ONOO^-$ resulted in a significant inhibition of the vasodilator actions of ACh, ISO, and SNAP. Oxyhemoglobin (Hb, 6 $\mu$M) unmasked the inhibitory effects of 30 $\mu$M $ONOO^-$ (*D*). Open bars show the effects of compounds before—and filled bars, after—administration of $ONOO^-$ in the presence or absence of Hb. Results are means ± SEM, $n = 3$. ns, Not significant.

Table 1. Effect of SNAP and prostacyclin ($PGI_2$) on $ONOO^-$-induced inhibition of vasodilatation elicited by ISO and SNAP

| Compound infused with $ONOO^-$ | Decrease in CPP (area, $cm^2$) | | | |
|---|---|---|---|---|
| | ISO | | SNAP | |
| | Before | After | Before | After |
| None | 1.38 ± 0.36 | 0.16 ± 0.05* | 1.35 ± 0.29 | 0.52 ± 0.10* |
| SNAP | 0.86 ± 0.24 | 0.89 ± 0.22 | 0.69 ± 0.06 | 0.76 ± 0.11 |
| $PGI_2$ | 0.96 ± 0.20 | 0.95 ± 0.20 | 1.13 ± 0.40 | 1.02 ± 0.30 |

$ONOO^-$ (3 μM) caused a significant reduction in the vasodilatation elicited by ISO and SNAP. This effect was prevented by coinfusion of $ONOO^-$ with SNAP (2 nM) or with $PGI_2$ (2 nM). Data are means ± SEM, $n = 3$.
*$P < 0.05$.

(20–22). We have recently shown that, in the presence of small concentrations of human plasma, serum albumin, or glutathione, $ONOO^-$ is converted to *S*-nitrosothiols or other NO donors which may subsequently release NO (11). Thus, the local formation of *S*-nitrosothiols may be responsible for the vasodilator action of $ONOO^-$ in the intact coronary vasculature.

We have, in addition, shown that on repeated exposure to $ONOO^-$ the coronary vasculature develops rapid tachyphylaxis in response to the vasodilator action of this compound. This is not likely to be due to tachyphylaxis to NO at the level of the soluble guanylate cyclase (23, 24), since such a phenomenon has recently been reported not to occur (25, 26). Moreover, nitrosothiols, which are likely to be formed from $ONOO^-$ (11), are not dependent on an exhaustable metabolic pathway for their generation of NO (27). That the responses to other vasodilators—including ISO, which dilates through an NO-independent mechanism (28)—are also substantially reduced by $ONOO^-$, and that this phenomenon persists throughout the period of observation, strongly suggests that $ONOO^-$ induces a generalized and permanent change in the reactivity of the vasculature. $ONOO^-$ is a strong, relatively long-lived oxidant which has been implicated in tissue injury (29–34). This compound is known to initiate lipid peroxidation (9), sulfhydryl oxidation (8), and nitration of aromatic amino acids such as tyrosine (10, 35). Some of these actions might lead to irreversible tissue damage, resulting in impaired function such as we have observed in the coronary circulation. Since $ONOO^-$ might be released in conditions such as chronic inflammatory diseases and atherosclerosis, the investigation of the cellular basis of its injurious action is likely to be relevant for the understanding of the pathophysiology of these conditions.

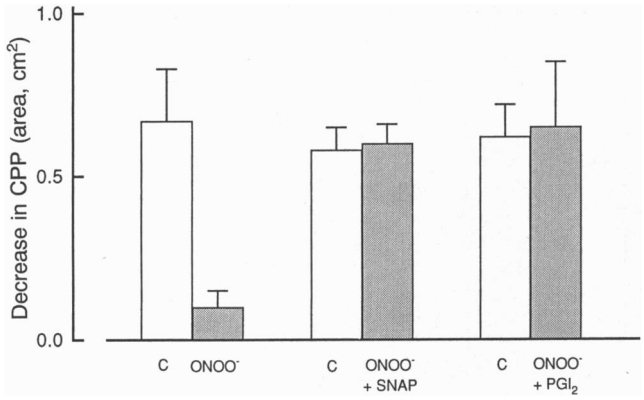


FIG. 4. Prevention by SNAP and prostacyclin (prostaglandin $I_2$, $PGI_2$) of $ONOO^-$-induced inhibition of the vasodilatation elicited by ACh. $ONOO^-$ (3 μM) decreased the vasodilatation elicited by ACh. The effect of $ONOO^-$ was prevented by SNAP (2 nM; $ONOO^-$ + SNAP) and by $PGI_2$ (2 nM; $ONOO^-$ + $PGI_2$). Open bars (control, C) show the effects of ACh before and filled bars after administration of $ONOO^-$, either alone or in combination with other agents. Results are means ± SEM, $n = 3$.

Interestingly, the lowest concentration of $ONOO^-$, as well as the highest used, significantly reduced the responses to other vasodilators, whereas intermediate concentrations inhibited the responses to other vasodilators only in the presence of oxyhemoglobin. These experiments suggest that $ONOO^-$ induced tissue damage in the coronary circulation but at the same time decomposed and generated NO donors which protected against this damage via the release of NO. However, at the highest concentration of $ONOO^-$ its damaging action is likely to overcome the protective action of the NO generated. The rate of conversion to *S*-nitrosothiols or other NO donors, their breakdown into NO, and the extent of $ONOO^-$-induced injury probably vary from one preparation to another; this may be the origin of the variability in the vasodilator responses that we observed with the higher concentrations of $ONOO^-$.

That NO can protect against the vascular dysfunction induced by $ONOO^-$ was confirmed by our demonstration that an *S*-nitrosothiol, SNAP, protects against this action of $ONOO^-$. Prostacyclin was also able to exert a protective effect, suggesting that this mechanism might be the consequence of activation of either cyclic GMP or cyclic AMP, the nucleotide systems through which these mediators act. This protective mechanism does not seem to be dependent on vasodilatation, since both SNAP and prostacyclin were protective at concentrations which did not induce vasodilatation. Both prostacyclin and NO are known to protect cells and tissues from various noxious stimuli. However, the mechanisms of this cytoprotection have not been clearly defined (28–30). One possible mechanism involves a cyclic nucleotide-dependent decrease in the resting and stimulated concentrations of $Ca^{2+}$ (36, 37). If this is the case, it would also be worth investigating whether the damage induced by $ONOO^-$ occurs through mechanisms which lead to high intracellular $Ca^{2+}$ (38).

These results and previous studies (6–11, 30, 31) provide experimental evidence to support the suggestion that $ONOO^-$, if generated *in vivo*, may indeed be involved in tissue damage. However, they also show that there are effective detoxification mechanisms designed to counteract its potential to injure tissues. Understanding the balance between these two opposing actions in biological systems is likely to provide important information regarding tissue damage in some pathological conditions. Furthermore, the beneficial effects of NO donors, prostacyclin, and reactive thiols suggest possible therapeutic strategies for protection against $ONOO^-$-induced cell and tissue damage.

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1. Moncada, S. & Higgs, A. (1993) *N. Engl. J. Med.* **329**, 2002–2012.

2. Hibbs, J. B., Jr., Taintor, R. R., Vavrin, Z., Granger, D. L., Drapier, J. C. & Amber, J. B. (1990) in *Nitric Oxide from L-arginine: A Bioregulatory System*, eds. Moncada, S. & Higgs, E. A. (Elsevier, Amsterdam), pp. 189–223.
3. Lepoivre, M., Flaman, J. M. & Henry, Y. (1992) *J. Biol. Chem.* **267,** 22994–23000.
4. Gryglewski, R. J., Palmer, R. M. J. & Moncada, S. (1986) *Nature (London)* **320,** 454–456.
5. McCall, T. B., Boughton-Smith, N. K., Palmer, R. M. J., Whittle, B. J. R. & Moncada, S. (1989) *Biochem. J.* **261,** 293–296.
6. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. & Freeman, B. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1620–1624.
7. Hogg, N., Darley-Usmar, V. M., Wilson, M. T. & Moncada, S. (1992) *Biochem. J.* **281,** 419–424.
8. Radi, R., Beckman, J. S., Bush, K. M. & Freeman, B. A. (1991) *J. Biol. Chem.* **266,** 4244–4250.
9. Radi, R., Beckman, J. S., Bush, K. M. & Freeman, B. A. (1991) *Arch. Biochem. Biophys.* **288,** 481–487.
10. Van der Vliet, A., O'Neil, C. A., Halliwell, B., Cross, C. E. & Kaur, H. (1994) *FEBS Lett.* **339,** 89–92.
11. Moro, M. A., Darley-Usmar, V. M., Goodwin, D. A., Read, N. G., Zamora-Pino, R., Feelisch, M., Radomski, M. W. & Moncada, S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6702–6706.
12. Drexler, H., Zeiher, A. M., Wollschlager, H., Meinertz, T., Just, H. & Bonzel, T. (1989) *Circulation* **80,** 466–474.
13. Amezcua, J. L., Palmer, R. M. J., de Souza, B. M. & Moncada, S. (1989) *Br. J. Pharmacol.* **97,** 1119–1124.
14. Amezcua, J. L., Dusting, G. J., Palmer, R. M. J. & Moncada, S. (1988) *Br. J. Pharmacol.* **95,** 830–834.
15. Lefroy, D. C., Crake, T., Uren, N. G., Davies, G. J. & Maseri, A. (1993) *Circulation* **88,** 43–54.
16. Lefer, A. M. & Lefer, D. J. (1991) *Basic Res. Cardiol.* **86,** Suppl. 2, 109–116.
17. Ely, D., Dunphy, G., Dollwet, H., Richter, H., Sellke, F. & Azodi, M. (1992) *Free Radical Biol. Med.* **12,** 479–485.
18. Blough, N. V. & Zafiriou, O. C. (1985) *Inorg. Chem.* **24,** 3504–3505.
19. Paterson, R. A., Eagles, P. A. M., Young, D. A. B. & Beddell, C. R. (1976) *Int. J. Biochem.* **7,** 117–118.
20. Ku, D. D., Lui, S. & Beckman, J. S. (1992) *Circulation* **86,** Suppl. 1, I-619.
21. Wu, M., Kaminski, P. M., Fayngersh, R. P., Groszec, L. L., Pritchard, K. A., Jr., Hintze, T. H., Stemerman, M. B. & Wolin, M. S. (1993) *Circulation* **88,** 1522.
22. Lui, S., Beckman, J. S. & Ku, D. D. (1994) *J. Pharmacol. Exp. Ther.* **268,** 1114–1121.
23. Axelsson, K. L. & Andersson, R. G. (1983) *Eur. J. Pharmacol.* **88,** 71–79.
24. Waldman, S. A., Rapaport, R. M., Ginsburg, R. & Murad, F. (1986) *Biochem. Pharmacol.* **35,** 3525–3531.
25. Rossaint, R., Falke, K. J., Lopez, F., Slama, K., Pison, U. & Zapol, W. M. (1993) *N. Engl. J. Med.* **328,** 399–405.
26. Kiff, R. J., Moss, D. W. & Moncada, S. (1994) *Br. J. Pharmacol.* **113,** 496–498.
27. McAninly, J., Williams, D. L. H., Askew, S. C., Butler, A. R. & Russell, C. (1993) *J. Chem. Soc. Chem. Commun.* **23,** 1758–1759.
28. Shaul, P. W., Muntz, K. H. & Buja, L. M. (1990) *J. Pharmacol. Exp. Ther.* **252,** 86–92.
29. Berti, F., Magni, F., Rossini, G., De Angeli, L. & Galli, G. (1990) *J. Cardiovasc. Pharmacol.* **16,** 727–732.
30. Beckman, J. S., Zu Ye, Y., Anderson, P. G., Chen, J., Accavitti, M. A., Tarpey, M. M. & White, C. R. (1994) *Biol. Chem. Hoppe-Seyler* **375,** 81–88.
31. Graham, A., Hogg, N., Kalyanaraman, B., O'Leary, V., Darley-Usmar, V. & Moncada, S. (1993) *FEBS Lett.* **330,** 181–185.
32. Matheis, G., Sherman, M. P., Buckberg, G., Haybron, D. M., Young, H. H. & Ignarro, L. J. (1992) *Am. J. Physiol.* **262,** H616–H620.
33. Beckman, J. S. (1991) *J. Dev. Physiol.* **15,** 53–59.
34. Yu, L., Gengaro, P. E., Niederberger, M., Buke, T. J. & Schrier, R. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1691–1695.
35. Beckman, J. S. & Crow, J. P. (1993) *Biochem. Soc. Trans.* **21,** 330–334.
36. Nakashima, S., Tohmatsu, T., Hattori, H., Okano, Y. & Nozawa, H. (1986) *Biochem. Biophys. Res. Commun.* **35,** 1099–1104.
37. Kaser-Glanzmann, R., Jakabova, M., George, J. N. & Luscher, E. F. (1977) *Biochim. Biophys. Acta* **466,** 429–440.
38. Stone, D. S., Darley-Usmar, V. M. & Martin, J. F. (1992) in *Myocardial Response to Acute Injury*, ed. Parratt, J. R. (Macmillan, London), pp. 67–79.