Potentiation of the cardiovascular effects of nitroglycerin by N-acetylcysteine


ABSTRACT The biochemical basis of the mechanism of vasodilatation by nitroglycerin (NTG) has not been previously investigated in man. However, evidence from in vitro studies suggests that NTG induces activation of guanylate cyclase via a series of enzymatic reactions that are modulated by the availability of sulphydryl groups. Cysteine appears to be particularly effective in potentiating guanylate cyclase activation by NTG. To determine whether hemodynamic responsiveness to NTG in man might be modulated by sulphydryl availability, concentration-response curves for effects of intravenously infused NTG on mean arterial pressure (MAP) and mean pulmonary capillary wedge pressure (PCW) were obtained in 10 patients undergoing cardiac catheterization for investigation of chest pain. NTG infusion was repeated 10 min after the intravenous infusion of 100 mg/kg of the cysteine source N-acetylcysteine (NAC). NAC induced no significant hemodynamic effect, but after NAC infusion there was a significant reduction both in the NTG infusion rate associated with a 10% fall from control values in MAP (25.8 ± 8.3 to 9.3 ± 2.7 μg/min; p < .01) and in the infusion rate inducing a 30% reduction in PCW (13.6 ± 4.6 to 4.2 ± 1.6 μg/min; p < .02). In a control group of five patients who received no NAC, there was no significant change in responsiveness to NTG between infusions. It is concluded that NAC potentiates the vasodilator effects of NTG in man. This suggests that sulphydryl availability and/or redox state may be determinants of in vivo responsiveness to NTG.

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Nitroglycerin (NTG) has been used as an antianginal agent for over 100 years. Although the efficacy of NTG in the treatment of myocardial ischemia and in some clinical settings of congestive heart failure is now well established, controversy remains concerning its mechanism of action at both the hemodynamic and cellular levels. Furthermore, the fundamental biochemical mechanisms of action of NTG are not well understood.

Needleman et al. observed that incubation of rabbit aortic strips with the sulphydryl alkylating agent ethacrynic acid led to a reduction in sensitivity to NTG. It was further suggested that tolerance to NTG could be induced by oxidation of sulphydryl groups. These observations have led to the suggestion that the vasodilator action of NTG is closely linked to the availability of critical SH groups in vascular smooth muscle.

More recent studies using isolated tissues have tended to support this postulated role of sulphydryl groups in modulating responses to NTG. There is now evidence consistent with the view that NTG indirectly activates guanylate cyclase and that the vasodilator effects of NTG are mediated by increased intracellular concentrations of guanosine 3', 5'-monophosphate (cyclic GMP). Although possible modulation of this process by sulphydryl availability may occur at several points, available data suggest that S-nitrosothiol compounds, formed by interaction of NTG with tissue sulphydryl, activate guanylate cyclase. Of a number of S-nitrosothiols tested, S-nitroso-cysteine was most effective in stimulating guanylate cyclase activation.

Thus production of S-nitrosothiols by interaction of NTG with tissue sulphydryl groups may be an essential step in the development of NTG-induced vasodilation. Variability in tissue sulphydryl availability might also offer an explanation for the wide disparity in hemodynamic responsiveness to NTG at any particular plasma NTG concentration. However, to date no studies have been carried out to determine whether availability of sulphydryl, or particularly cysteine, modulate responsiveness to NTG in intact animals or man. The current-
ly reported series of studies were carried out to test the hypothesis that this interaction may be operative in patients with ischemic heart disease.

Methods

Patients. The study population consisted of 15 patients (12 men, three women) undergoing diagnostic cardiac catheterization and coronary arteriography for evaluation of chest pain. Presence of unstable angina pectoris, hemodynamically significant stenosis of the left main coronary artery, significant valvular heart disease, requirement for nitrate administration during the routine component of cardiac catheterization, or previous adverse reactions to NTG or N-acetylcysteine (NAC) excluded patients from entry to the study.

Informed consent was obtained in writing before cardiac catheterization. Administration of long-acting nitrate preparations or cutaneously administered NTG was halted 12 to 24 hr before catheterization; other prophylactic antianginal agents were continued in unchanged dosages and sublingually administered NTG was used as required.

Protocol. Left and right heart catheterizations were performed from the femoral approach. Cardiac index was determined by the Fick method. After completion of the diagnostic procedures, a No. 7F balloon-tipped catheter was advanced into the pulmonary artery. Baseline levels of pulmonary arterial and pulmonary capillary wedge pressures were recorded, with determination of mean pressures over a 30 sec period to minimize errors due to respiratory variation. Femoral artery pressure was recorded via the side-arm of a femoral arterial sheath.

After determination of baseline pressures, NTG infusion was initiated into a peripheral vein by means of a Model 2206 Harvard infusion pump and nonabsorbent tubing (McGaw Laboratories, Irvine, CA). The initial rate of NTG infusion was 1 mcg/min. Effects on heart rate and on femoral arterial, pulmonary arterial, and pulmonary capillary wedge pressures were determined at 5 min intervals. Infusion rates of NTG were increased every 5 min, with successive rates of 1, 2.5, 5, 10, 25, and 50 mcg/min. Infusion was terminated after any particular rate had induced a greater than 10% fall in mean arterial pressure (MAP) or a greater than 30% fall in mean pulmonary capillary wedge pressure (PCW).

After a further 5 min, 10 patients (the NAC group) received an infusion of 100 mg/kg body weight of NAC in 200 ml of 5% dextrose via a peripheral vein over 15 min. After a further 10 min, infusion of NTG was repeated, with the same hemodynamic end points.

To assess the magnitude of spontaneous changes in responsiveness to NTG during the time required for this infusion protocol, a control group of five subjects receiving only 5% dextrose (200 ml) between the first and second NTG infusions was also studied.

Results

Patients. Characteristics of the patients are summarized in table 1. There was no significant difference between the NAC patients and control subjects with respect to ages or weight; previous exposure to long-acting nitrates and ß-adrenoceptor antagonists was similar in the two groups.

Findings at cardiac catheterization and baseline hemodynamic values are summarized in table 2. Two
subjects, one in each group, were found to have no hemodynamically significant coronary artery stenoses. In one patient only systemic artery pressure was measured. There was no significant difference between baseline values of MAP, mean pulmonary arterial, or PCW pressures, cardiac index, or heart rate between the NAC and control groups.

No patient had an initial MAP ≤75 mm Hg or an initial PCW ≤5 mm Hg, and the highest initial PCW was 17 mm Hg. Only one patient (No. 6, NAC group) had a left ventricular ejection fraction below 50%.

**Initial response to NTG infusion.** Threshold infusion rates of NTG inducing detectable falls in MAP or PCW varied considerably among the 10 NAC patients and five control subjects. The end points of a 10% reduction in systemic MAP or a 30% fall in PCW occurred at NTG infusion rates as low as 1.6 μg/min (table 3). In only one patient was the infusion rate associated with a 10% reduction in MAP greater than 50 μg NTG/min. However, two control patients (Nos. 3 and 4) developed less than 20% reductions in PCW at NTG infusion rates that reduced MAP by greater than 10%.

In the NAC group, mean maximal reduction in MAP was 8.3 ± 0.7%; maximal fall in PCW was 30.8 ± 3.2% (excluding patients 3 and 4). There was no significant difference in the NTG infusion rates initially producing a 10% reduction in MAP in the NAC and control groups. A Wilcoxon rank-sum test revealed no evidence of correlation between previous exposure of patients to long-acting nitrate preparations and initial sensitivity to infused NTG.

**Effects of NAC or 5% dextrose infusion.** No consistent hemodynamic effects were noted during infusion of either NAC or 5% dextrose in the NAC and control groups, respectively. Comparisons of hemodynamic parameters before the first and second NTG infusions (table 4) revealed no statistically significant fluctuations in baseline induced by NAC.

**Effects of NAC on responsiveness to NTG.** After infusion of NAC there was increased responsiveness to NTG, as determined by comparison of NTG infusion rates required to produce a 10% reduction of MAP or 30% reduction in PCW (table 3). Changes in responsiveness were determined separately for both of these hemodynamic end points (table 3). Because it appeared that changes in NTG responsiveness in the NAC group did not follow a Gaussian distribution,
However there were consistent trends with patients showing a reduction and two patients a mild increase in responsiveness (table 3), suggesting that significant hemodynamic tolerance to NTG was not induced by the first infusion.

Comparison of changes in responsiveness to NTG between the NAC and control groups indicated a statistically significant \((p < .05)\) difference between the two groups for both the MAP and PCW end points.

**Adverse effects.** NTG was well tolerated by most patients. However, one patient (No. 1, control group) experienced mild headache during infusion at maximum rate. One patient (No. 1, NAC group) developed transient cough and production of mucus 20 min after termination of the NAC infusion.

**Discussion**

The results of these experiments demonstrate that NAC potentiates hemodynamic responsiveness to NTG, as measured by changes in MAP and PCW per unit NTG infusion rate. This potentiation contrasts with the lack of spontaneous variability in responses to NTG in the control group. A number of possible mechanisms for the effects of NAC are revealed by consideration of the results of in vitro experiments related to the mechanisms of action of NTG.

The initially proposed model of the NTG "receptor," was based on studies in preparations of rabbit aortic strips which indicated that specific tolerance to nitrates could be induced by prolonged exposure to NTG, potentiated by alkaline pH, but reversed by the reducing agent dithiothreitol. It was suggested that NTG (and organic nitrates) reversibly oxidizes one or more sulphydryl groups in the "receptor," leading to the formation of a disulfide form of the receptor that has a much lower affinity for NTG. The inhibitory effects of ethacrylic acid, which alkylates sulphydryl groups, on responsiveness to NTG in vitro provided further support for this hypothesis.\(^3\)

The postulated role of cyclic GMP as an intracellular mediator of NTG-induced vasodilatation\(^5,6\) makes the potential mechanism of modulation of NTG sensitivity more complex. First, a number of compounds have been shown to be potent activators of guanylate cyclase in arterial smooth muscle. These include several S-nitrosothiols, which would be formed when NO\(_3\) or nitric oxide react with a tissue sulphydryl source.\(^7\) In these experiments, it was found that cysteine was far more effective in stimulating activation of guanylate cyclase by NTG than other sulphydryl-containing materials such as dithiothreitol, penicillamine, and reduced glutathione. Ascorbate was totally ineffective. This suggests that the interaction between cysteine and NTG is not purely a matter of increased availability of sulphydryl groups or of alteration in tissue redox state.
Further modulation of NTG responsiveness by variation in sulfhydryl availability is possible at least at two other points. NTG inactivation, catalyzed by the hepatic enzyme organic nitrate ester reductase, induces oxidation of glutathione; depletion of hepatic reduced glutathione can be demonstrated in vitro after prolonged NTG infusion. Inhibition of hepatic organic nitrate ester reductase activity may produce prolongation of the plasma half-life of NTG.

A final mechanism of sulfhydryl modulation of NTG responsiveness is apparent from the finding that hepatic and pulmonary guanylate cyclase is rapidly inactivated by molecular oxygen, sulfhydryl oxidants, or thiol alkylating agents and that the stimulation of guanylate cyclase activity by nitric oxide and S-nitrosocysteine is prevented by sulfhydryl oxidants. This suggests that sulfhydryl groups on the guanylate cyclase catalytic site are critical in its activation. Thus guanylate cyclase activity is likely to be susceptible to variations in tissue redox state.

The study reported here was performed in an attempt to evaluate, in a clinical setting, some of the issues raised from these in vitro studies. We chose to investigate the effects of short-term administration of NAC on hemodynamic response to NTG in patients with ischemic heart disease. NAC was chosen as the sulfhydryl source for a number of reasons. It was possible that a cysteine-containing material would produce optimal potentiation of responses to NTG. NAC is extensively hydrolyzed to cysteine in vivo, although plasma concentrations of unchanged NAC become detectable after long-term administration. Furthermore, NAC has proved to be safe and rapidly effective when administered intravenously as a cysteine source in the emergency treatment of acetaminophen overdose.

We used a commercially available sterile preparation of NAC for intravenous administration (Parvolex),

### TABLE 3

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>First NTG infusion</th>
<th>Second NTG infusion</th>
<th>Change in infusion rate (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
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<tr>
<td></td>
<td>1 MAP 10</td>
<td>I PCW 30</td>
<td>1 MAP 10</td>
</tr>
<tr>
<td>NAC group</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>5</td>
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<tr>
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<td>8.5</td>
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<td>25.7</td>
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<tr>
<td>SD</td>
<td>12.5</td>
<td>22.3</td>
<td>17.4</td>
</tr>
</tbody>
</table>

1 MAP 10 = NTG infusion rate (µg/min) inducing 10% fall in MAP; 1 PCW 30 = NTG infusion rate (µg/min) inducing 30% fall in mean PCW.

<sup>a</sup>Percent of first infusion required to achieve hemodynamic end point during second infusion.

### TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP 1st</th>
<th>MAP 2nd</th>
<th>PCW 1st</th>
<th>PCW 2nd</th>
<th>PA 1st</th>
<th>PA 2nd</th>
<th>HR 1st</th>
<th>HR 2nd</th>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>101.1</td>
<td>10.9</td>
<td>11.1</td>
<td>19.1</td>
<td>19.0</td>
<td>70.7</td>
<td>70.1</td>
</tr>
<tr>
<td>SD</td>
<td>12.3</td>
<td>11.7</td>
<td>3.5</td>
<td>3.5</td>
<td>4.4</td>
<td>4.7</td>
<td>12.0</td>
<td>11.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>105.4</td>
<td>11.4</td>
<td>10.8</td>
<td>19.6</td>
<td>18.2</td>
<td>78.4</td>
<td>76.8</td>
</tr>
<tr>
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<td>5.4</td>
<td>5.4</td>
<td>6.2</td>
<td>15.4</td>
<td>13.6</td>
</tr>
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</table>

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which was infused at a rate similar to that used by Prescott et al. The lack of significant hemodynamic effects of NAC in our study was in accordance with previous experience with the drug.

In view of the potential risk of inducing substantial decreases in MAP, the major end points chosen to assess patient responsiveness to NTG were the infusion rates that produced a 10% reduction in MAP and a 30% reduction in PCW. However, it must be recognized that the hemodynamic end points chosen are representative only of the lower end of the NTG dose-response curve and that assessment of the extent of fluctuations in responsiveness to NTG cannot be extrapolated beyond the chosen end points.

Although each patient who received NAC served as his or her own control, a small control group of patients who did not receive NAC was included because of the possibility that changes in sensitivity to NTG could occur between infusions as a result of absorption of NTG onto infusion tubing, onset of true hemodynamic tolerance or potentiation, or changes in baseline values induced by infusion of 5% dextrose between NTG infusions. Although minor fluctuations in responsiveness to NTG were observed in the control group, there was no major change between infusions. This, together with the initial responsiveness of the NAC group to NTG infusion rates of less than 100 μg/min, suggests that the infusion system used was effective in minimizing losses of NTG.

Administration of NAC induced a clear-cut potentiation of hemodynamic responsiveness to NTG, assessed both on the basis of changes in MAP and PCW. The hypotensive effects of NTG were more markedly increased in those patients who initially had been least responsive to NTG. The most probable explanation of these findings is that NAC potentiated the vasodilator effects of NTG, presumably in both arteries and veins. Although this cannot be stated with certainty in the absence of more extensive assessment of hemodynamic changes, the only other explanation (attenuation of the effects of NTG on cardiac output) seems unlikely.

It is possible that the greater potentiation after NAC of NTG effects in patients who were initially less responsive to the drug reflects variations among patients in initial availability of sulfhydryl groups and/or in tissue redox state. Although, the present study was not designed to evaluate this possibility, no evidence was found of effects of previous administration of long-acting nitrates on either initial responsiveness or the degree of NAC-induced potentiation. Thus, while tolerance to NTG may under some circumstances be induced by long-acting nitrates, this did not appear to be the major determinant of responsiveness in the present series.

The results of our study suggest that, particularly in initially insensitive patients, enhanced hemodynamic responsiveness to NTG may be induced after NAC. This finding is in accordance with the results of previous in vitro studies. Whether this altered responsiveness is of clinical value in the management of ischemic heart disease and/or congestive heart failure remains to be addressed in future studies.

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