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The Reaction between Organic Nitrates and Sulfhydryl Compounds

A Possible Model System for the Activation of Organic Nitrates

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SUMMARY

The rate of loss of the sulfhydryl group, determined with the Ellman reagent, was used to derive second order rate constants for the reaction of a series of organic nitrates with a series of sulfhydryl compounds. For the organic nitrates, increases in the rate of reaction with cysteine, in general, ran parallel both with increases in pharmacological potency (flow in the Langendorff heart) and with increases in total clearance. Cysteine was the most active sulfhydryl compound examined, which is compatible with a possible role as an important nitrate receptor. Under some conditions the rate of loss of the sulfhydryl group was much greater than the rate of formation of nitrite ion. This indicates the presence of a reaction intermediate, probably a thionitrate. It is suggested that, *in vivo*, a thionitrate could function as an important intermediate in the activation of guanylate cyclase.

INTRODUCTION

There is now rather good evidence that the relaxation of vascular smooth muscle by the nitrovasodilators is mediated by cGMP (reviewed in Ref. 1). This must occur through activation of the enzyme, guanylate cyclase, and, to achieve this, it appears that organic nitrates must themselves first be chemically activated. Ignarro *et al.* (2) found that nitroglycerin activated partially purified guanylate cyclase, but only in the presence of cysteine. An involvement of sulfhydryl compounds in the mechanism of action of nitroglycerin was also suggested by the earlier work of Needleman *et al.* (3), using chemical modification of intact rabbit aortas.

Although it is probable that sulfhydryl compounds are involved in the pharmacological action of organic nitrates, the details remain uncertain. Ignarro *et al.* (2) discovered that organic thionitrites activate guanylate cyclase and reduce arterial blood pressure. They suggested that organic nitrates are reduced by sulfhydryl compounds to inorganic nitrite. This slowly forms nitric oxide, which then reacts with sulfhydryl compounds to form thionitrites. These then act as the proximal activators of guanylate cyclase. A corollary of this theory is that organic nitrates function solely through formation of intracellular nitrite. This is also true if, not thionitrites, but nitric oxide is the proximal activator of guanylate cyclase (4).

Most of the work of the reaction between organic nitrates and sulfhydryl compounds has been on the enzymatic reaction. Heppel and Hilmoe (5) demonstrated that an enzyme present in liver extracts catalyzes the reaction of glutathione with nitroglycerin or erythritol tetranitrate to give oxidized glutathione and nitrite. Needleman and Hunter (6) and Needleman *et al.* (7) measured this activity with a series of organic nitrates and demonstrated that the di- and polynitrates lose nitrite groups during the reaction. Keen *et al.* (8) demonstrated that the enzyme that catalyzed the reaction between glutathione (GSH) and organic nitrates is identical with glutathione *S*-transferase. They established the stoichiometry of the reaction and for mechanistic reasons proposed that it proceeds through an intermediate, glutathione thionitrate:

$$GSH + RONO_2 \rightarrow GSNO_2 + ROH \quad \text{enzymic}$$

$$GSNO_2 + GSH \rightarrow GSSG + HNO_2 \quad \text{nonenzymic}$$

Addition of a second sulfhydryl compound reduced the formation of glutathione disulfide. This was taken as evidence that the intermediate thionitrate had been trapped.

Very little is present in the literature on the nonenzymic reaction between organic nitrates and sulfhydryl compounds. Klason and Carlson (9) demonstrated that the reaction between thiophenol and nitroglycerin gives diphenyl disulfide and glycerol. Heppel and Hilmoe (5) demonstrated that glutathione, cysteinylglycine, and cysteine react nonenzymically with nitroglycerin. During this reaction the sulfhydryl compounds are oxidized and nitrite is formed. Needleman and Hunter (6) showed that the nonenzymatic reaction between glutathione and nitroglycerin gives inorganic nitrite and glycerol dinitrates.

The present paper contains a study of the nonenzymic

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reaction between organic nitrates and sulfhydryl compounds. It was felt that this would be chemically interesting and could perhaps suggest ideas about the pharmacological activation of organic nitrates, which probably proceeds both enzymically and nonenzymically (10). The study concentrates on glycerol-1-nitrate, which recently has been shown in this laboratory to be a long-acting compound with typical nitrate activity (11).

MATERIALS AND METHODS

All solutions were prepared in 0.1 M sodium phosphate buffer, pH 7.2, and all incubations were carried out at 37°. At various times after mixing a solution of a sulfhydryl compound with a solution of an organic nitrate, samples were taken out and either the sulfhydryl concentration or the concentration of nitrite ion was determined. The sulfhydryl group assay employed the spectrophotometric determination of 2-nitro-5-thiobenzoic acid, liberated by the reaction between sulfhydryl compounds and 5,5′-dithiobis-(2-nitrobenzoic acid) (12).

The nitrite assay employed the spectrophotometric determination of the diazo dye formed by the reaction between nitrite ion, sulfanilic acid, and *N*-(1-naphthyl)-ethylenediamine dihydrochloride at acid pH (13).

For measurements of the decrease of sulfhydryl concentration, a blank without nitrate was included and corrected for. For experiments with tissue extracts or plasma the samples were diluted 1:200 before the sulfhydryl groups were determined. For measurements of the formation of nitrite, a blank without sulfhydryl compound was included and corrected for. Each incubation was carried out in duplicate. For experiments in which isomeric nitrates were compared, incubations of the two nitrates were always carried out simultaneously and in parallel. Each incubation was carried out in duplicate. The concentrations of sulfhydryl compounds used were too low to interfere with the determination of nitrite.

The results were analyzed with the help of calibration curves, prepared either for inorganic nitrite or for the appropriate sulfhydryl compound. In all experiments at least a 100-fold excess of organic nitrate over sulfhydryl compound was used, thus allowing the disappearance of sulfhydryl groups to be analyzed as a pseudo-first order decay. Semilogarithmic plots of the data were fitted by eye and gave second order rate constants, which were determined at least three times for each pair of substances.

The sulfhydryl compounds were obtained from the purest available commercial sources. Nitroglycerin was purchased as a 5% ethanolic solution from Dynamite Nobel Ltd. The other organic nitrates were synthesized in our laboratories.

RESULTS

The second order rate constants for the disappearance of the sulfhydryl group were measured for the reaction between cysteine and a group of organic nitrates (Table 1). Fig. 1 shows a typical experiment, in which isosorbide-2- and -5-nitrates were compared. The second order rate constants for the disappearance of the sulfhydryl group were also measured for the reaction between glycerol-1-nitrate and a series of sulfhydryl compounds; the results are summarized in Table 2.

In preliminary experiments, the rate of loss of sulfhydryl groups was measured from either rat liver homogenate (50 mg/ml) or rat plasma, in the presence of 0.1 M glycerol-1-nitrate. With liver homogenate the decline was very slow and monophasic ($k = 0.8$ M^{1-} hr^{-1}). However, with plasma the decline was clearly and reproducibly biphasic (Fig. 2). The two phases were approximately separated, giving second order rate constants of 2.8 and 0.2 M^{-1} hr^{-1}.

TABLE 1

Second order rate constants, with standard deviations, for the loss of sulfhydryl in the reaction between cysteine and organic nitrates

Organic nitrates were incubated with 40 μM cysteine in 0.1 M sodium phosphate buffer (pH 7.2) at 37°. At various times aliquots of the incubation mixture were taken out and the concentration of sulfhydryl groups was determined, as described in "Materials and Methods." The concentrations of the organic nitrates were selected so that about 50% of the cysteine was consumed within 30 min.

Organic nitrate	Rate constant
	M^{-1} hr^{-1}
Glycerol trinitrate	310 ± 38
Glycerol-1,2-dinitrate	37 ± 4
Glycerol-1,3-dinitrate	23 ± 3
Glycerol-1-mononitrate	6.1 ± 1.2
Isosorbide dinitrate	250 ± 14
Isosorbide-2-mononitrate	13.1 ± 1.1
Isosorbide-5-mononitrate	10.1 ± 0.7

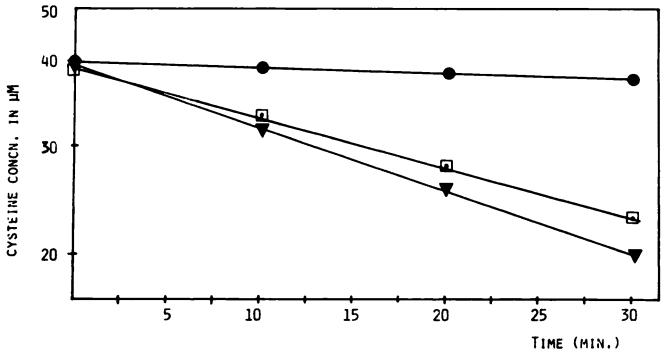


FIG. 1. *The reaction between 96 mM isosorbide-5-mononitrate (□) and 96 mM isosorbide-2-mononitrate (▼) and cysteine (40 μM)*

Semilogarithmic plot of cysteine concentration (μM) against time (min) is given. Control with cysteine alone (●) is indicated.

TABLE 2

Second order rate constants, with standard deviations, for the loss of sulfhydryl in the reaction between glycerol-1-nitrate and sulfhydryl compounds

Glycerol-1-nitrate was incubated with 40 μM concentrations of the sulfhydryl compounds in 0.1 M sodium phosphate buffer (pH 7.2) at 37°. At various times aliquots of the incubation mixture were taken out and the concentration of sulfhydryl groups was determined, as described in "Materials and Methods."

Sulfhydryl compound	Rate constant
	M^{-1} hr^{-1}
Cysteine	6.1 ± 1.2
Cysteine methyl ester	5.6 ± 0.8
Cysteamine	4.0 ± 0.3
N-Acetylcysteine	0.6 ± 0.2
Glutathione	0.5 ± 0.1

The rate of nitrite formation was compared with the rate of sulfhydryl loss for the reaction between glycerol-1-nitrate and cysteine. With 0.1 M glycerol-1-nitrate and 40 μM cysteine, the rate of sulfhydryl loss was, within experimental error, twice that of nitrite formation (Fig. 3). However, with 1.0 M glycerol-1-nitrate and 40 μM cysteine, the processes were much more clearly separated, the initial rate of loss of sulfhydryl being at least 10 times that of the formation of nitrite (Fig. 4).

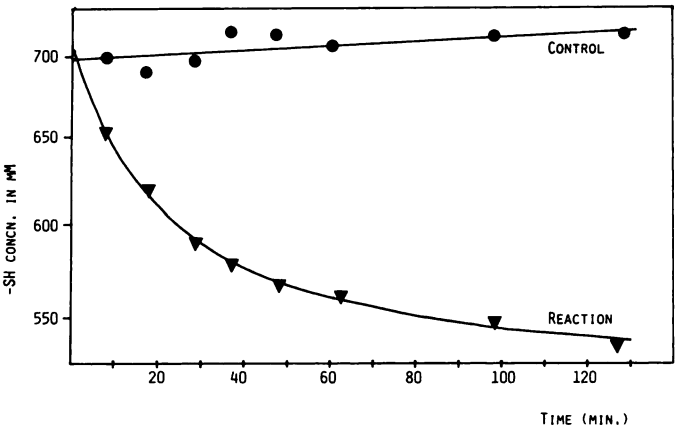


FIG. 2. *Loss of sulfhydryl groups from rat plasma on reaction with glycerol-1-nitrate*

Equal volumes of 0.2 M glycerol-1-nitrate and plasma were mixed. A semilogarithmic plot of sulfhydryl concentration (mM) against time (min) is given. Control without nitrate is included.

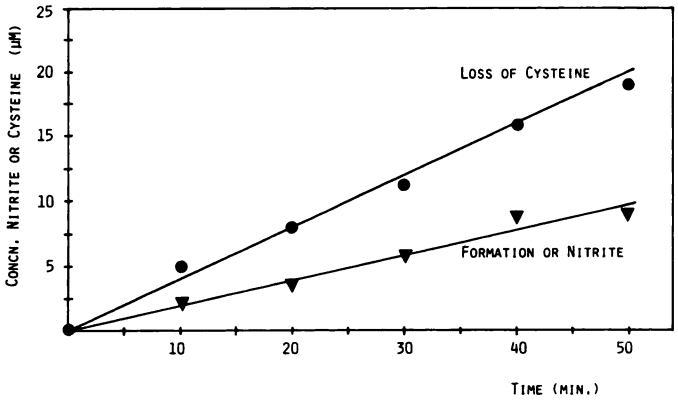


FIG. 3. *Comparison of the loss of sulfhydryl groups (●) with the formation of nitrite (▼)*

Forty μM cysteine was reacted with 0.1 M glycerol-1-nitrate.

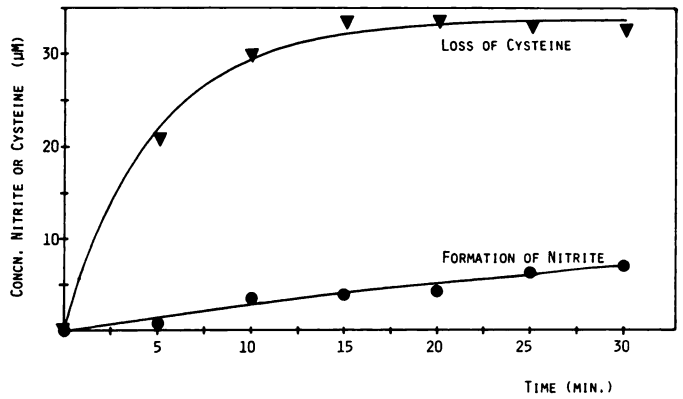


FIG. 4. *Comparison of the loss of sulfhydryl groups (▼) with the formation of nitrite (●)*

Forty μM cysteine was reacted with 1.0 M glycerol-1-nitrate.

It is well established that nitrite ion can react with thiols to give unstable thionitrites (*S*-nitrosothiols) (2). The importance of this reaction under our conditions was examined by incubating 40 μM cysteine in the presence of 40 μM nitrite for 30 min. The nitrite had no effect on the concentration of sulfhydryl groups, indicating that the formation of thionitrite was negligible.

DISCUSSION

Reactivity of the organic nitrates. There are relatively few publications in which the pharmacological properties of a series of organic nitrates are systemically compared. In the most careful, recent study, Noack (14) measured the ability of glycerol trinitrate, glycerol-1,2-dinitrate, glycerol-1,3-dinitrate, glycerol-1-mononitrate, isosorbide dinitrate, and isosorbide-2- and -5-mononitrates to increase coronary flow in the Langendorff heart preparation. With the exception of isosorbide-5-mononitrate, Noack found an excellent correlation between potency and lipid solubility. His data are replotted in Fig. 5, but with lipid solubility replaced by the rate constant for the reaction with cysteine. Again there is an excellent correlation, with the exception of isosorbide-5-mononitrate. The correlations suggest that either lipophilicity or sulfhydryl reactivity can influence the pharmacological activity of organic nitrates. Further work is needed to separate the two contributions. Needleman *et al.* (7) have already noted a general correlation between sulfhydryl activity (measured with glutathione *S*-transferase plus glutathione) and vasodilatory potency.

The low activity of isosorbide-5-mononitrate in the Langendorff heart preparation apparently can be explained neither on the basis of lipophilicity nor on the basis of sulfhydryl reactivity. This may be taken as indicating some additional specificity in nitrate activation, perhaps in the form of an activating enzyme.

It is known that mononitrates (15–17) and polynitrates (6, 18, 19) are rapidly broken down in the body, giving the corresponding alcohol and inorganic nitrite or nitrate. The processes by which the mononitrates are broken down are totally unknown. Most work with the polynitrates has focused on their breakdown by glutathione *S*-transferase in the liver (6). It is known, however,

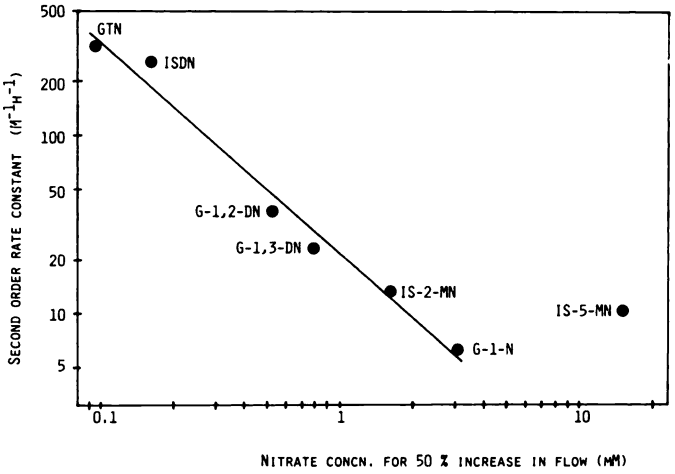


FIG. 5. *Correlation between the rate of reaction with cysteine and the vasodilator potency for six nitrates*

The log of the second order rate constant (M^{-1} hr^{-1}) was plotted against the log of the concentration of organic nitrate (mmol/liter) required for a 50% increase in coronary flow in the Langendorff heart preparation (the latter data from Noack (14)). Coefficient of correlation = 0.985, excluding isosorbide-5-mononitrate. *GTN*, glycerol trinitrate; *G-1,2-DN*, glycerol-1,2-dinitrate; *G-1,3-DN*, glycerol-1,3-dinitrate; *G-1-N*, glycerol-1-nitrate; *ISDN*, isosorbide dinitrate; *IS-2-MN*, isosorbide-2-mononitrate; *IS-5-MN*, isosorbide-5-mononitrate.

that extrahepatic metabolism is important for both glycerol trinitrate (20) and isosorbide dinitrate (21). These extrahepatic processes are not understood, although Bennett *et al.* (22) have shown that hemoglobin can break glycerol trinitrate down to dinitrates. In spite of these uncertainties, and bearing in mind the known activity of organic nitrates toward the sulfhydryl group, it is a reasonable hypothesis that this activity could give some sort of general guide to the breakdown of organic nitrates *in vivo*. Because of the disparate volumes of distribution of the organic nitrates, it will be desirable to use total clearance as a parameter of breakdown.

The total clearance in humans of nitroglycerin is given as 38–80 liters/min (18), of isosorbide dinitrate as 6–7 liters/min (23), of isosorbide-2-mononitrate as 0.39 liter/min (24), and of isosorbide-5-mononitrate as 0.14 liter/min (24). These values are in the same sequence as the rate constants for the reaction of the nitrates with cysteine (Table 1). There are no published values for the clearance of glycerol-1-nitrate or of the glycerol dinitrates. However, in a recent study (11), the duration of action of the glycerol nitrates was shown to lie in the order mononitrates > 1,2-dinitrate $\doteq$ 1,3-dinitrate > trinitrate. This too is compatible with the present results. Nevertheless, it is quite clear that much further work on the mechanism of nitrate breakdown will be necessary before the significance, if any, of this correlation can be assessed.

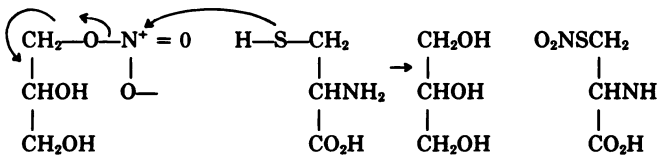
Mechanism of the reaction. In Fig. 3 it is shown that two molecules of cysteine are consumed per mole of nitrite produced. This would point to the formation of cystine, although the disulfide was not directly identified.

2 cysteine + 1 glycerol-1-nitrate

$\rightarrow$ 1 cystine + 1 glycerol + 1 nitrite

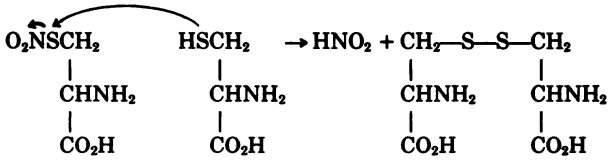
This stoichiometry has not been established previously for the nonenzymic reaction between organic nitrates and sulfhydryl compounds, although it is known for the enzymic reaction (8).

In Fig. 4 it is shown that when a large excess of glycerol-1-nitrate is reacted with cysteine, the rate of consumption of —SH is much greater than the rate of formation of nitrite. The only possible explanation for this observation is that a reaction intermediate is formed. The strongest candidate for a reaction intermediate would be a thionitrate formed by nucleophilic attack of the —SH group of the positively charged N atom of the nitrate group:



cysteine thionitrate

The thionitrate would then be subject to further nucleophilic attack by a second cysteine molecule, giving nitrite and cystine:



Such a mechanism has already been proposed for the enzymic reaction by Keen *et al.* (8), who succeeded in chemically trapping the intermediate. It has also been suggested (25) that thionitrates can be hydrolyzed slowly to sulphenic acids, although no evidence was obtained for this in the present study. Formation of thionitrite (*S*-nitrosothiol) by the reaction between cysteine and nitrite was negligible at the pH, temperature, and concentration ratio examined. It follows that there will also be negligible formation of nitric oxide from the decomposition of thionitrite.

In a recent review, Bennett and Marks (26) have pointed out some difficulties in current ideas about the mechanism by which organic nitrates activate guanylate cyclase. The two proximal activators most often considered are nitric oxide (4) and thionitrites (2). Both can be formed by the reaction between organic nitrates and sulfhydryl compounds and both activate guanylate cyclase. The problem lies in the fact that both compounds can be formed only through inorganic nitrite, which is itself of very low activity as a vasodilator. One must then postulate that cells are of low permeability to inorganic nitrite, which must be formed intracellularly to be effective. The problem is that vascular cells are, in fact, permeable to inorganic nitrite (27). A possible way round this difficulty could be provided by the involvement of thionitrates. These might be reduced intracellularly to thionitrites or nitric oxide without the intermediacy of nitrite ion. Alternatively, and perhaps less probably, thionitrate itself could act as the proximal activator. The organic chemistry of the thionitrates has been studied (reviewed in Ref. 28) and they have been found, at least in some cases, to be more stable than the thionitrites.

Table 2 contains a comparison of the rate constants for the reaction of glycerol-1-nitrate with five different sulfhydryl compounds. The most striking finding is that acetylation of the amino group of cysteine reduces the rate of the reaction by a factor of 10. This may be explained by the presence of a neighboring group effect. Possibly —NH_2 (or —NH_3^+) is capable of polarizing the nitrate group by binding to an oxygen atom. The susceptibility of the nitrate group to attack by —SH would then be increased. Another possibility is that the inductive effect of the ammonium group increases the nucleophilicity or ionization of the sulfhydryl group.

Methylation of the carboxylate group of cysteine produces no significant change in reaction rate. However, total removal of the carboxylate group (in cysteamine) does decelerate the reaction, but not by nearly so much as does modification of the amino group. It therefore appears that the carboxyl group of cysteine is capable of exerting a weak positive neighboring group effect on the reaction with nitrates. The mechanism of this remains obscure. A direct inductive effect from the carboxyl group

probably plays no important role, as this could be more likely to decelerate the reaction.

The low rate of reaction between glutathione and glycerol-1-nitrate can be adequately explained by the acylation of the amino group in the cysteine residue in glutathione. The fast phase of the reaction between rat plasma and glycerol-1-nitrate (Fig. 2) might be explained by the presence in plasma of significant levels of cysteine and, perhaps, of peptides with amino terminal cysteine. It has been reported previously that cysteine is much more active than glutathione in enhancing the activation of guanylate cyclase by glycerol trinitrate (29, 30). The latter findings are clearly compatible with the present results.

The present and previous findings are clearly compatible with the idea that cysteine plays a role as an important nitrate receptor. However, whether this is the case or not will clearly be decided by the relative concentrations of cysteine and of other possible nitrate receptors, and also by the activities of appropriate enzymes.

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REFERENCES

1. Rapoport, R. M., and F. Murad. Endothelium-dependent and nitrovasodilator-induced relaxation of vascular smooth muscle: role of cyclic GMP. *J. Cyclic Nucleotide Protein Phosphor. Res.* **9**:281–296 (1983).
2. Ignarro, L. J., H. Lippton, J. C. Edwards, W. H. Baricos, A. L. Hyman, P. I. Kadowitz, and C. A. Gruetter. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of *S*-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* **218**:739–749 (1981).
3. Needleman, P., B. Jakschik, and E. M. Johnson, Jr. Sulfhydryl requirement for relaxation of vascular smooth muscle. *J. Pharmacol. Exp. Ther.* **187**:324–331 (1973).
4. Katsuki, S., W. Arnold, E. Mittal, and F. Murad. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucleotide Res.* **3**:23–35 (1977).
5. Heppel, L. A., and R. J. Hilmoe. Metabolism of inorganic nitrite and nitrate esters. II. The enzymatic reduction of nitroglycerin and erythritol tetranitrate by glutathione. *J. Biol. Chem.* **183**:129–138 (1950).
6. Needleman, P., and F. E. Hunter, Jr. The transformation of glyceryl trinitrate and other nitrates by glutathione—organic nitrate reductase. *Mol. Pharmacol.* **1**:77–86 (1965).
7. Needleman, P., D. J. Blehm, and K. S. Rotskoff. Relationship between glutathione-dependent denitration and the vasodilator effectiveness of organic nitrates. *J. Pharmacol. Exp. Ther.* **165**:286–288 (1969).
8. Keen, J. H., W. H. Habig, and W. B. Jakoby. Mechanism of the several activities of the glutathione *S*-transferases. *J. Biol. Chem.* **251**:6183–6188 (1976).
9. Klason, P., and T. Karlson. Zur Kenntnis der organischen Nitrate. *Ber. Dtsch. Chem. Ges.* **39**:2752–2754 (1906).
10. Axelsson, K. L., and J.-O. G. Karlsson. Nitroglycerin tolerance *in vitro*: effect on cGMP turnover in vascular smooth muscle. *Acta Pharmacol. Toxicol.* **55**:203–210 (1984).
11. Leitold, M., H. Laufen, and R. A. Yeates. Vergleichende Pharmakologie von Glycerol-1-Nitrat (G-1-N) und Glycerol-trinitrat (GTN) an verschiedenen Tierarten. *Arzneim. Forsch.* in press.
12. Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**:70–77 (1959).
13. Bell, F. K., J. J. O'Neill, and R. M. Burgison. Determination of the oil/water distribution coefficients of glyceryl trinitrate and two similar nitrate esters. *J. Pharm. Sci.* **52**:637–639 (1963).
14. Noack, E. Investigation on structure-activity relationship in organic nitrates. *Methods Find. Exp. Clin. Pharmacol.* **6**:583–586 (1984).
15. Hodgson, J. R., J. P. Glennon, J. C. Dacre, and C.-C. Lee. Metabolism and disposition of isomers of dinitro- and mono-nitroglycerin in the rat. *Toxicol. Appl. Pharmacol.* **40**:65–70 (1977).
16. Wood, S. G., B. A. John, L. F. Chasseaud, R. M. Major, M. E. Forrest, R. Bonn, A. Darragh, and R. F. Lambe. Metabolic fate of ^{14}C-isosorbide 5-mononitrate in humans. *Arzneim. Forsch.* **34**:1031–1035 (1984).
17. Clark, D. G., and M. H. Litchfield. Metabolism of ethylene glycol dinitrate and its influence on the blood pressure of the rat. *Br. J. Ind. Med.* **24**:320–325 (1967).
18. McNiff, E. F., A. Yacobi, F. M. Young-Chang, L. H. Golden, A. Goldfarb, and H. L. Fung. Nitroglycerin pharmacokinetics after intravenous infusion in normal subjects. *J. Pharm. Sci.* **70**:1054–1058 (1981).
19. Assinder, D. F., L. F. Chasseaud, and T. Taylor. Plasma isosorbide dinitrate concentrations in human subjects after administration of standard and sustained release formulations. *J. Pharm. Sci.* **66**:775–778 (1977).
20. Blei, A. T., J. Gottstein, and H.-L. Fung. Role of the liver in the disposition of intravenous nitroglycerin in the rat. *Biochem. Pharmacol.* **33**:2681–2686 (1984).
21. Morrison, R. A., U.-W. Wiegand, E. Jähnchen, E. Höhmann, W. Kasper, T. Meinertz, and H.-L. Fung. Hepatic extraction of isosorbide dinitrate in cardiac patients. *Clin. Pharm. Ther.* **34**:724–731 (1983).
22. Bennett, B. M., K. Nakatsu, J. F. Brien, and G. S. Marks. Biotransformation of glyceryl trinitrate to glyceryl dinitrate by human hemoglobin. *Can. J. Physiol. Pharmacol.* **62**:704–706 (1984).
23. Fung, H.-L., and J. O. Parker. Prolonged plasma half-life after oral isosorbide dinitrate in patients with angina pectoris. *Br. J. Clin. Pharmacol.* **15**:746–748 (1983).
24. Straehl, P., R. L. Galeazzi, and M. Soliva. Isosorbide 5-mononitrate and isosorbide 2-mononitrate kinetics after intravenous and oral dosing. *Clin. Pharm. Ther.* **36**:485–492 (1984).
25. Sokolovsky, M., D. Harell, and J. F. Riordan. Reaction of tetranitromethane with sulfhydryl groups in proteins. *Biochemistry* **8**:4740–4745 (1969).
26. Bennett, B. M., and G. S. Marks. How does nitroglycerin induce vascular smooth muscle relaxation? *Trends Pharmacol. Sci.* **5**:329–332 (1984).
27. Parks, N. J., K. A. Krohn, C. A. Mathis, J. H. Chasko, K. R. Geiger, M. E. Gregor, and N. F. Peck. Nitrogen-13-labeled nitrite and nitrate: distribution and metabolism after intratracheal administration. *Science* **212**:58–61 (1981).
28. Oae, S., and K. Shinhama. Organic thionitrites and related substance. A review. *Org. Prep. Proced. Int.* **15**:165–198 (1983).
29. Laustiola, K., P. Vuorinen, H. Vapaatalo, and T. Metsä-Ketelä. Effects of cysteine and nitroglycerin on bovine heart guanylate cyclase and on tissue cyclic GMP and lactate of rat atria. *Eur. J. Pharmacol.* **91**:301–304 (1983).
30. Ignarro, L. J., and C. A. Gruetter. Requirement of thiols for activation of coronary arterial guanylate cyclase by glyceryl trinitrate and sodium nitrite. Possible involvement of *S*-nitrosothiols. *Biochim. Biophys. Acta* **631**:221–231 (1980).

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