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EFFECT OF ETHACRYNIC ACID, A GLUTATHIONE-*S*-TRANSFERASE INHIBITOR, ON NITROGLYCERIN-MEDIATED cGMP ELEVATION AND VASORELAXATION OF RABBIT AORTIC STRIPS

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Abstract—The effects of ethacrynic acid (ECA), an inhibitor of glutathione-*S*-transferase, on both the pharmacologic and biochemical responses of aortic tissue to nitroglycerin (GTN) were evaluated. Using the rabbit aortic strip model, relaxation responses to 0.6 μM GTN were measured with and without ECA (0.2 mM) pretreatment. These same strips were frozen, and the concentrations of cGMP in the strips were measured using a ^{3}H-labeled radioimmunoassay. Both the relaxation response and the increase in cGMP upon GTN treatment were reduced significantly by pretreatment of the strips with ECA. A correlation was observed between the decreases in the pharmacodynamic and biochemical responses upon ECA pretreatment. cGMP levels in strips treated with sodium nitroprusside, which generates nitric oxide by mechanisms distinct from that for organic nitrates, were not decreased by ECA pretreatment. These observations suggest that the mechanism of GTN action involves a glutathione-*S*-transferase-mediated metabolic step for GTN and that the isozyme(s) involved in this activation process may be inhibited by ECA.

Although nitroglycerin (GTN†) has been a drug of choice in the treatment of angina for over a century, its mechanism of action is still not clearly elucidated. It is agreed that the site of GTN action is the vasculature, and that the drug enters vascular smooth muscle cells where it is metabolized. The final product of this metabolism is nitric oxide, which itself [1–3] or via the formation of an *S*-nitrosothiol acts as a first messenger and activates the enzyme guanylate cyclase (GC). Activation of GC leads to the conversion of GTP to cGMP, which serves as a second messenger triggering a cascade of events leading to vasorelaxation ([4, 5]; Fig. 1). Although the role of cGMP in organic nitrate-induced vasorelaxation has been well established, and the necessity of a metabolic step has been agreed upon, the exact nature of the metabolic process and the particular pathway (enzymatic or nonenzymatic) responsible for the "productive" (leading to effect) metabolism of GTN have not yet been established.

Studies carried out in our laboratory [6–9] have provided evidence for the possible role of glutathione-*S*-transferases (GSTs) in this metabolic process. On the basis of inhibitor studies in homogenates of rabbit liver [6, 7], rabbit aorta [8] and bovine aorta [9], we have shown successfully that GST catalyzes the metabolic conversion of GTN to its denitrated products in these tissues. We have also demonstrated that when rabbit aortic strips were pretreated with ethacrynic acid (ECA), an inhibitor of GST, the relaxation response to GTN was reduced and this was accompanied by reduced metabolism of GTN [8]. This led us to conclude that the enzyme involved in the pharmacologic activation of GTN was GST. To obtain further evidence for our hypothesis regarding the role of GST in the productive metabolism of GTN, we investigated the effect of ECA (GST substrate-inhibitor) on a primary biochemical response (cGMP levels) and a simultaneously measurable secondary pharmacologic response (relaxation) to GTN in rabbit aortic strips.

MATERIALS AND METHODS

Materials. GTN was purchased as 10-mL vials of Tridil® [in 30% alcohol (v/v), 30% propylene glycol and water for injection q.s.] from Du Pont Pharmaceuticals (Wilmington, DE). ECA, *l*-phenylephrine hydrochloride (PE) and sodium nitroprusside (SNP) were obtained from the Sigma Chemical Co. (St. Louis, MO). Diethyl ether was purchased from the Fischer Scientific Co. (Fair Lawn, NJ). The tritium-labeled radioimmunoassay (RIA) kit was purchased from Amersham (Arlington Heights, IL). All solutions and dilutions were made in the Krebs buffer used for the vasorelaxation study.

Isolation of rabbit aorta for the vasorelaxation study. These techniques followed procedures described previously [7]. Briefly, New Zealand White rabbits (Nitabell Rabbitry, Hayward, CA; male, 2–3 kg) were anesthetized with an intramuscular injection (40 mg/kg) as 1.5 mL of a 100 mg/mL solution of ketamine in water for injection and decapitated 15 min later. The thoracic cavity of the

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† Abbreviations: GTN, nitroglycerin; GST, glutathione-*S*-transferase; ECA, ethacrynic acid; GC, guanylate cyclase; PE, *l*-phenylephrine hydrochloride; TCA, trichloroacetic acid; SBP, sulfobromophthalein; and SNP, sodium nitroprusside.

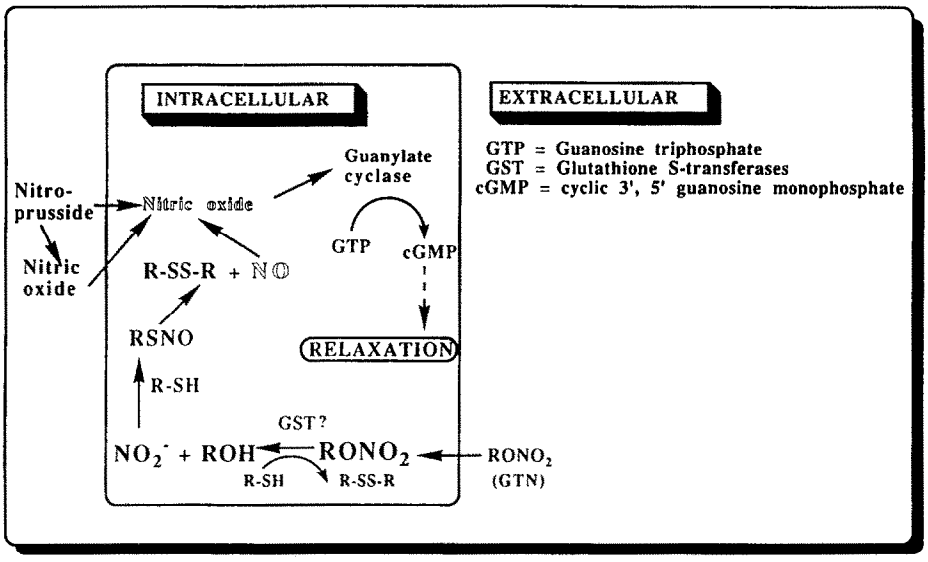

Fig. 1. Mechanism of action of organic nitrates in the vasculature as hypothesized by Ignarro [5].

animal was opened and the descending aorta was surgically removed and immediately placed in a beaker containing Krebs buffer (NaCl, 119 mM; KCl, 4.8 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; $CaCl_2$, 2.5 mM; glucose, 11.1 mM), which had been gassed continuously with carbogen (95% oxygen and 5% carbon dioxide). After isolation, excess fat and connective tissues were removed carefully; during this process the buffer was replaced constantly with fresh buffer. The endothelium was not disturbed.The helically prepared tissue was cut into four strips (each 3 cm × 4 mm) that were blotted dry and weighed.

Vasorelaxation measurements. The aortic strips were suspended individually in Krebs buffer (composition described above) contained in 25-mL jacketed circulating water tissue baths maintained at 37°. One of these strips was assigned for the buffer control, a second for the GTN treatment in the absence of inhibitor, and the third and the fourth as the inhibitor-treated strips. The contraction and relaxation of the strips were recorded via transducers, coupled to a Grass model 7 polygraph (Quincy, MA). The tension on the strips was maintained at 1 g. The strips were washed every 20 min during the initial equilibration period with fresh buffer. They were then allowed to equilibrate for at least 30 min to 1 hr before addition of any chemicals.

Upon stabilization, 100 μM PE was added to contract the strips maximally. The maximum tension produced in the strips was recorded. The strips were then returned to baseline tension by washing with fresh Krebs buffer four to six times, approximately 3 min apart. This was followed by two longer washes for 15 min, and the strips were then allowed to stabilize. Upon stabilization, the strips were treated with consecutive additions of 0.1 μM PE, until the tension in the strips reached 60–80% of the maximal contraction previously recorded. The inhibitor studies were then carried out on these strips.

Inhibitor studies. Upon attaining a state of submaximal contraction, the strips that were assigned for the inhibitors were incubated for 10 min with ECA at a final concentration of 0.2 mM. These inhibitor conditions were chosen based on previous results from this laboratory [7]. The same volume of Krebs buffer solution was added to the other noninhibitor strips. At the end of the 10 min, GTN solution was added to all strips, except the buffer control strips, at a final concentration of 0.6 μM, while the same volume of buffer was added to the control strips; the relaxation was recorded for 5 min after GTN addition. The strips were then freeze clamped with tongs precooled in liquid nitrogen and immediately frozen on dry ice. Analysis of cGMP was carried out in each of these strips.

Analysis of cGMP levels. The frozen strips were homogenized in 1 mL of 6% cold trichloroacetic acid (TCA) to precipitate proteins; the homogenate was then centrifuged at 4000 *g* for 10 min at 2–4° to remove the protein precipitate. The supernatant was transferred into a fresh test tube and washed with a 4-fold volume of water-saturated diethyl ether. This procedure was repeated at least four times. Each time the ether layer was removed carefully, after the tubes were vortexed, and allowed to stand for some time. After removing the residual ether by evaporation under a stream of nitrogen, the TCA-free samples were then assayed for cGMP using a ^{3}H-labeled cGMP RIA kit. Each time a new kit was used standard curves were made with six concentrations (0, 0.5, 1, 2, 4 and 8 pmol cGMP/100 μL) prepared from the standard solutions of cGMP.

Control experiments. SNP, a compound known to produce nitric oxide by a mechanism distinct from that for organic nitrates, was used as a control to test the effect of ECA on the production of cGMP in rabbit aortic strips. Concentrations of 10^{-6} M and 10^{-5} M SNP were added to the strips (PE-treated)

Table 1. PE-induced contraction and GTN (0.6 μM)-induced relaxation in control and ECA-pretreated groups at 5 min

Treatment	N	PE-induced contraction (g)	GTN-induced relaxation (g)	% Relaxation
(A) Control	9	3.72 ± 0.31	3.22 ± 0.53	86.3 ± 10.0
(B) ECA-pretreated (0.2 mM)	9	3.14 ± 0.66	1.21 ± 0.39	35.7 ± 2.9
Statistics		A = B	A > B*	A > B*

Values are means ± SD.
* Significant at $P < 0.05$.

with or without ECA, and cGMP levels were measured.

Calculations and statistical analysis of data. Relaxation was measured as the percent decrease in tension below the elevated tension elicited by precontracting the aortic strips with phenylephrine. Contraction was measured as the increase in tension above the resting base line, and is expressed as the percent of maximal contraction produced by phenylephrine under a given set of conditions. The percent change in relaxation due to ECA inhibition of GTN effect was calculated as [1 − (% relaxation in the presence of inhibitor/% relaxation in the absence of the inhibitor)] × 100. The percent change in cGMP levels was calculated as [1 − (cGMP in the presence of GTN plus ECA − cGMP in ECA controls)/(cGMP in the presence of GTN − cGMP in controls)] × 100. Statistical comparisons were made for the relaxation data and the cGMP data between groups with and without GTN and within groups with and without ECA. For this purpose, a one-way ANOVA was used followed by the Student Newman Keuls test. Data are expressed as means ± standard deviation. The level for statistical significance was set at $P < 0.05$.

RESULTS

Relaxation measurements. On the basis of preliminary studies, a 0.6-μM GTN concentration was chosen to test the effect of ECA. This GTN concentration produced about 80% relaxation of PE-contracted rabbit aortic strips. A 59% decrease in the relaxation response to GTN was observed in the ECA-pretreated strips (35.7 ±2.9% relaxation, N = 9) compared with the control strips (86.3 ± 10.0% relaxation, N = 9) that were not pretreated with ECA (Table 1). This difference was statistically significant ($P < 0.05$). ECA (0.2 mM) by itself did not cause a decrease or an increase in the tension of strips produced by PE. A comparison was also made between the extent of PE-induced contraction in the two groups of strips tested. No significant difference was found between strips pretreated with ECA and strips not pretreated with ECA (Table 1, column 3), whereas both the GTN-induced relaxation (g) as well as % relaxation by GTN were significantly less in the strips with ECA pretreatment (Table 1, columns 4 and 5).

cGMP analysis. The cGMP levels were measured using the RIA method described in Materials and Methods and are expressed in picomoles cGMP produced per gram wet weight of the tissue (pmol/g wet wt). As presented in Table 2, 0.6 μM GTN produced a significant rise in the level of cGMP in the strips as compared with buffer controls ($P < 0.05$). Strips pretreated with ECA showed much lower levels of cGMP upon GTN treatment than untreated strips with GTN treatment ($P < 0.05$), although cGMP levels in the ECA-pretreated strips were still significantly higher than buffer controls ($P < 0.05$). The levels of cGMP in GTN-treated strips were about 5-fold higher than the buffer control, and upon preincubation with ECA they were decreased about 2-fold (Table 2).

Table 2. cGMP levels in strips incubated in buffer, upon treatment with GTN and preincubated with ECA prior to GTN treatment

Treatment	N	cGMP levels (pmol/g wet wt)
(A) Control (buffer)	4	13.2 ± 1.5
(B) GTN (0.6 μM)	9	64.4 ± 8.7
(C) ECA (0.2 mM) preincubation + GTN (0.6 μM)	9	29.1 ± 6.2
(D) ECA treatment only	3	13.7 ± 2.3
Statistics A < B* B > C*		A < C* A = D

Values are means ± SD.
* Significant at $P < 0.05$.

The 70% decrease in the cGMP response that was observed in the strips pretreated with ECA correlated well with the 59% decrease in relaxation response to GTN upon pretreatment with ECA (Fig. 2).

Control experiments with SNP. At the two concentrations of SNP tested here, no significant difference was observed in cGMP levels measured in the strips pretreated with ECA as compared with those not pretreated with the inhibitor. At 10^{-6} M SNP, the levels of cGMP were 56.0 ± 0.2 pmol/g wet weight in control strips versus 50.6 ± 5.6 pmol/g wet weight in the ECA-pretreated strips (N = 3) and at 10^{-5} M SNP, the levels of cGMP were 72.3 ± 13.0 pmol/g wet weight in control strips versus 70.7 ± 3.6 pmol/g wet weight in the ECA-pretreated strips (N = 3).

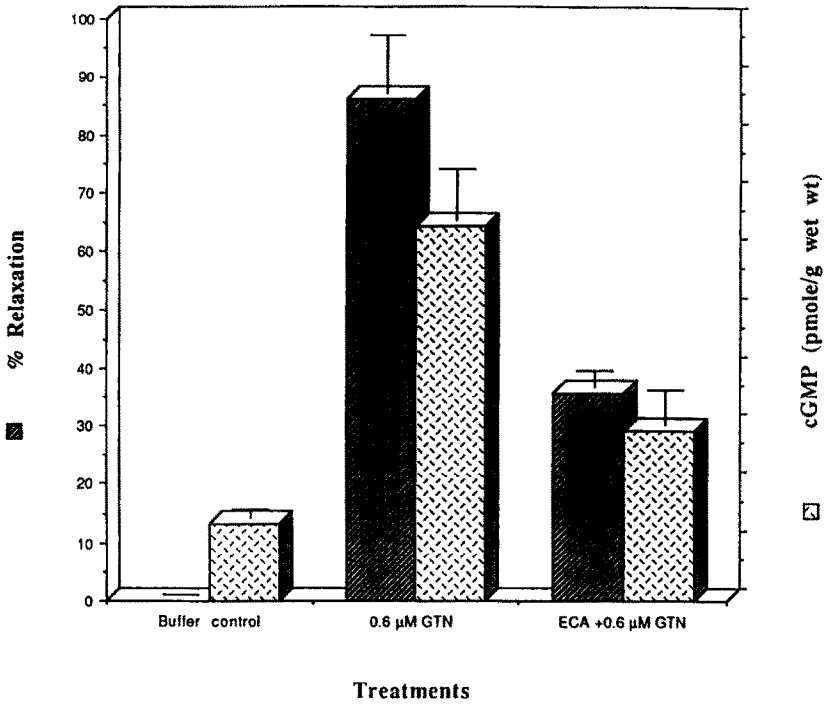

Fig. 2. Correlation between decrease in the relaxation and cGMP response of the strips to 0.6 μM GTN upon preincubating with ECA (0.2 mM). Values are means ± SD (N = 9).

DISCUSSION

The currently accepted hypothesis for the mechanism of action of nitroglycerin and all other organic nitrates emphasizes the necessity of a biotransformation process for GTN to produce its effect ([5]; Fig. 1). Brien and co-workers [10, 11] previously demonstrated a relationship between nitrate metabolism and cGMP elevation in rabbit aortic strips, where the increase in cGMP levels was found to correlate with the extent of GTN metabolism, as measured by the formation of dinitrate metabolites. Although the exact nature of the enzyme responsible for the activation of GTN has not been identified, various theories have been suggested. Feelisch and Noack [2] proposed that the nonenzymatic decomposition of organic nitrates in the presence of cysteine is of importance for the pharmacologic effect. Servent *et al.* [12], McDonald and Bennett [13], Schröder and Schror [14] and Schröder [15] have suggested that cytochromes P450 may be involved in the formation of nitric oxide from GTN. Brien *et al.* [10] have proposed that the biotransformation of GTN in rabbit aorta occurs due to direct interaction of one of the nitrate groups of GTN with the iron (Fe^{2+}) of guanylate cyclase bound heme. Fung and co-workers [16, 17] proposed that the enzyme responsible for the activation of GTN to nitric oxide is located in the plasma membrane and is thiol dependent. Most recently, Fung *et al.* [17] reported that this enzyme is a 160 kDa protein that is distinct from GSTs. We and others have suggested that GST plays an important role in the activation process [6–9, 18, 19].

In bovine aorta cytosol [9], we found that metabolism of GTN was a cytosolic thiol-dependent enzymatic process and that ECA and SBP significantly reduced the rate of this process. In an earlier publication, Yeates *et al.* [19] reported that GTN-induced relaxation could be antagonized by sulfobromophthalein (SBP) in rabbit aortic strips in muscle bath studies. However, we were unable to reproduce this inhibition of GTN-induced relaxation by SBP, whereas ECA produced a significant inhibition [8]. Needleman *et al.* [20] previously reported a decrease in the relaxation due to GTN in rabbit aortic strips upon preincubation with ECA. Similar observations were also made by Moffat *et al.* [21] in canine dorsal pedal artery rings. Here, we chose to measure not only the relaxation response to GTN but also a primary biochemical response, cGMP levels in the tissue, which leads to the relaxation process. We hoped that since the two responses were measurable in the same strips we would have stronger evidence for the role of GST in the GTN bioactivation process by studying the effect of a GST inhibitor on this process.

In this study, the addition of ECA led to a decrease in the GTN-induced vasorelaxation in all strips tested. This was accompanied by a parallel decrease in the levels of cGMP in the strips pretreated with ECA compared with those without pretreatment. In fact we found a correlation between the decrease in the relaxation response and the biochemical response to GTN (Fig. 2). The results here can be used to address two important points. First, the results highlight the potential role of GST in the GTN

activation process. If ECA specifically inhibits the activity of a GST isozyme, or a group of GST isozymes, then we would expect that by preincubating strips with this compound, the response to GTN would be affected only if the isozyme (or group of isozymes) is responsible for the metabolic activation of GTN. Second, the correlation between the decrease in the two responses provides evidence against a non-specific effect of ECA on a point in the process (Fig. 1) after cGMP production. However, these results do not rule out the possibility that ECA may have produced its effect on the guanylate cyclase enzyme itself. To test this possibility, we investigated the effect of ECA on cGMP levels in rabbit aortic strips exposed to SNP, a compound for which it has previously been believed there was no requirement for an enzymatic conversion to activate GC but which more recently has been suggested to yield nitric oxide by a mechanism distinct from that for organic nitrates [22–24]. At the two concentrations of SNP tested, no significant differences were observed in the cGMP levels induced by SNP in the presence and absence of ECA. This is consistent with the previous findings of Rapaport and Murad [25] who reported that 0.1 mM ECA did not affect either cGMP levels or GC activation in such tissues. We also studied the effect of ECA on the tension and cGMP levels in the PE-treated strips. ECA did not change the basal level of cGMP nor did it cause any changes in the tension of the tissue (Table 1).

Cytosolic GSTs consist of a family of isozymes broadly classified into three groups described as alpha, mu and pi on the basis of their subunit compositions, kinetic properties and immunoreactivities. These isozymes are expressed to different extents in different organs in the body, as well as in different species [26]. Since it is clear that the vasculature is the site of action of GTN, the expression of the GST isozymes in these tissues is of interest to us. The limitation of our study is that we cannot rule out possible cross-reactivities of ECA with different GST isozymes. However, Ploeman *et al.* [27] demonstrated that ECA most strongly inhibits the mu class GST. Furthermore, Tsuchida *et al.* [28] successfully purified GSTs with activities towards GTN from the human heart and aorta. Two of these seven purified forms belonged to the GST pi family while the other five were immunologically related to the GST mu family. Two of the five isozymes (mu) were found to exhibit high activities towards ECA and one of these two (pI = 8.3) also possessed high activity to GTN. Furthermore, recent studies, reported in abstract form [29], found that the metabolism of GTN correlates with the activity of the mu class of GST. Affinity chromatography, immunoprecipitation and immunoblots indicated that in rabbit aorta cytosol the mu class GST mediates the metabolism of GTN [30].

In conclusion, we observed a correlation between the decrease in GTN-induced cGMP levels and the decrease in GTN-induced vasorelaxation. These results, together with our previous metabolism studies [6, 9], indicate that GSTs may be involved in the productive metabolism of GTN. Furthermore, the apparent preferential mu specificity of ECA inhibition [27, 28] and the results of Chern *et al.* [29] and Lanzo *et al.* [30] point to a possible involvement of the mu isozyme of GST. Further testing of this hypothesis is the subject of continuing studies in our laboratory.

REFERENCES

1. Katsuki S, Arnold W, Mittal C and Murad F, 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.
2. Feelisch M and Noack EA, Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *Eur J Pharmacol* **139**: 19–30, 1987.
3. Slack CJ, McLaughlin BE, Nakatsu K, Marks GS and Brien JF, Nitric oxide-induced vasodilation of organic nitrate-tolerant rabbit aorta. *Can J Physiol Pharmacol* **66**: 1344–1346, 1988.
4. Ignarro LJ, Lippton H, Edwards JC, Baricos WH, Hyman AL, Kadowitz PJ and Gruetter CA, 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.
5. Ignarro LJ, Endothelium-derived nitric oxide: Pharmacology and relationship to the actions of organic nitrate esters. *Pharm Res* **6**: 651–659, 1989.
6. Lau DTW and Benet LZ, Nitroglycerin metabolism in subcellular fractions of rabbit liver. Dose dependency of GDN formation and possible involvement of multiple isozymes of glutathione *S*-transferases. *Drug Metab Dispos* **18**: 292–297, 1990.
7. Lau DTW and Benet LZ, Differential formation of dinitrate metabolism from glyceryl trinitrate in subcellular fractions of rabbit liver. *Biochem Pharmacol* **38**: 543–546, 1989.
8. Lau DTW and Benet LZ, Effects of sulfobromophthalein and ethacrynic acid on glyceryltrinitrate relaxation. *Biochem Pharmacol* **43**: 2247–2254, 1992.
9. Lau DTW, Chan EK and Benet LZ, Glutathione *S*-transferase mediated metabolism of glyceryl trinitrate in subcellular fractions of bovine coronary arteries. *Pharm Res* **9**: 1460–1464, 1992.
10. Brien JF, McLaughlin BE, Breedon TH, Bennett BM, Nakatsu K and Marks GS, Biotransformation of glyceryl trinitrate occurs concurrently with relaxation of rabbit aorta. *J Pharmacol Exp Ther* **237**: 608–614, 1986.
11. Brien JF, McLaughlin BE, Kobus SM, Kawamoto JH, Nakatsu K and Marks GS, Mechanism of glyceryl trinitrate-induced vasodilation. I. Relationship between drug biotransformation, tissue cyclic GMP elevation and relaxation of rabbit aorta. *J Pharmacol Exp Ther* **244**: 322–327, 1988.
12. Servent D, Delaforge M, Ducrocq C, Mansuy D and Lenfant M, Nitric oxide formation during microsomal hepatic denitration of glyceryl trinitrate: Involvement of cytochrome P-450. *Biochem Biophys Res Commun* **163**: 1210–1216, 1989.
13. McDonald PJ and Bennett BM, Cytochrome P-450 mediated biotransformation of organic nitrates. *Can J Physiol Pharmacol* **68**: 1552–1557, 1990.
14. Schröder H and Schror K, Inhibitors of cytochrome P-450 reduce cyclic GMP stimulation by glyceryl trinitrate in LLC-PK1 kidney epithelial cells. *Naunyn Schmiedebergs Arch Pharmacol* **342**: 616–618, 1990.
15. Schröder H, Cytochrome P-450 mediates bioactivation

of organic nitrates. *J Pharmacol Exp Ther* **262**: 298–302, 1992.

16. Chung SJ and Fung HL, Identification of the subcellular site for nitroglycerin metabolism to nitric oxide in bovine coronary smooth muscle cells. *J Pharmacol Exp Ther* **253**: 614–619, 1990.
17. Fung HL, Chung SJ, Bauer JA, Chong S and Kowaluk EA, Biochemical mechanism of organic nitrate action. *Am J Cardiol* **70**: 4B–10B, 1992.
18. Kukovetz WR and Holzmann S, Mechanism of nitrate induced vasodilation and tolerance on a biochemical base. *Z Kardiol* **74** (Suppl 1): 39–44, 1985.
19. Yeates RA, Schmid M and Leitold M, Antagonism of glyceryl trinitrate activity by an inhibitor of glutathione *S*-transferase. *Biochem Pharmacol* **38**: 1749–1753, 1989.
20. Needleman P, Jakschik B and Johnson EM, Sulfhydryl requirement for relaxation of vascular smooth muscle. *J Pharmacol Exp Ther* **187**: 324–331, 1973.
21. Moffat JA, Abdollah H, Rollwage D and Armstrong PW, Ethacrynic acid: Acute hemodynamic effects and influence on the *in vivo* and *in vitro* response to nitroglycerin in the dog. *J Cardiovasc Pharmacol* **7**: 637–642, 1985.
22. Kowaluk EA, Seth P and Fung HL, Metabolic activation of sodium nitroprusside to nitric oxide in vascular smooth muscle. *J Pharmacol Exp Ther* **262**: 916–922, 1992.
23. Marks GS, McLaughlin BE, Brown LB, Beaton DE, Booth BP, Nakatsu K and Brien JF, Interaction of glyceryl trinitrate and sodium nitroprusside with bovine pulmonary vein homogenate and 10,000 × *g* supernatant: Biotransformation and nitric oxide formation. *Can J Physiol Pharmacol* **69**: 889–892, 1991.
24. Bates JN, Baker MT, Guerra R Jr and Harrison DG, Chemical release of nitric oxide from sodium nitroprusside requires chemical reduction and cyanide loss. *FASEB J* **4**: A456, 1990.
25. Rapaport RM and Murad F, Effects of ethacrynic acid and cystamine on sodium nitroprusside-induced relaxation, cGMP levels and guanylate cyclase activity in rat aorta. *Gen Pharmacol* **19**: 61–65, 1988.
26. Vos RME and van Bladeren PJ, Glutathione *S*-transferases in relation to their role in the biotransformation of xenobiotics. *Chem Biol Interact* **75**: 241–265, 1990.
27. Ploemen JHTM, van Ommen B and van Bladeren PJ, Inhibition of rat and human glutathione *S*-transferase isoenzymes by ethacrynic acid and its glutathione conjugate. *Biochem Pharmacol* **40**: 1631–1635, 1990.
28. Tsuchida S, Maki T and Sato K, Purification and characterization of glutathione transferases with an activity towards nitroglycerin from human aorta and heart.Multiplicity of the human class *mu* forms. *J Biol Chem* **265**: 7150–7157, 1990.
29. Chern WH, Serabjit-Singh CJ, Lanzo CA, Han BJ, Shaffer JE and Lee FW, The metabolism of nitroglycerin (GTN) in rabbit aorta correlates with the activity of the mu class glutathione *S*-transferase (GST), but not the appearance of nitric oxide. *FASEB J* **5**: A1220, 1991.
30. Lanzo CA, Chern WH, Burkhart W, Moseley MA, Lee FW and Serabjit-Singh CJ, Isolation and characterization of the cytosolic mu class glutathione *S*-transferase (GST) active in the metabolism of nitroglycerin (NTG). *FASEB J* **6**: A1588, 1992.