ELSEVIER

European Journal of Pharmacology 276 (1995) 239–245

ejp

Bioactivation of nitroglycerin in vascular smooth muscle cells is different from that in non-vascular tissue

Marina Braun [1], Tilo Grosser, Karsten Schrör *

Institut für Pharmakologie, Heinrich-Heine-Universität Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany

Received 22 November 1994; revised 3 January 1995; accepted 10 January 1995

Abstract

The mechanism of biotransformation of nitroglycerin into the pharmacologically active radical nitric oxide (NO) or a related compound is still unclear. Different enzymes have been discussed to be involved in the bioactivation process. The effects of inhibition of glutathione-*S*-transferase and cytochrome *P*-450 enzymes were investigated on nitroglycerin-induced relaxation of bovine and porcine coronary arteries and on nitroglycerin-induced activation of guanylyl cyclase in cultivated porcine aortic smooth muscle cells. The glutathione-*S*-transferase inhibitor sulfobromophthalein had no effect on nitroglycerin-induced vascular relaxation, nor on nitroglycerin-induced elevation of cGMP levels in porcine coronary artery smooth muscle cells. The modulation of cytochrome *P*-450 activity by selective inhibitors as well as inducers did not alter the bioactivity of nitroglycerin in both systems. The data demonstrate that the isoenzymes of both enzyme families, which have been shown to be involved in the metabolism of nitroglycerin in different non-vascular tissues, do not play a role in bioactivation of nitroglycerin in the vascular system.

Keywords: Nitroglycerin bioactivation; Glutathione-*S*-transferase; Cytochrome *P*-450; Coronary artery; Cultivated smooth muscle cell; cGMP

1. Introduction

It is generally accepted that the biotransformation of nitroglycerin to nitric oxide (NO) or a related compound is necessary for its vasodilating effect (Ahlner et al., 1991). Several mechanisms for the metabolic activation of nitroglycerin have been proposed. Studies on cell-free preparations have shown that thiols such as cysteine directly interact with organic nitrates resulting in NO or nitrosothiol formation (Feelisch and Noack, 1987; Ignarro et al., 1981). In intact cells or tissues, however, this reaction does not seem to play a crucial role. There is rather evidence for metabolization by a membrane-bound enzyme (Chung and Fung, 1990). In a rabbit aortic preparation inhibition of nitroglycerin-induced relaxation by sulfobromophthalein was observed, suggesting a role for glutathione-*S*-transferases in the bioactivation process (Yeates et al., 1989). A recent study, however, showed that there is no correlation between glutathione-*S*-transferase activity and NO formation (Kurz et al., 1993). Some reports focussed on the role of cytochrome *P*-450 (CYP) isoenzymes in the metabolization of nitroglycerin. Whereas in non-vascular tissue and cultivated cells the bioactivation of nitroglycerin by specific cytochrome *P*-450 inhibitors has clearly been demonstrated (Schröder, 1992; Servent et al., 1989), studies on vascular preparations mostly failed to achieve similar results (Liu et al., 1993; Salvemini et al., 1993). A recent study, however, showed an inhibition of nitroglycerin-induced rat aortic relaxation by 7-ethoxyresorufin, a specific inhibitor of CYP IA1 and IA2, suggesting that these isoenzymes might be responsible for the bioactivation of nitrates (Bennett et al., 1993).

The aim of the present study was to investigate the effects of different inhibitors of CYP families and subfamilies in vascular tissue more systematically by using a broad range of different substances. Vascular

[1] Present address: 1. Medizinische Klinik, Charité, Alexander von Humboldt Universität zu Berlin, Berlin, Germany.

* Corresponding author. Tel. (+49-211) 311-2500, fax (+49-211) 311-4781.

SSDI 0014-2999(95)00031-3

relaxation studies were performed on coronary preparations from various species. In addition, nitroglycerin-induced elevation of cGMP levels were studied on cultivated vascular smooth muscle cells.

2. Materials and methods

2.1. Vessel preparation

Bovine and porcine hearts were obtained at a local slaughterhouse and transported in cold Krebs-Henseleit buffer to the laboratory where the coronary arteries were prepared immediately. Rings of coronary arteries (inner diameter: 3–4 mm) of 3–4 mm length were prepared. The endothelial layer was removed mechanically by a pipe cleaner.

2.2. Relaxation studies

Coronary artery rings from bovine or porcine hearts were mounted in a 10 ml organ bath, containing oxygenated (20% O_2, 5% CO_2, 75% N_2) Krebs-Henseleit solution (pH 7.4; 37°C) supplemented with indomethacin (3 μM) in order to prevent endogenous prostaglandin synthesis. The pO_2 obtained under these conditions was approximately 160 mm Hg and no signs of hypoxia were observed (Braun and Schrör, 1992).

Bovine and porcine coronary arteries were adjusted to a vascular tension of 20 mN and 40 mN, respectively. After equilibration, the coronary rings were precontracted with prostaglandin $F_{2\alpha}$ (10 μM). Successful removal of endothelium was assessed by the loss of the relaxing effect of acetylcholine (1 nM–10 μM). Nitroglycerin was added cumulatively in concentrations of 0.1 nM up to 10 μM either in the absence (control) or presence of different inhibitors. The following substances were used: (1) nitric oxide scavenger: oxyhemoglobin (3 μM), (2) glutathione-*S*-transferase-inhibitor: sulfobromophthalein (100 μM), (3) cytochrome *P*-450 inhibitors: cimetidine (100 μM), 7-ethoxyresorufin (1 μM), metyrapone (100 μM), miconazole (10 μM), proadifen (100 μM). All these agents were added 30 min before addition of nitroglycerin. In some experiments, linsidomine (0.1 nM–100 μM), which spontaneously releases NO, was used for relaxation. All measurements were performed in duplicate. Relaxations are given in percentage of contractile tension.

2.3. Smooth muscle cells of porcine aorta

Cultivated smooth muscle cells from porcine aorta were kindly provided by P. Pietsch, PhD, Düsseldorf, Germany. The cells were isolated and cultivated using the explant technique described by Ross (1971). Briefly, the vessels were opened longitudinally and the endothelium was removed mechanically. Explants from the media (1 × 1 mm) were placed into culture dishes. The cells were cultivated in Dulbecco's modified Eagle medium (DMEM) containing penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), fetal calf serum (15%). The cells were characterized as smooth muscle cells by the typical hill and valley growth pattern and by the presence of specific α-actin using a selective monoclonal antibody (Boehringer, Mannheim, Germany).

2.4. Measurement of cyclic GMP

Smooth muscle cells from passages 4–6 were cultivated to confluence in 35 mm dishes. Cells were washed twice with a balanced salt solution (mM) NaCl 130; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 0.8; glucose 5.5, and Hepes-NaOH 20, pH 7.3, and preincubated with isobutylmethylxanthine (0.5 mM), a non-selective phosphodiesterase inhibitor, for 10 min at 37°C. If indicated, an enzyme inhibitor was added at the same time. The following substances were tested: cimetidine (100 μM), 7-ethoxyresorufin (1 μM), proadifen (100 μM). Nitroglycerin was added at concentrations of 0.01 μM up to 100 μM. In some experiments, linsidomine (0.1–100 μM) was used to stimulate guanylyl cyclase. After an incubation period of 10 min, the reaction was stopped by the addition of ice-cold ethanol (96%). Ethanol was evaporated and Tris-buffered (50 mM) solution was added. The dishes were stored at −70°C. cGMP content was measured by radioimmunoassay using a specific rabbit antibody developed in our laboratory. The protein concentration of the suspensions were determined according to Bradford using a commercially available kit (Boehringer, Mannheim, Germany). All measurements were performed in duplicate. cGMP concentrations are given in pmol/mg protein.

2.5. Statistical analysis

The effects of nitroglycerin in the absence and presence of the different inhibitors were evaluated in parallel on n different preparations. The data are presented as mean and standard error of the mean ($x \pm$ S.E.M.) of n observations. Comparisons between the effects of nitroglycerin in the absence and presence of enzyme inhibitors were performed by Student's t-test for paired observations, evaluating the right shift of concentration-response curves, i.e. the difference of the logarithmic IC_{50} values. P values of < 0.05 were considered significant.

2.6. Drugs and solutions

Cimetidine, isobutylmethylxanthine, methylcholanthrene, metyrapone, miconazole, phenobarbital and

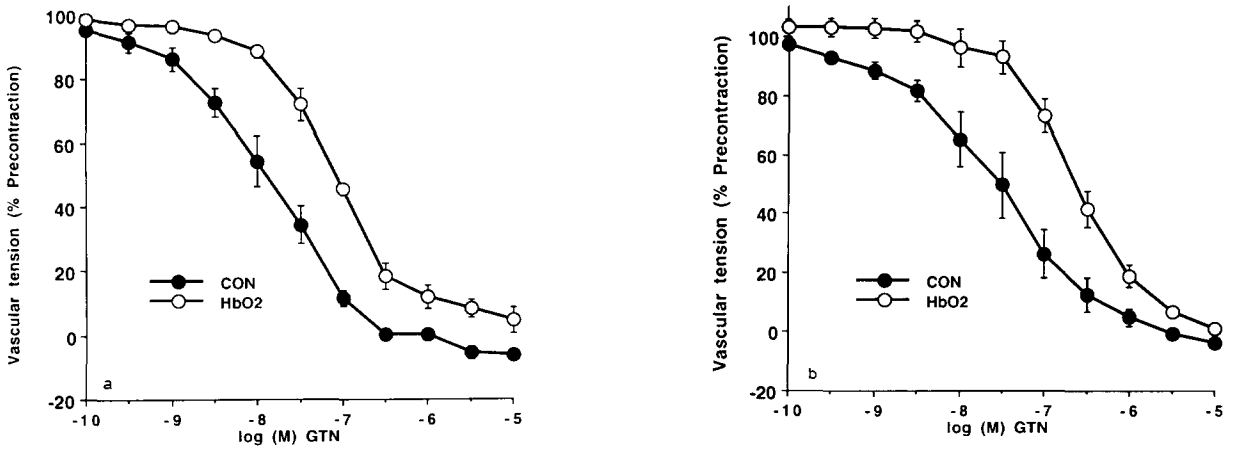

Fig. 1. Concentration-dependent relaxation of bovine (a) and porcine (b) coronary arteries by nitroglycerin (GTN) in the absence (CON) or presence of oxyhemoglobin (HbO_2; 3 μM). The nitric oxide (NO) scavenger inhibits significantly nitroglycerin-induced relaxation in both species (IC_{50} value; $P < 0.05$ CON vs. HbO_2; $n = 4$; for further explanation see text).

sulfobromophthalein were purchased from Sigma (Deisenhofen, Germany), 7-ethoxyresorufin from Molecular Probes (Eugene, OR, USA), indomethacin (Luitpold Pharma, Munich, Germany), nitroglycerin (Schwarz Pharma, Monheim, Germany), linsidomine (Cassella/Riedel, Frankfurt/M., Germany) and proadifen (SmithKline and Beecham Laboratories, King of Prussia, PA, USA) were kindly provided by the respective manufacturers. Cell culture medium and supplements were obtained from Life Technologies (Eggenstein, Germany).

Stock solutions of 7-ethoxyresorufin, miconazole and proadifen were prepared in dimethyl sulfoxide. Stock solutions of cimetidine and metyrapone were made in methanol. Dilutions were prepared in Krebs-Henseleit buffer. The solvents had no direct effects in the concentrations used in this study (not shown).

3. Results

3.1. Vasorelaxation studies

Effect of nitric oxide scavenger oxyhemoglobin

Nitroglycerin induced a concentration-dependent relaxation of bovine and porcine coronary arteries. The IC_{50} values were 18 ± 5 nM and 70 ± 24 nM, respectively. Preincubation of vascular rings with oxyhemoglobin (3 μM) caused a significant rightward shift of the concentration-response curves by a factor of 4 in both cases (bovine coronary arteries: $IC_{50} = 69 \pm 11$ nM; porcine coronary arteries: $IC_{50} = 292 \pm 57$ nM; $n = 4$; $P < 0.05$) (Fig. 1a and b). Similar effects were obtained with linsidomine (1 nM–100 μM) as relaxing agent (data not shown). In contrast, oxyhemoglobin

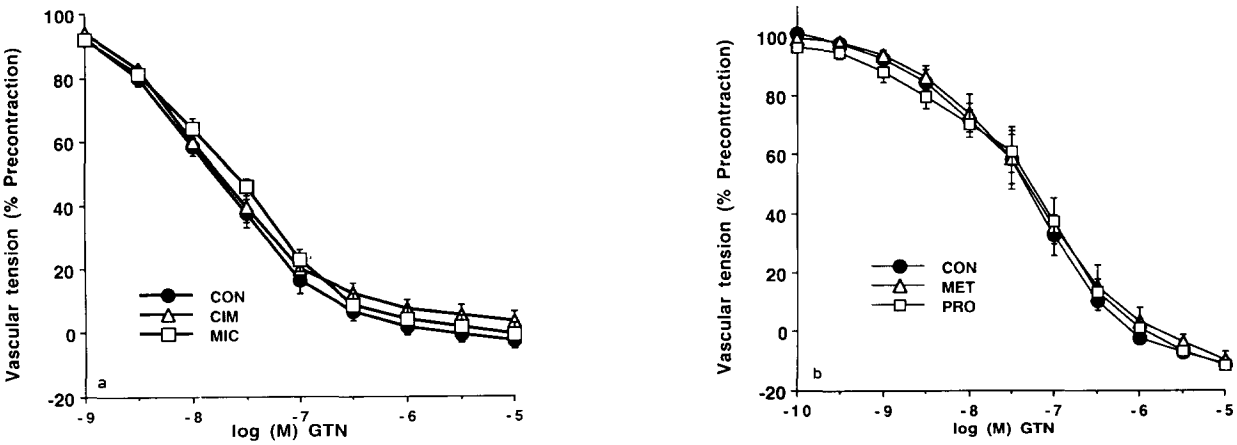

Fig. 2. (a) Concentration-dependent relaxation of bovine coronary arteries by nitroglycerin (GTN) in the absence (CON) or presence of one of the cytochrome *P*-450 inhibitors cimetidine (CIM) or miconazole (MIC) (100 μM). (b) Concentration-dependent relaxation of porcine coronary arteries by nitroglycerin (GTN) in the absence (CON) or presence of one of the cytochrome *P*-450 inhibitors metyrapone (MET) or proadifen (PRO) (100 μM). In both coronary preparations the cytochrome *P*-450 inhibitors did not change the concentration-response curve for nitroglycerin (IC_{50} value; $P > 0.05$; $n = 4$; for further explanation see text).

had no effect on isoprenaline-induced vasorelaxation (1 nM–10 μM; $n = 3$; data not shown).

Inhibition of glutathione-S-transferases

Nitroglycerin-induced relaxation of bovine coronary arteries was not altered after the incubation of the vascular rings with sulfobromophthalein (100 μM), the glutathione-*S*-transferase inhibitor, for 30 min ($P > 0.05$; $n = 4$).

Inhibition of cytochrome P-450 isoenzymes

The different cytochrome *P*-450 inhibitors cimetidine, metyrapone, proadifen and miconazole showed no inhibitory effect on nitroglycerin-induced relaxation of bovine or porcine coronary arteries ($P > 0.05$; $n = 4$; Fig. 2a and b). 7-Ethoxyresorufin (1 μM), a specific inhibitor of cytochrome *P*-450 enzymes of the IA subfamily, also had no significant inhibitory effect on bovine and porcine coronary arteries ($P > 0.05$; $n = 4$; Fig. 3a and b). Higher concentrations of 7-ethoxyresorufin (10 μM) were tested on porcine coronary arteries and had also no significant inhibitory effect. In addition, linsidomine-induced relaxation was not modified by 7-ethoxyresorufin (1 μM) in bovine and porcine coronary arteries, respectively (data not shown).

3.2. Guanylyl cyclase stimulation

Inhibition of glutathione-S-transferases

Basal cGMP levels of cultivated porcine aortic smooth muscle cells were 7.3 ± 0.9 pmol/mg protein ($n = 10$). Nitroglycerin induced a concentration-dependent stimulation of the guanylyl cyclase resulting in a 16-fold increase of cGMP levels at 10 μM. The concentration-response relationship was not altered by preincubation of the cells with the glutathione-*S*-transferase inhibitor sulfobromophthalein (100 μM) (Table 1).

Table 1
Effect of sulfobromophthalein (SBP; 100 μM) on nitroglycerin-induced activation of soluble guanylyl cyclase in cultivated porcine aortic smooth muscle cells

Treatment	Basal	Nitroglycerin				
		0.01 μM	0.1 μM	1 μM	10 μM	100 μM
Control	9±3	13±3	16±4	33±4	117±10	147± 8
SBP	11±2	12±3	15±2	35±5	133±12	123±12

Inhibition of cytochrome P-450 isoenzymes

None of the cytochrome *P*-450 inhibitors used, namely cimetidine, proadifen and 7-ethoxyresorufin, had any significant effect on the nitroglycerin-induced stimulation of guanylyl cyclase in cultivated porcine aortic smooth muscle cells (Fig. 4 and Fig. 5a and b). Neither methylcholanthrene nor phenobarbital, both known as inducers of cytochrome *P*-450, enhanced the effect of nitroglycerin on guanylyl cyclase (Table 2).

4. Discussion

Previous studies have shown that cultivated vascular smooth muscle cells and isolated vascular tissue release NO from the organic nitrate nitroglycerin (Feelisch and Kelm, 1991; Schrör et al., 1991; Kurz et al., 1993). The precise mechanism of biotransformation of nitroglycerin, however, remains unclear. Protein denaturation virtually eliminates NO formation, suggesting the involvement of one or more enzymes (Feelisch and Kelm, 1991; Kurz et al., 1993).

In the present study we have chosen two models for the vascular effects of nitroglycerin, namely the functional level (vasorelaxation) and the signal transduction pathway (cGMP). Nitroglycerin-induced relaxations of bovine and porcine coronary arteries were significantly

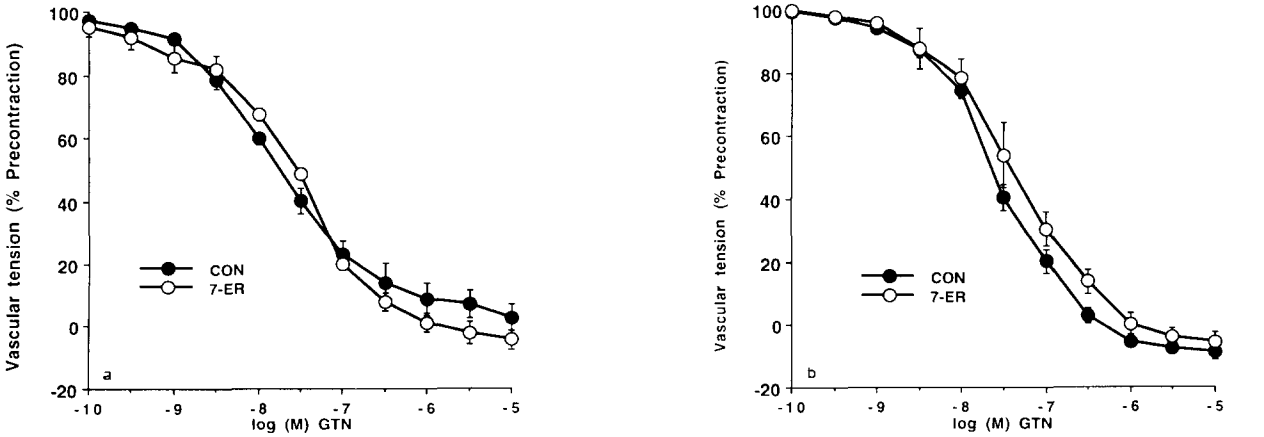

Fig. 3. Concentration-dependent relaxation of bovine (a) and porcine (b) coronary arteries by nitroglycerin (GTN) in the absence (CON) or presence of 7-ethoxyresorufin (7-ER; 1 μM). In both species the cytochrome *P*-450 inhibitor did not change the concentration-response curve for nitroglycerin (IC_{50} value; $P > 0.05$; $n = 4$; for further explanation see text).

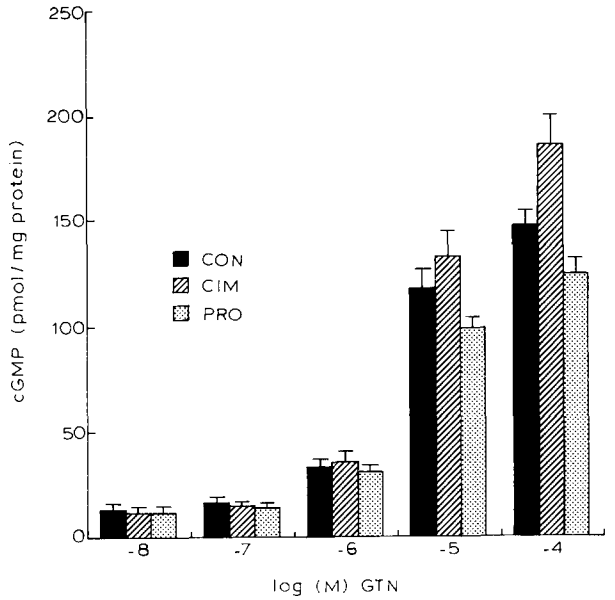

Fig. 4. Nitroglycerin-induced elevation of cGMP levels in cultivated porcine aortic smooth muscle cells in the absence (CON) or presence of one of the cytochrome *P*-450 inhibitors cimetidine (CIM) or proadifen (PRO) (100 μM). The activation of guanylyl cyclase was not changed by any of the substances (IC_{50} value; $P > 0.05$; $n = 4$; for further explanation see text).

inhibited by oxyhemoglobin (3 μM), a well-known scavenger of NO, but were unaffected by all cytochrome *P*-450 inhibitors tested.

One enzyme system that has been extensively studied is the glutathione-*S*-transferase. Data in the literature on the effects of the enzyme inhibitor sulfobromophthalein on vascular relaxation are controversial.

Table 2
Effect of cytochrome *P*-450 inducers phenobarbital (PB) and methylcholanthrene (MC) (100 μM) on nitroglycerin-induced activation of soluble guanylyl cyclase in cultivated porcine aortic smooth muscle cells

Treatment	Basal	Nitroglycerin				
		0.01 μM	0.1 μM	1 μM	10 μM	100 μM
Control	4±1	4±1	7±1	19±2	83±7	127±15
PB	4±1	4±1	7±1	19±2	81±9	120±11
MC	3±1	4±1	6±1	14±2	60±5	87±13

Whereas Yeates and co-workers reported a significant inhibition of nitroglycerin-induced relaxation of KCl-precontracted rabbit aortic rings by sulfobromophthalein (Yeates et al., 1989), other investigators did not observe an effect of this compound after precontraction with phenylephrine (Lau and Benet, 1992). Similar negative results were obtained in bovine coronary arteries (Chung and Fung, 1993). In the present study, sulfobromophthalein (100 μM) had no effect on nitroglycerin-induced relaxation in bovine as well as porcine coronary arteries and did not inhibit the resulting elevation of cGMP levels in cultivated porcine aortic smooth muscle cells. It has been suggested, that specific isoforms of glutathione-*S*-transferase, not sensitive to sulfobromophthalein, may be responsible for the bioactivation of nitroglycerin (Lau and Benet, 1992; Chung and Fung, 1993). 1-Chloro-2,4-dinitrobenzene, suggested to be more specific, however, could not be tested in our study because of direct effects on vascular tone (not shown). Glutathione-*S*-transferases were purified from vascular tissue and have been shown to

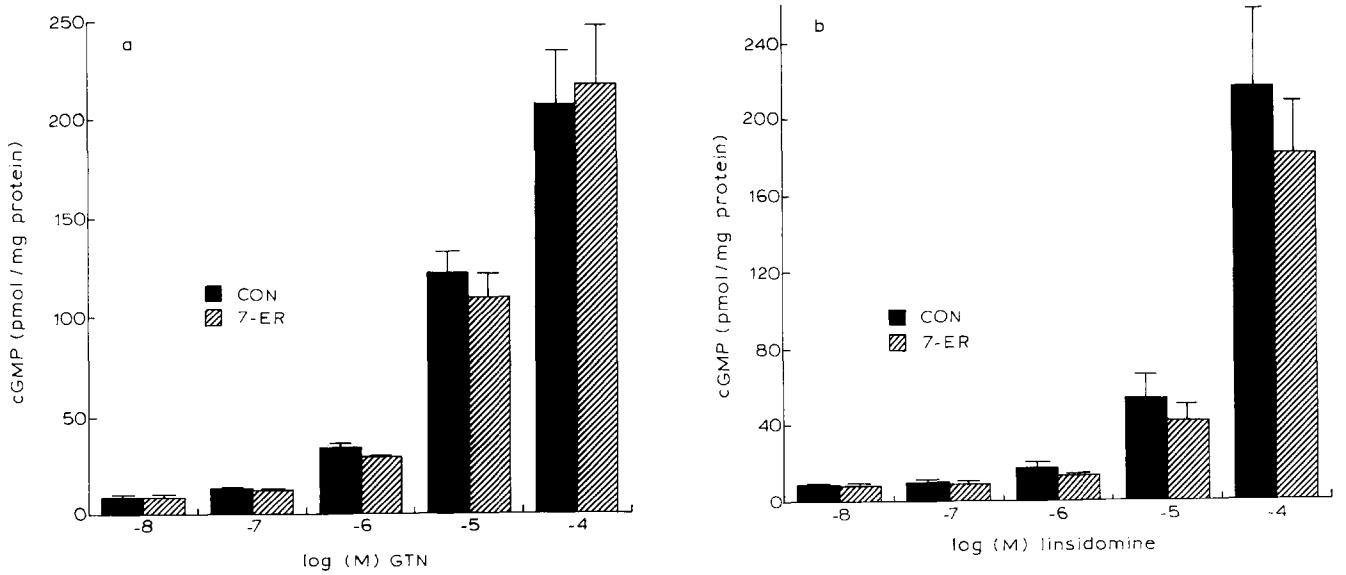

Fig. 5. Elevation of cGMP levels in cultivated porcine aortic smooth muscle cells induced by nitroglycerin (GTN) (a) or linsidomine (b) in the absence (CON) or presence of 7-ethoxyresorufin (7-ER; 100 μM). The cytochrome *P*-450 inhibitor had no effect on the concentration-response curve for nitroglycerin or linsidomine (IC_{50} value; $P > 0.05$; $n = 4$; for further explanation see text).

metabolize nitroglycerin to dinitrate and NO_2^-, similar to the hepatic enzyme (Tsuchida et al., 1990), but only minor amounts of NO were formed. There is a positive correlation between glutathione activity and NO_2^- but not glutathione activity and NO formation, suggesting that glutathione-*S*-transferases are not involved in the bioactivation of nitroglycerin but rather may play a role in the degradation of the substance (Kurz et al., 1993).

A series of recent publications has focussed on the role of the cytochrome *P*-450 enzyme family in the bioactivation of nitroglycerin. In rat liver microsomes, a NADPH-dependent denitration of nitroglycerin was found and was inhibited by different cytochrome *P*-450 inhibitors, namely cimetidine, metyrapone, miconazole and proadifen, active against different isoforms of the enzyme family (Servent et al., 1989; McDonald and Bennett, 1993). Similar results were obtained in rat lung fibroblasts (RFL-6) and porcine kidney epithelial cells (LLC-PK1) (Schröder and Schrör, 1990; Schröder, 1992). In these cell lines nitroglycerin-induced elevation of cGMP levels could be modulated by inhibitors and inducers of cytochrome *P*-450 enzymes. These data suggest that at least in these non-vascular cell lines the denitration of nitroglycerin is paralleled by the formation of NO. This seems also to be the case in hepatic microsomes, where the transformation resulted in a product which stimulated purified guanylyl cyclase from rat aorta (Bennett et al., 1992).

Cytochrome *P*-450 activity was also detected in the vessel wall (Jurchau et al., 1976), both in endothelial and smooth muscle cells (Abraham et al., 1985; Serabjit-Singh et al., 1988). The present study systematically investigates the role of various isoenzymes of cytochrome *P*-450 on nitroglycerin bioactivation in the vascular system using different classes of enzyme inhibitors: (1) members of the imidazole family, namely miconazole, which is a rather non-specific inhibitor and cimetidine, which has been reported to be an inhibitor of the 2C11 isoform and partly 1A1/2, but not 2A1, 2B1/2 and 3A1/2 (Bennett et al., 1992; Chang et al., 1991); (2) proadifen which interacts with the reduced form of IIB1, IIC1 and IIA1/2 isoforms; (3) metyrapone which interacts with the oxidized form of cytochromes that are not identified in detail; (4) 7-ethoxyresorufin, a substrate inhibitor of the IA1/2 isoforms (Gonzalez, 1992; Murray and Reidy, 1990). We could not observe any effect of all these compounds, neither on the relaxing activity of nitroglycerin on coronary segments nor on the elevation of cGMP levels in cultivated smooth muscle cells. In addition, the cytochrome *P*-450-inducing agents phenobarbital and methylcholanthrene did not augment nitroglycerin activity.

These data demonstrate that all the isoforms of the cytochrome *P*-450 family affected by these inhibitors are not involved in the vascular biotransformation, at least in the preparations tested here. This is in line with two recent reports demonstrating no effect of metyrapone and proadifen on nitroglycerin-induced relaxation in rabbit aorta and on cGMP levels in bovine aortic smooth muscle cells (Liu et al., 1993; Salvemini et al., 1993). In contrast, Bennett and co-workers reported a significant effect of 7-ethoxyresorufin on nitroglycerin-induced relaxation of rat aorta (Bennett et al., 1993). Interestingly, the cytochrome *P*-450 inhibitor also had a small but significant effect on the relaxation induced by sodium nitroprusside, that is also activated at the membrane site but by a distinct mechanism (Kowaluk et al., 1992; Seth and Fung, 1993). A recent paper demonstrated that some characteristics of the nitrate-metabolizing enzyme from bovine coronary arteries do not correspond with the cytochrome *P*-450 family, including the molecular size, its stability towards detergents like sodium dodecylsulfate, its apparent need for thiol and the lack of dependency on NAD(P)H (Seth and Fung, 1993).

In summary, this study demonstrates that modulation of cytochrome *P*-450 by several enzyme inhibitors and inducers, reported to be effective in liver and non-vascular cell lines, did not affect the activation of guanylyl cyclase in cultivated porcine aortic smooth muscle cells and the relaxation of bovine or porcine coronary arteries induced by nitroglycerin. These data suggest that cytochrome *P*-450 does not play a major role in the vascular bioactivation of nitroglycerin. Nitroglycerin metabolism obviously differs between various tissues. However, the involvement of one or more specific isoforms which were not affected by the substances used in this study, cannot be ruled out.

Acknowledgements

The authors are grateful to Peggy Rüter and Christine Machunsky for technical assistance, to Dr. D. Hafner for his advice and support in performing the statistical evaluation and to Erika Lohmann for competent secretarial assistance.

References

Abraham, N.G., A. Pinto, K.M. Mullane, R.D. Levere and E. Spokas, 1985, Presence of cytochrome *P*450-dependent monooxygenase in intimal cells of the dog aorta, Hypertension 7, 899.

Ahlner, J., R.G.G. Andersson, K. Torfgard and K.L. Axelsson, 1991, Organic nitrate esters: clinical use and mechanisms of action, Pharmacol. Rev. 43, 351.

Bennett, B.M., B.J. McDonald and M.J. St. James, 1992, Hepatic cytochrome *P*-450-mediated activation of rat aortic guanylyl cyclase by glyceryl trinitrate, J. Pharmacol. Exp. Ther. 261, 716.

Bennett, B.M., B.J. McDonald, R. Nigam, P.G. Long and W.C. Simon, 1993, Inhibition of nitrovasodilator- and acetylcholine-induced relaxation and cyclic GMP accumulation by the cy-

tochrome *P*-450 substrate, 7-ethoxyresorufin, Can. J. Physiol. Pharmacol. 70, 1297.

Braun, M. and K. Schrör, 1992, Prostaglandin D_2 relaxes bovine coronary arteries by endothelium-dependent nitric-oxide mediated cGMP formation, Circ. Res. 71, 1305.

Chang, T., M. Levine, S.M. Bandiera and G.D. Bellward, 1991, In vivo cimetidine inhibits rat hepatic cytochromes *P*-450 IIC11 but not cytochromes *P*-450 IIA1, *P*-450 IIB1/2 or *P*-450 III A1/2, FASEB J. 5, A478 (Abstract).

Chung, S.-J. and H.-L. Fung, 1990, Identification of the subcellular site for nitroglycerin metabolism to nitric oxide in bovine coronary smooth muscle cells, J. Pharmacol. Exp. Ther. 253, 614.

Chung, S.-J. and H.-L. Fung, 1993, Relationship between nitroglycerin-induced vascular relaxation and nitric oxide production, Biochem. Pharmacol. 45, 157.

Feelisch, M. and M. Kelm, 1991, Biotransformation of organic nitrates to nitric oxide by vascular smooth muscle and endothelial cells, Biochem. Biophys. Res. Commun. 180, 286.

Feelisch, M. and E. Noack, 1987, Correlation between nitric oxide formation and activation of guanylate cyclase, Eur. J. Pharmacol. 139, 19.

Gonzalez, F.J., 1992, Human cytochromes *P*450: problems and prospects, Trends Pharmacol. Sci. 13, 346.

Ignarro, L.J., H. Lippton, J.C. Edwards, W.H. Baricos, A.L. Hyman, P.J. Kadowitz and C.A. Gruetter, 1981, 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.

Jurchau, M.R., J.A. Bond and E.P. Benditt, 1976, Aryl 4-monooxygenase and cytochrome *P*450 in the aorta: possible role in atherosclerosis, Proc. Natl. Acad. Sci. USA 73, 3723.

Kowaluk, E.A., P. Seth and H.L. Fung, 1992, Metabolic activation of sodium nitroprusside to nitric oxide in vascular smooth muscle, J. Pharmacol. Exp. Ther. 262, 916.

Kurz, M.A., T.D. Boyer, R. Whalen, T.E. Peterson and D.G. Harrison, 1993, Nitroglycerin metabolism in vascular tissue: role of glutathione *S*-transferases and relationship between NO^- and NO_2^- formation, Biochem. J. 292, 545.

Lau, D.T.-W. and L.Z. Benet, 1992, Effects of sulfobromophthalein and ethacrynic acid on glyceryl trinitrate relaxation, Biochem. Pharmacol. 43, 2247.

Liu, Z., J.F. Brien, G.S. Marks, B.E. McLaughlin and K. Nakatsu, 1993, Lack of evidence for the involvement of cytochrome *P*-450 or other hemoproteins in metabolic activation of glyceryl trinitrate in rabbit aorta, J. Pharmacol. Exp. Ther. 264, 1432.

McDonald, B.J. and B.M. Bennett, 1993, Biotransformation of glyceryl trinitrate by rat aortic cytochrome *P*450, Biochem. Pharmacol. 45, 268.

Murray, M. and G.F. Reidy, 1990, Selectivity in the inhibition of mammalian cytochromes *P*-450 by chemical agents, Pharmacol. Rev. 42, 85.

Ross, R., 1971, The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibres, J. Cell Biol. 50, 172.

Salvemini, D., A. Pistelli and J. Vane, 1993, Conversion of glyceryl trinitrate to nitric oxide in tolerant and non-tolerant smooth muscle and endothelial cells, Br. J. Pharmacol. 108, 162.

Schröder, H., 1992, Cytochrome *P*-450 mediates bioactivation of organic nitrates, J. Pharmacol. Exp. Ther. 262, 298.

Schröder, H. and K. Schrör, 1990, Inhibitors of cytochrome *P*-450 reduce cyclic GMP stimulation by glyceryl trinitrate in LLC-PK1 kidney epithelial cells, Naunyn-Schmied. Arch. Pharmacol. 342, 616.

Schrör, K., S. Förster and I. Woditsch, 1991, On-line measurement of nitric oxide release from organic nitrates in the intact coronary circulation, Naunyn-Schmied. Arch. Pharmacol. 344, 240.

Serabjit-Singh, C.-J., S.J. Nishio, R.M. Philpot and C.G. Plopper, 1988, The distribution of cytochrome *P*-450 monooxygenase in cells of the rabbit lung: an ultrastructural immunocytochemical characterization, Mol. Pharmacol. 33, 279.

Servent, D., M. Delaforge, C. Durcocq, D. Mansuy and M. Lenfant, 1989, Nitric oxide formation during microsomal hepatic denitration of glyceryl trinitrate: involvement of cytochrome *P*-450, Biochem. Biophys. Res. Commun. 163, 1210.

Seth, P. and H.L. Fung, 1993, Biochemical characterization of a membrane-bound enzyme responsible for generating nitric oxide from nitroglycerin in vascular smooth muscle cells, Biochem. Pharmacol. 46, 1481.

Tsuchida, S., T. Maki and K. Sato, 1990, Purification and characterization of glutathione transferases with an activity toward nitroglycerin from human aorta and heart. Multiplicity of the human class Mu forms, J. Biol. Chem. 265, 7150.

Yeates, R.A., M. Schmid and M. Leitold, 1989, Antagonism of glyceryl trinitrate activity by an inhibitor of glutathione *S*-transferase, Biochem. Pharmacol. 38, 1749.