JPP Journal of Pharmacy and Pharmacology

2004, 56: 271–275

Received August 07, 2003
Accepted October 31, 2003
DOI 10.1211/0022357023088
ISSN 0022-3573

# Endothelin $ET_A$ receptor antagonist did not affect development of tolerance to glyceryl trinitrate in rat

Aarati Rai, Shaifali Bhalla and Anil Gulati

## Abstract

Glyceryl trinitrate (GTN), extensively used for the treatment of cardiovascular disorders, is associated with rapid development of tolerance. The exact mechanism responsible for tolerance development to GTN is still not known. Recently, it has been demonstrated that GTN tolerance is associated with increased expression of endothelin (ET). This study was carried out to determine the effect of $ET_A$ receptor antagonist, BMS182874, on the development of tolerance to GTN in urethane-anaesthetized rats. Diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate (HR) were continuously recorded in vehicle- and BMS182874 (3 mg $kg^{-1}$, i.v.)-treated rats. GTN was infused at the rate of 10 $\mu$g $min^{-1}$, intravenously for 4 h. Tolerance to GTN was determined using challenge doses of GTN (10, 30 and 90 $\mu$g, i.v.). GTN produced a fall in DBP, SBP and an increase in HR. In vehicle-treated rats, the fall in SBP before induction of GTN tolerance was $28 \pm 2$, $43 \pm 4$ and $52 \pm 4$ mmHg with 10, 30 and 90 $\mu$g GTN, respectively. However, following GTN infusion (10 $\mu$g $min^{-1}$, i.v. for 4 h) a rapid development of tolerance was observed and the fall in SBP was $1 \pm 1$, $9 \pm 4$ and $15 \pm 4$ mmHg with 10, 30 and 90 $\mu$g GTN, respectively. Similarly, in BMS182874-treated rats the fall in SBP in non-tolerant rats was $28 \pm 4$, $42 \pm 4$ and $48 \pm 5$ mmHg with 10, 30 and 90 $\mu$g GTN, respectively. In BMS182874-treated rats following GTN infusion (10 $\mu$g $min^{-1}$, i.v. for 4 h) a rapid development of tolerance was observed and the fall in SBP was $4 \pm 3$, $10 \pm 2$ and $13 \pm 4$ mmHg with 10, 30 and 90 $\mu$g GTN, respectively. The decrease in DBP and SBP in vehicle- and BMS182874-treated GTN-tolerant rats was statistically similar. These results suggest that $ET_A$ receptor antagonist BMS182874 did not affect development of tolerance to GTN in rats.

Department of Biopharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL 60612, USA

Aarati Rai, Shaifali Bhalla, Anil Gulati

Department of Bioengineering, University of Illinois at Chicago, Chicago, IL 60612, USA

Anil Gulati

Department of Neurology & Rehabilitation Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA

Anil Gulati

**Correspondence:** A. Gulati, Department of Biopharmaceutical Sciences (M/C 865), The University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA.
E-mail: gulati@uic.edu

**Funding:** This study was partially funded by Chicago Labs, Inc.

## Introduction

Glyceryl trinitrate (GTN) has been extensively used in the treatment of ischaemic heart disease. However, the usefulness of organic nitrates is limited by tolerance, which develops shortly after onset of treatment. This imposes a major limitation of efficacy on nitrate therapy for stable angina pectoris, congestive heart failure and acute myocardial infarction. The mechanisms responsible for tolerance remain controversial. Multiple hypotheses have been proposed (Parker & Parker 1998). The major proposed mechanisms are either impaired nitrate bioconversion resulting in decreased nitric oxide (NO) release (Agvald et al 1999) or increased NO clearance mediated by the incremental generation of superoxide ($O_2^-$) (Munzel et al 1995a). It has been shown that vascular $O_2^-$ production was increased only in blood vessels of animals treated in-vivo with GTN but not in vessels treated in-vitro, even with high concentration of GTN (Munzel et al 1995b). Down regulation of the target enzyme, vascular smooth muscle soluble guanylate cyclase, as a mechanism of GTN tolerance has also been proposed (Axelsson & Andersson 1983; Waldman et al 1986). Recent experimental and clinical studies suggest that concomitant treatment with high doses of angiotensin converting enzyme (ACE) inhibitors prevents nitrate tolerance (Katz et al 1991; Munzel & Bassenge 1996). Losartan, an angiotensin ($AT_1$) receptor blocker, was also found to prevent nitrate tolerance (Kurz et al 1999), indicating that the angiotensin system may be involved. However, it has also been demonstrated that nitrate tolerance is associated with increased expression of endothelin (ET)-1 in the vascular media (Munzel et al 1995b) and it has been found that a high dose of non-selective ET-1

receptor antagonist, bosentan, modestly improved GTN-induced relaxations (Kurz et al 1999). Losartan was found to be much more effective than bosentan in preventing nitrate tolerance (Kurz et al 1999). If losartan was acting through the endothelin system, then bosentan should have been equally effective as losartan, suggesting that the angiotensin and endothelin systems may be acting independently of each other. Bosentan is a non-specific endothelin antagonist and blocks both $ET_A$ and $ET_B$ receptors (Clozel et al 1994). Studies suggest that most of the cardiovascular effects of endothelin are mediated through $ET_A$ receptors (Gulati et al 1995; Kumar et al 1997; Bagnall & Webb 2001; Wimalasundera et al 2002). Therefore, we selected an $ET_A$ receptor antagonist to determine the involvement of $ET_A$ receptors in GTN tolerance. We propose that inhibition of $ET_A$ receptors will attenuate GTN tolerance. This study was conducted to determine the effect of specific $ET_A$ receptor antagonist BMS182874 on GTN tolerance in rats.

## Materials and Methods

### Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed three per cage in a room with controlled ambient temperature ($23 \pm 1^\circ$ C), humidity ($50 \pm 10\%$) and 12-h light–dark cycle (0600–1800 h). Food and water were freely available. Experiments were carried out after the rats had been acclimatized to this environment for at least 4 days. Studies were approved and were carried out according to the guidelines established by the Animal Care Committee of University of Illinois at Chicago.

### Drugs

BMS182874 (Tocris Pharmaceuticals Inc., Ellisville, MO) was dissolved in dimethyl sulfoxide DMSO (20%) and administered intravenously. GTN (50 mg/10 mL vials; American Regent Laboratories Inc., Shirely, NY) was diluted in dextrose saline and injected intravenously.

### Surgical preparations

Male Sprague-Dawley rats, 330–370 g, were used in the study. Rats were anaesthetized with urethane ($1.5\,g\,kg^{-1}$, i.p.). The left femoral vein was cannulated (PE 50 tubing) for drug administration. The left femoral artery was cannulated (PE 50 tubing) and connected to a Gould P23 ID pressure transducer for recording the blood pressure (BP) on a Grass P7D polygraph (Grass Instrument Co., Quincy, MA) through a 7PI preamplifier. Heart rate (HR) was recorded through a 7P4B Grass tachograph (Grass Instrument Co., Quincy, MA) triggered from BP signals. The right femoral vein was cannulated to infuse GTN at the rate of $10\,\mu g\,min^{-1}$ for 4 h using an infusion pump (Model 22; Harvard Apparatus, South Natick, MA). The rate of infusion was kept at $0.5\,mL\,h^{-1}$, so that the total amount infused over 4 h was 2.0 mL. To keep the blood $pO_2$, $pCO_2$ and pH constant, and to avoid the effect of respiration on BP and HR, rats were kept on constant-rate artificial respiration by inserting an endotracheal cannula connected to a rodent ventilator (Model 683; Harvard Apparatus Inc., South Natick, MA).

### Experimental design

Rats were divided into two groups: group 1 received vehicle ($1\,mL\,kg^{-1}$, i.v., $n = 5$); group 2 received BMS182874 ($3\,mg\,kg^{-1}$, i.v.; $n = 5$).

GTN was infused at the rate of $10\,\mu g\,min^{-1}$, intravenously, for 4 h after baseline measurement of systolic BP (SBP), diastolic BP (DBP) and HR responses to intravenous doses of 0, 10, 30 and 90 $\mu$g GTN (Wang et al 2002). Tolerance to GTN was determined by measuring SBP, DBP and HR responses to 0, 10, 30 and 90 $\mu$g of intravenous GTN before and after GTN infusion. Modification of tolerance with BMS182874 was measured by changes in SBP, DBP and HR in response to GTN challenge doses in vehicle- and BMS182874-treated rats.

### Data analysis

The data is presented as the mean ± s.e.m. and was analysed by Student's *t*-test. Data were compared with the baseline values and the values obtained following 4 h of GTN infusion. The number of rats in each group was 5 and a value of $P < 0.05$ was considered significant.

## Results

### Effect of GTN infusion on development of tolerance in vehicle-treated rats

In non-tolerant rats, GTN 10, 30 and 90 $\mu$g (i.v.) produced a fall of −2.2%, −31.4% and −37.3%, respectively, in DBP and −18.1%, −26.8% and −30.9%, respectively, in SBP. However, in tolerant vehicle-treated rats, GTN 10, 30 and 90 $\mu$g (i.v.) produced a change of 0.79%, −9.16% and −14.7%, respectively, in DBP and −0.75%, −7.0% and −11.4%, respectively, in SBP (Figures 1 and 2). In non- tolerant rats GTN 10, 30 and 90 $\mu$g (i.v.) increased HR by 4.5%, 6.2% and 9.2%, respectively, whereas in tolerant vehicle-treated rats GTN 10, 30 and 90 $\mu$g (i.v.) increased HR by 1.2%, 2.8% and 3.2%, respectively (Figure 3)

### Effect of GTN infusion on development of tolerance in BMS182874-treated rats

In non-tolerant rats, GTN 10, 30 and 90 $\mu$g (i.v.) produced a fall of −23.3%, −35.4%, and –41.9%, respectively, in DBP and −17.7%, −27.1% and –33.2%, respectively, in SBP. However, in tolerant BMS182874-treated rats, GTN 10, 30 and 90 $\mu$g (i.v.) produced a fall of −1.9%, −10.3% and –15.9%, respectively, in DBP and −3.1%, −7.1% and –9.9%, respectively, in SBP (Figures 1 and 2). In non-tolerant rats, GTN 10, 30 and 90 $\mu$g (i.v.) increased HR by 5.1%, 8.1% and 11%, respectively, whereas in tolerant BMS182874-treated rats GTN 10, 30 and 90 $\mu$g (i.v.)

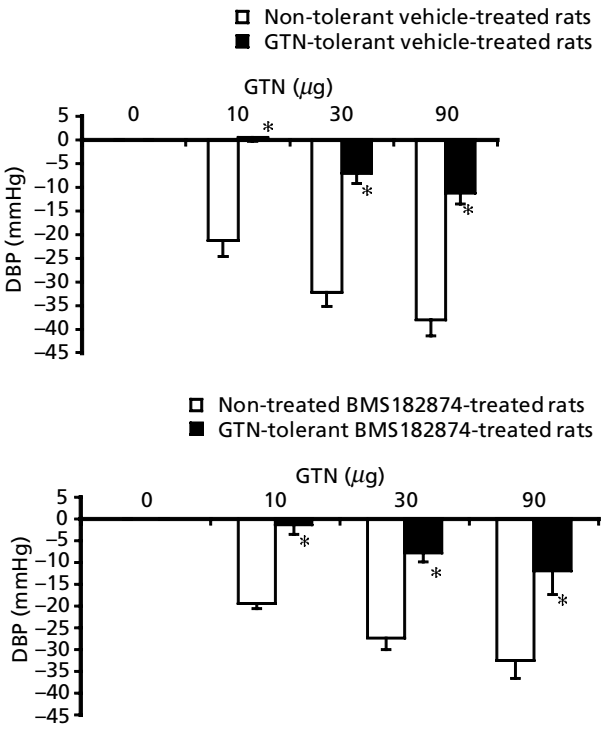


**Figure 1** Change in DBP (mmHg) with vehicle ($1\,mL\,kg^{-1}$, i.v.; upper panel) and BMS182874 treatment ($3\,mg\,kg^{-1}$, i.v.; lower panel) in GTN-tolerant and non-tolerant rats. Tolerance to GTN was determined by measuring DBP responses to 10, 30 and 90 $\mu$g (i.v.) of GTN before, and 4 h after, GTN infusion ($10\,\mu g\,min^{-1}$, i.v. for 4 h). Values are mean ± s.e.m.; n = 5. $^*P < 0.05$ compared with non-tolerant GTN response.

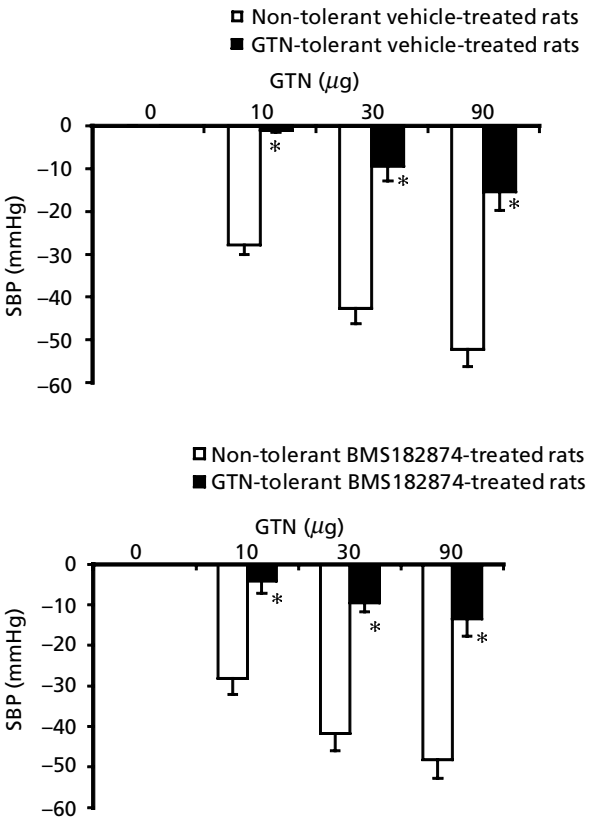


**Figure 2** Change in SBP (mmHg) with vehicle ($1\,mL\,kg^{-1}$, i.v.; upper panel) and BMS182874 treatment ($3\,mg\,kg^{-1}$, i.v.; lower panel) in GTN-tolerant and non-tolerant rats. Tolerance to GTN was determined by measuring SBP responses to 10, 30 and 90 $\mu$g (i.v.) of GTN before, and 4 h after, GTN infusion ($10\,\mu g\,min^{-1}$, i.v. for 4 h). Values are mean ± s.e.m.; n = 5. $^*P < 0.05$ compared with non-tolerant GTN response.

increased HR by 0.48%, 0.55% and 1.8%, respectively (Figure 3).

The decrease in DBP in GTN-tolerant rats was similar in vehicle-treated (−14.7%) and BMS182874-treated (−15.9%) rats. Similarly, decrease in SBP in GTN-tolerant rats was statistically similar in vehicle-treated (−11.4%) and BMS182874-treated (−9.9%) rats.

## Discussion

We proposed that inhibition of $ET_A$ receptors would attenuate GTN tolerance. In accordance with our hypothesis we anticipated that an $ET_A$ receptor specific antagonist, BMS182874, would attenuate the development of GTN tolerance. But, we found that BMS182874 (Webb et al 1995) did not affect GTN tolerance in rats. GTN tolerance was induced in rats by constant infusion of GTN at a rate of $10\,\mu g\,min^{-1}$ for 4 h. A dose–response effect of GTN (10, 30 and 90 $\mu$g) on blood pressure was obtained before, and 4 h after, GTN infusion. The fall in BP induced by GTN was significantly attenuated following GTN infusion.

The development of GTN tolerance was tested by measuring DBP and SBP responses to 10, 30 and 90 $\mu$g GTN in rats that received continuous infusion of $10\,\mu g\,min^{-1}$ of GTN for 4 h. This method produces a high degree of tolerance to GTN in rats within 1 h (Wang et al 2002). In another study using in-vitro left coronary arteries of Japanese monkeys, tolerance to GTN was induced by 1 h of treatment with GTN (Omura et al 2001).

Tolerance to GTN was first documented in 1867 (Marsh & Marsh 2000) as a clinical condition associated with a serious impairment of nitrovasodilator function due to its continuous administration. GTN tolerance has been associated with decrease in systemic and pulmonary arterial pressure and decreased cardiac preload and afterload (Abrams 1987). Several mechanisms have been proposed to account for the development of tolerance to GTN, including depletion of essential thiols (Needleman et al 1972), decreased biotransformation of GTN to NO (Chung & Fung 1993), desensitization of soluble guanylate cyclase (Waldman et al 1986), enhanced phosphodiesterase activity (Bohyn et al 1991) and production of vascular superoxide (Munzel et al 1995a).

Recent reports indicate involvement of $AT_1$ and endothelin in the development of tolerance to GTN. It

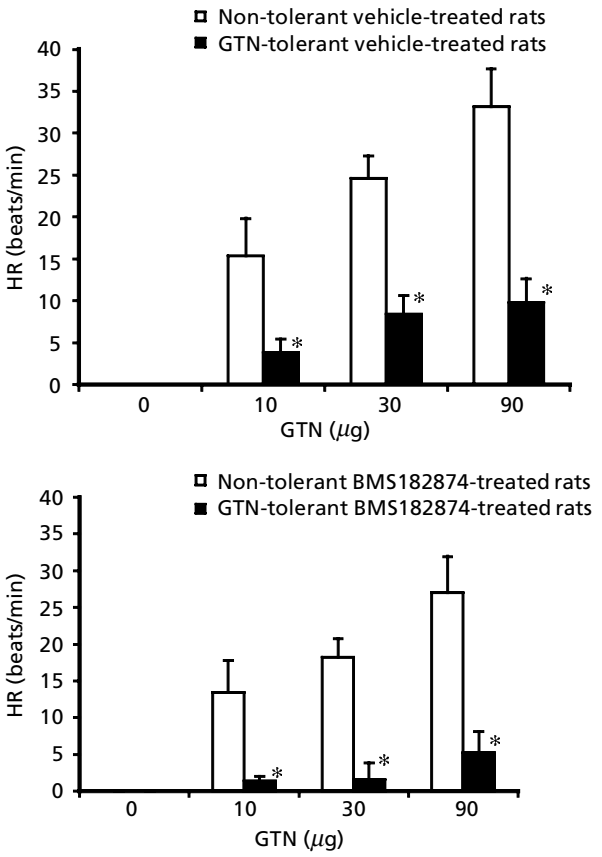


**Figure 3** Change in HR (beats/min) with vehicle (1 mL $kg^{-1}$, i.v.; upper panel) and BMS182874 treatment (3 mg $kg^{-1}$, i.v.; lower panel) in GTN-tolerant and non-tolerant rats. Tolerance to GTN was determined by measuring HR responses to 10, 30 and 90 $\mu$g (i.v.) of GTN before, and 4 h after, GTN infusion (10 $\mu$g $min^{-1}$, i.v. for 4 h). Values are mean ± s.e.m.; n = 5. *$P < 0.05$, compared with non-tolerant GTN response.

was found that losartan, an $AT_1$ receptor blocker, significantly prevented the development of tolerance to GTN (Kurz et al 1999). It is known that human myocardium expresses all three ET isoforms. Both $ET_A$ and $ET_B$ receptors are found in smooth muscle of human coronary vessels (Davenport et al 1993). ET-1 is known to promote chronotropy (Ishikawa et al 1988a) and inotropy in the heart (Ishikawa et al 1988b). A non-selective ($ET_A$/$ET_B$) endothelin receptor antagonist, bosentan, has also been shown to moderately improve GTN tolerance (Kurz et al 1999). Ratz et al (2000), however, found that the non-selective endothelin antagonist, ZD 2574, had no effect on the chronic development of GTN tolerance. Nevertheless, $ET_A$ and $ET_B$ receptors could be differentially involved in the development of GTN tolerance. It is possible that $ET_A$ receptors could act in an opposing manner to $ET_B$ receptors in the development of GTN tolerance. An increase in $ET_A$-receptor-mediated vasoconstriction could attenuate GTN's pharmacological actions. BMS182874, an $ET_A$ receptor specific antagonist, has been shown to decrease blood pressure (Bird et al 1995a, b; Holm et al 1998; Mercier et al 2002). It has also been shown to decrease pulmonary artery pressure (Snapper et al 1998). We did not measure pulmonary artery pressure in this study but focused on systemic pressure, which is commonly measured for GTN tolerance. The dose of BMS182874 (3 mg $kg^{-1}$, i.v.) used in this study has been shown to attenuate the pressor response to ET-1 by blocking $ET_A$ receptors (Webb et al 1995). This study, using this selective $ET_A$ receptor antagonist, shows lack of effect on GTN tolerance in rats. Our results show that $ET_A$ receptors do not play any role in the development of GTN tolerance. However, it is possible that $ET_B$ receptors might play a role. The haemodynamic response to ET-1 is mediated by $ET_B$ receptors and $ET_A$ receptors. $ET_B$ receptors mediate an initial transient decrease in systemic pressure, which is then followed by a sustained increase in blood pressure mediated by $ET_A$ receptors. Both $ET_A$ and $ET_B$ receptors are present in blood vessels. $ET_A$ receptors are usually located on vascular smooth muscle cells and are responsible for vasoconstriction (Gulati et al 1995), while $ET_B$ receptors are usually located on vascular endothelial cells and are predominantly responsible for vasodilatation (Karaki et al 1993; Schilling et al 1995; Rai & Gulati 2003). It is clear from our results that $ET_A$ receptors are not involved in GTN tolerance. However, it has also been found that some $ET_B$ receptors may also be located on vascular smooth muscle cells (Gellai et al 1996; Mickley et al 1997; Pierre & Davenport 1998) and are capable of producing vasoconstriction. Since bosentan, a non-selective ($ET_A$/$ET_B$) endothelin receptor antagonist, attenuated tolerance development while BMS182874, an $ET_A$ receptor antagonist, did not affect development of tolerance to GTN, it could be possible that $ET_B$ receptors located either on vascular smooth muscle cells or on endothelial cells may be involved in GTN tolerance. Therefore, further studies on the role of $ET_B$ receptors in the development of GTN tolerance need to be done.

In summary, this study indicates that an $ET_A$ receptor antagonist did not affect the development of tolerance to GTN in rats.

## References

Abrams, J. (1987) Glyceryl trinitrate (nitroglycerin) and the organic nitrates. Choosing the method of administration. *Drugs* **34**: 391–403

Agvald, P., Adding, L. C., Gustafsson, L. E., Persson, M. G. (1999) Nitric oxide generation, tachyphylaxis and cross-tachyphylaxis from nitrovasodilators in vivo. *Eur. J. Pharmacol.* **385**: 137–145

Axelsson, K. L., Andersson, R. G. (1983) Tolerance towards nitroglycerin, induced in vivo, is correlated to a reduced cGMP response and an alteration in cGMP turnover. *Eur. J. Pharmacol.* **88**: 71–79

Bagnall, A., Webb, D. (2001) Are selective endothelin A receptor antagonists better than mixed antagonists? *J. Cardiovasc. Pharmacol.* **38** (Suppl. 2): S43–S46

Bird, J. E., Moreland, S., Waldron, T. L., Powell, J. R. (1995a) Antihypertensive effects of a novel endothelin-A receptor antagonist in rats. *Hypertension* **25**: 1191–1195

Bird, J. E., Webb, M. L., Giancarli, M. R., Chao, C., Dorso, C. R., Asaad, M. M. (1995b) A role for endothelin ETA receptors in regulation of renal function in spontaneously hypertensive rats. *Eur. J. Pharmacol.* **294**: 183–189

Bohyn, M., Berkenboom, G., Fontaine, J. (1991) Effect of nitrate tolerance and dipyridamole on the response to SIN1 in the human isolated saphenous vein. *Cardiovasc. Drugs Ther.* **5**: 457–461

Chung, S. J., Fung, H. L. (1993) Relationship between nitroglycerin-induced vascular relaxation and nitric oxide production. Probes with inhibitors and tolerance development. *Biochem. Pharmacol.* **45**: 157–163

Clozel, M., Breu, V., Gray, G. A., Kalina, B., Loffler, B. M., Burri, K., Cassal, J. M., Hirth, G., Muller, M., Neidhart, W. (1994) Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J. Pharmacol. Exp. Ther.* **270**: 228–235

Davenport, A. P., O'Reilly, G., Molenaar, P., Maguire, J. J., Kuc, R. E., Sharkey, A., Bacon, C. R., Ferro, A. (1993) Human endothelin receptors characterized using reverse transcriptase-polymerase chain reaction, in situ hybridization, and subtype-selective ligands BQ123 and BQ3020: evidence for expression of ETB receptors in human vascular smooth muscle. *J. Cardiovasc. Pharmacol.* **22** (Suppl, 8): S22–S25

Gellai, M., Fletcher, T., Pullen, M., Nambi, P. (1996) Evidence for the existence of endothelin-B receptor subtypes and their physiological roles in the rat. *Am. J. Physiol.* **271**: R254–R261

Gulati, A., Sharma, A. C., Robbie, G., Saxena, P. R. (1995) Endothelin ETA receptor antagonist, BQ-123, blocks the vasoconstriction induced by sarafotoxin 6b in the heart but not in other vascular beds. *Gen. Pharmacol.* **26**: 183–193

Holm, P., Liska, J., Franco-Cereceda, A. (1998) The ETA receptor antagonist, BMS-182874, reduces acute hypoxic pulmonary hypertension in pigs in vivo. *Cardiovasc. Res.* **37**: 765–771

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., Masaki, T. (1988a) Positive chronotropic effects of endothelin, a novel endothelium-derived vasoconstrictor peptide. *Pflugers Arch.* **413**: 108–110

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., Masaki, T. (1988b) Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. *Am. J. Physiol.* **255**: H970–H973

Karaki, H., Sudjarwo, S. A., Hori, M., Takai, M., Urade, Y., Okada, T. (1993) Induction of endothelium-dependent relaxation in the rat aorta by IRL 1620, a novel and selective agonist at the endothelin ETB receptor. *Br. J. Pharmacol.* **109**: 486–490

Katz, R. J., Levy, W. S., Buff, L., Wasserman, A. G. (1991) Prevention of nitrate tolerance with angiotensin converting enzyme inhibitors. *Circulation* **83**: 1271–1277

Kumar, A., Morrison, S., Gulati, A. (1997) Effect of ETA receptor antagonists on cardiovascular responses induced by centrally administered sarafotoxin 6b: role of sympathetic nervous system. *Peptides* **18**: 855–864

Kurz, S., Hink, U., Nickenig, G., Borthayre, A. B., Harrison, D. G., Munzel, T. (1999) Evidence for a causal role of the renin-angiotensin system in nitrate tolerance. *Circulation* **99**: 3181–3187

Marsh, N., Marsh, A. (2000) A short history of nitroglycerine and nitric oxide in pharmacology and physiology. *Clin. Exp. Pharmacol. Physiol.* **27**: 313–319

Mercier, I., Pham-Dang, M., Clement, R., Gosselin, H., Colombo, F., Rouleau, J. L., Calderone, A. (2002) Elevated mean arterial pressure in the ovariectomized rat was normalized by ET(A) receptor antagonist therapy: absence of cardiac hypertrophy and fibrosis. *Br. J. Pharmacol.* **136**: 685–692

Mickley, E. J., Gray, G. A., Webb, D. J. (1997) Activation of endothelin ETA receptors masks the constrictor role of endothelin ETB receptors in rat isolated small mesenteric arteries. *Br. J. Pharmacol.* **120**: 1376–1382

Munzel, T., Bassenge, E. (1996) Long-term angiotensin-converting enzyme inhibition with high-dose enalapril retards nitrate tolerance in large epicardial arteries and prevents rebound coronary vasoconstriction in vivo. *Circulation* **93**: 2052–2058

Munzel, T., Sayegh, H., Freeman, B. A., Tarpey, M. M., Harrison, D. G. (1995a) Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A novel mechanism underlying tolerance and cross-tolerance. *J. Clin. Invest.* **95**: 187–194

Munzel, T., Giaid, A., Kurz, S., Stewart, D. J., Harrison, D. G. (1995b) Evidence for a role of endothelin 1 and protein kinase C in nitroglycerin tolerance. *Proc. Natl Acad. Sci. USA* **92**: 5244–5248

Needleman, P., Lang, S., Johnson, E. M. (1972) Organic nitrates: relationship between biotransformation and rational angina pectoris therapy. *J. Pharmacol. Exp. Ther.* **181**: 489–497

Omura, T., Matsumoto, T., Nakae, I., Takahashi, M., Kinoshita, M. (2001) Two possible mechanisms underlying nitrate tolerance in monkey coronary arteries. *Clin. Exp. Pharmacol. Physiol.* **28**: 259–265

Parker, J. D., Parker, J. O. (1998) Nitrate therapy for stable angina pectoris. *N. Engl. J. Med.* **338**: 520–531

Pierre, L. N., Davenport, A. P. (1998) Relative contribution of endothelin A and endothelin B receptors to vasoconstriction in small arteries from human heart and brain. *J. Cardiovasc. Pharmacol.* **31** (Suppl. 1): S74–S76

Rai, A., Gulati, A. (2003) Evidence for the involvement of ET(B) receptors in ET-1-induced changes in blood flow to the rat breast tumor. *Cancer Chemother. Pharmacol.* **51**: 21–28

Ratz, J. D., Fraser, A. B., Rees-Milton, K. J., Adams, M. A., Bennett, B. M. (2000) Endothelin receptor antagonism does not prevent the development of in vivo glyceryl trinitrate tolerance in the rat. *J. Pharmacol. Exp. Ther.* **295**: 578–585

Schilling, L., Feger, G. I., Ehrenreich, H., Wahl, M. (1995) Endothelin-3-induced relaxation of isolated rat basilar artery is mediated by an endothelial ETB-type endothelin receptor. *J. Cereb. Blood Flow Metab.* **15**: 699–705

Snapper, J. R., Thabes, J. S., Lefferts, P. L., Lu, W. (1998) Role of endothelin in endotoxin-induced sustained pulmonary hypertension in sheep. *Am. J. Respir. Crit. Care Med.* **157**: 81–88

Waldman, S. A., Rapoport, R. M., Ginsburg, R., Murad, F. (1986) Desensitization to nitroglycerin in vascular smooth muscle from rat and human. *Biochem. Pharmacol.* **35**: 3525–3531

Wang, E. Q., Lee, W. I., Brazeau, D., Fung, H. L. (2002) cDNA microarray analysis of vascular gene expression after nitric oxide donor infusions in rats: implications for nitrate tolerance mechanisms. *AAPS Pharm. Sci.* **4**: E10

Webb, M. L., Bird, J. E., Liu, E. C., Rose, P. M., Serafino, R., Stein, P. D., Moreland, S. (1995) BMS-182874 is a selective, nonpeptide endothelin ETA receptor antagonist. *J. Pharmacol. Exp. Ther.* **272**: 1124–1134

Wimalasundera, R. C., McG. Thom, S. A., Regan, L., Hughes, A. D. (2002) Action of the endothelin receptor (ETA) antagonist BQ-123 on forearm blood flow in young normotensive subjects. *Clin. Sci.* (*Lond.*) **102**: 661–666