Arch Toxicol (2005) 79: 705–710
DOI 10.1007/s00204-005-0003-y

ORGAN TOXICITY AND MECHANISMS

**Yang Sun · Motoyuki Iemitsu · Nobutake Shimojo
Takashi Miyauchi · Masahiro Amamiya · Daigo Sumi
Toshio Hayashi · Guifan Sun · Nobuhiro Shimojo
Yoshito Kumagai**

2,4,6-Trinitrotoluene inhibits endothelial nitric oxide synthase activity and elevates blood pressure in rats

Received: 12 March 2005 / Accepted: 01 June 2005 / Published online: 16 July 2005

Abstract 2,4,6-Trinitrotoluene (TNT), which is widely used in explosives, is an important occupational and environmental pollutant. Human exposure to TNT has been reported to be associated with cardiovascular dysfunction, but the mechanism is not well understood. In this study, we examine the endothelial nitric oxide synthase (eNOS) activity and blood pressure value following TNT exposure. With a crude enzyme preparation, we found that TNT inhibited the enzyme activity of eNOS in a concentration-dependent manner (IC_{50} value = 49.4 μM). With an intraperitoneal administration of TNT (10 and 30 mg/kg) to rats, systolic blood pressure was significantly elevated 1 h after TNT exposure (1.2- and 1.3-fold of that of the control, respectively). Under the conditions, however, experiments with the inducible NOS inhibitor aminoguanidine revealed that an adaptive response against hypertension caused by TNT occurs. These results suggest that TNT is an environmental chemical that acts as an uncoupler of constitutive NOS isozymes, resulting in decreased nitric oxide formation associated with hypertension in rats.

Keywords 2,4,6-Trinitrotoluene · Nitric oxide · Endothelial nitric oxide synthase · Blood pressure

Abbreviations TNT: 2,4,6-Trinitrotoluene · NO: Nitric oxide · nNOS: Neuronal nitric oxide synthase · iNOS: Inducible nitric oxide synthase · eNOS: Endothelial nitric oxide synthase · CaM: Calmodulin · BH_4: (6R)-5,6,7,8-Tetrahydro-L-biopterin · DMSO: Dimethyl sulfoxide · AG: Aminoguanidine

Y. Sun · N. Shimojo · T. Miyauchi · D. Sumi · N. Shimojo
Y. Kumagai (✉)
Doctoral Programs in Medicine Sciences,
Graduate School of Comprehensive Sciences,
University of Tsukuba, 1-1-1 Tennodai, Tsukuba,
Ibaraki 305-8575, Japan
E-mail: yk-em-tu@md.tsukuba.ac.jp
Tel.: +81-29-8533133
Fax: +81-29-8533133

M. Iemitsu
Institute of Health and Sport Sciences/Center
for Tsukuba Advanced Research Alliance,
University of Tsukuba, 1-1-1 Tennodai,
Tsukuba, Ibaraki 305-8575, Japan

M. Amamiya
Department of Environmental Medicine,
Master's Program in Environmental Sciences,
University of Tsukuba, 1-1-1 Tennodai,
Tsukuba, Ibaraki 305-8575, Japan

T. Hayashi
Department of Geriatrics, Graduate School of Medicine,
Nagoya University, 65 Tsuruma-cho, Showa-ku,
Nagoya 466-8550, Japan

G. Sun
Department of Occupational Health, School of Public Health,
China Medical University, Shenyang 110001, China

Introduction

2,4,6-Trinitrotoluene (TNT) (Fig. 1), a widely used explosive is a common contaminant at military bases and munitions production and handling facilities. Acute and chronic exposure of workers to TNT has been shown to cause toxicity in the liver, the hematopoietic system, the eye and cardiovascular system (Voegtin et al. 1921; Snyder et al. 1942; Vychub 1970; Hathaway 1977; Liu et al. 1988). However, mechanistic details of pathophysiological actions caused by TNT remain to be elucidated.

Nitric oxide (NO), which is synthesized by NO synthase (NOS), plays an important role in neurotransmission, vasorelaxation and immune response (Moncada et al. 1991). This gas produced in endothelial cells is involved in the regulation of blood pressure, inhibition of platelet aggregation, inhibition of smooth muscle migration and ischemic protection (Moncada et al. 1991; Nathan 1992; Schmidt et al. 1994). NOS are hemeproteins that catalyze oxidation of L-arginine to NO and L-citrulline. Three main isozymes exist in mammals that are regulated by distinct genes: a constitutive neuronal NOS (nNOS) (Schmidt et al. 1991), an endotoxin- and

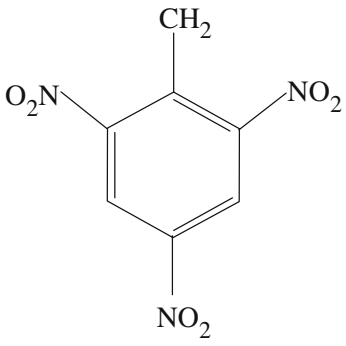

Fig. 1 Structure of 2,4,6-trinitrotoluene (TNT)

cytokine-inducible NOS (iNOS) (Stuehr et al. 1991) and a constitutive endothelial NOS (eNOS) (Pollock et al. 1991). Reduction in NO formation by NOS inhibitors or disruption of the gene encoding eNOS caused blood pressure elevation (Huang et al. 1995; Rees et al. 1989; Sander et al. 1999). It has been shown that impairment of NO production in the endothelium is implicated in the phathophysiological actions of vascular diseases (Kelly et al. 1996; Umans et al. 1995).

It is well recognized that nitroaromatic chemicals, such as TNT, are good substrates for flavin enzymes such as NADPH-cytochrome P450 (P450 reductase), resulting in one-electron reduction of these compounds (Kappus et al. 1986; Biaglow et al. 1986). We have recently reported that TNT has a potent inhibitory action on the enzyme activity of nNOS by shunting electrons away from the normal catalytic pathway, resulting in a decrease in NO production (Kumagai et al. 2004). Since the sequence of the P450 reductase domain of nNOS is similar to that of eNOS (Bredt et al. 1991; Lamas et al. 1992), we hypothesized that TNT would suppress not only nNOS activity, but also eNOS activity via a similar mechanism, thereby leading to hypertension. In the present study, we examined the effects of TNT on eNOS activity and blood pressure of rats.

Materials and methods

Materials

2,4,6-Trinitrotoluene was synthesized by the method of McGookin et al. (1940). Chemicals were obtained as follows: L-arginine, dimethyl sulfoxide (DMSO) and aminoguanidine (AG) from Sigma Chemical (St. Louis, MO, USA); ethyl carbamate and α-chloralose from Wako; L-[2,3−^{3}H]-arginine from DuPont/NEN Research Products (Boston, MA, USA) and AG50W-X8 resin from Bio-Rad Laboratories (Richmond, CA, USA). Calmodulin (CaM) was purified from the bovine brain by the method of Gopalakrishna and Anderson (Gopalakrishna et al. 1982). All other chemicals used were of the highest grade available.

Animals

Male Wistar rats (8 weeks), weighing 240-290 g were used in the measurement of blood pressure.

Enzyme preparation

Bovine aortic endothelial cells (BAEC) were obtained from Dainippon Pharmaceutical Industrial (Tokyo, Japan). BAEC were maintained in Dulbecco's modified Eagle's medium-nutrient mixture F-12 (1:1, vol/vol)-15% heat-inactivated fetal bovine serum–penicillin (100 U/ml)–streptomycin (100 μg/ml), fibroblast growth factor-acidic (5 ng/ml)–heparin (10 U/ml). Cells were incubated in a humidified atmosphere of 95% air–5% CO_2. The medium was changed every 2-3 days, and cells were routinely passaged by trypsin-EDTA with a split ratio of 1:4. BAEC between passages 3 and 7 were scraped from culture plates and homogenized in 50 mM phenylmethylsulfonly fluoride–leupeptin (1 μg/ml). The homogenate was centrifuged at 100,000 *g* for 60 min. The total membrane fractions obtained were suspended in the homogenate buffer containing 2.5 mM $CaCl_2$ according to the method of Patel and Block (Patel et al. 1995). Suspensions obtained were frozen under liquid nitrogen and kept at –80°C until use.

Enzyme activity

Incubation mixtures (0.1 ml) consisted of a suspension of the membrane fraction BAEC (0.8–0.10 mg of protein), TNT (10–200 μM), complete medium (20 nM 2,3-[^{3}H]arginine, 50 μM L-arginine, 100 μM NADPH, 10 μg BH_4, 2 mM $CaCl_2$, 1 μg of CaM) and 20 mM HEPES (pH 7.4). After the enzyme solution was preincubated with TNT at 37°C for 5 min, reactions were initiated by the addition of the complete medium and carried out at 37°C for 30 min. Under these conditions, NO production determined by formation of citrulline was linear with time and protein concentration. TNT was dissolved in DMSO, and the maximal volume of DMSO was maintained at 20 μl/ml of the assay mixture, because DMSO slightly affected NOS activity. Production of [^{3}H]citrulline from L-[^{3}H]arginine was performed by the method of Bredt and Snyder (Bredt et al. 1990). Briefly, each incubation was terminated by addition of 2 ml of cold stop buffer [20 mM sodium acetate buffer (pH 5.5)–1 mM citrulline–2 mM EDTA–0.2 mM EGTA]. A portion (2 ml) of the mixture was applied to a column packed with AG50W-X8 resin (1 ml), which had been extensively equilibrated with the stop buffer, and then the column was washed with 2 ml of water. The sample (1 ml) of eluate collected was mixed with 5 ml of scintillation cocktail, and radioactivity was determined using a Beckman LS-600 scintillation counter. Protein concentration was measured by the method of Bradford (Bradford 1976), with bovine serum albumin as the

standard. To calculate the IC_{50} value of TNT on eNOS activity, we analyzed the values obtained from eNOS activity in the presence of TNT by a nonlinear regression program using PRISM version 3.0 (Graph Pad Software, San Diego, CA, USA).

Measurement of blood pressure

Rats were anesthetized with urethane (2 mg/kg) (35% ethyl carbamate and 4% α-chloralose). A polyethylene catheter (SP-31, Natsume, Tokyo, Japan) was inserted into the right carotid artery to measure arterial blood pressure. TNT (10 or 30 mg/kg) was administered to rats by intraperitoneal injection, as a vehicle (corn oil) was injected in control rats. In the further experiment, aminoguanidine (AG) (100 mg/kg) was injected with TNT (30 mg/kg) in rats. Hemodynamic measurements were recorded by means of a polygraph system (AP-601G amplifier and WT-687G thermal pen recorder, Nihon Koden, Tokyo, Japan).

Statistics

Data are expressed as the mean ± SDs for each group. A *t* test was carried out on a personal computer using a biomedical program (HALBAU, Japan).

Results

Inhibition of eNOS activity

When the membrane fraction of BAEC was used as an enzyme source for eNOS, L-citrulline formation from L-arginine was inhibited by TNT exposure in a concentration-dependent manner with an IC_{50} value of 49.4 μM (Fig. 2).

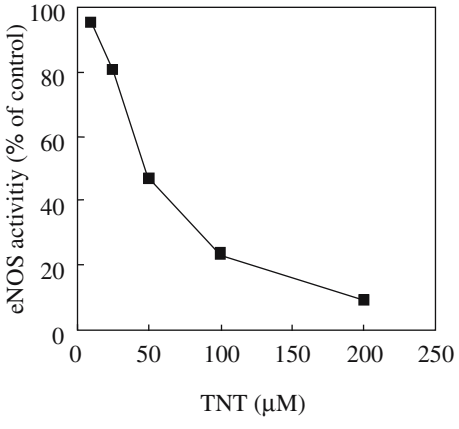

Fig. 2 Effect of TNT on eNOS activity in BAEC membrane fraction. eNOS activity was determined as L-citrulline formation from L-arginine. Each point is the average of two determinations

Changes in arterial blood pressure

2,4,6-Trinitrotoluene (10 mg/kg and 30 mg/kg) dissolved in corn oil (3 ml/kg), was administered to rats by intraperitoneal injection; in control rats, a vehicle (corn oil) was injected. Subsequently, arterial pressure and heart rate (HR) were measured in the conscious state for 1 h. Administration of TNT (10 and 30 mg/kg) to rats had no obvious effect on HR (Fig. 3). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) were elevated 1 h after TNT exposure. SBP was elevated to 1.2-fold that of the control (10 mg/kg of TNT, $P < 0.05$) and 1.3-fold that of the control (30 mg/kg of TNT, $P < 0.01$) (Fig. 4). With exposure to TNT (30 mg/kg), DBP and MBP increased to 1.3- and 1.4-fold that of the control ($P < 0.05$), respectively (Fig. 4). However, there was no change in blood pressure by vehicle administration (corn oil) in rats.

To explore whether there is an initial response against such hypertension during TNT exposure, we examined the effect of aminoguanidine (AG), a specific inhibitor of iNOS, on TNT-mediated blood pressure elevation. As shown in Fig. 5, an intraperitoneal injection of AG (100 mg/kg) into rats had a slight effect on blood pressure. However, simultaneous administration of TNT (30 mg/kg) with AG (100 mg/kg) resulted in a significant increase in blood pressure compared to treatment with either TNT or AG alone (Fig. 5). The elevations of SBP, DBP and MBP following co-administration of TNT and AG were 1.3-, 1.7- and 1.4-fold that of the control, respectively ($P < 0.005$, < 0.01 and < 0.05) (Fig. 5).

Discussion

This study indicates that TNT inhibits the enzyme activities of eNOS, as well as nNOS (Kumagai et al. 2004), and thus elevates blood pressure. In TNT-exposed humans, the notable toxic manifestations have included toxic hepatitis, cataracts and aplastic anemia

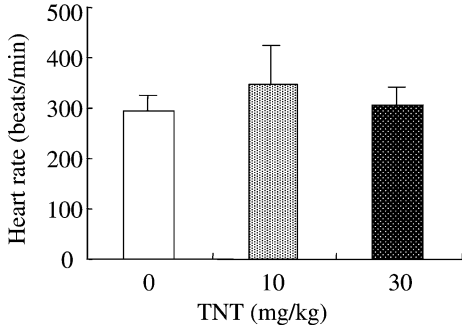

Fig. 3 Effect of TNT on heart rate in rats. Effect of TNT on heart rate in rats at 1 h after administration is shown in this figure. TNT (10 and 30 mg/kg) was administered to rats. Heart rate was measured for 1 h after injection of TNT. Each value is the mean ± SE of six rats

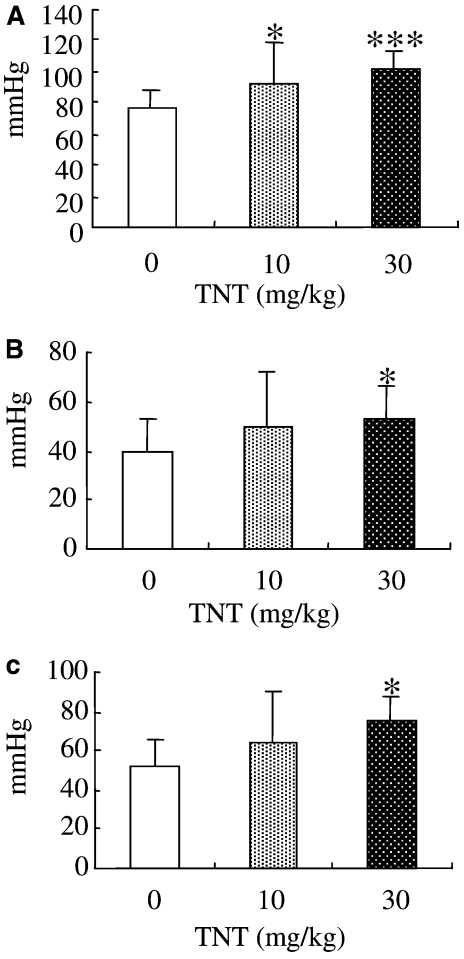

Fig. 4 Effects of TNT on blood pressure in rats. Effect of TNT on blood pressure in rats at 1 h after administration is shown in this figure. TNT (10 and 30 mg/kg) was injected into rats. Blood pressure was measured for 1 h after injection of TNT. (**a**) Systolic blood pressure (SBP); (**b**) Diastolic blood pressure (DBP); (**c**) Mean blood pressure (MBP). Each value is the mean ± SE of six rats. $P < 0.05$ (*) and $P < 0.001$ (***) compared to the control

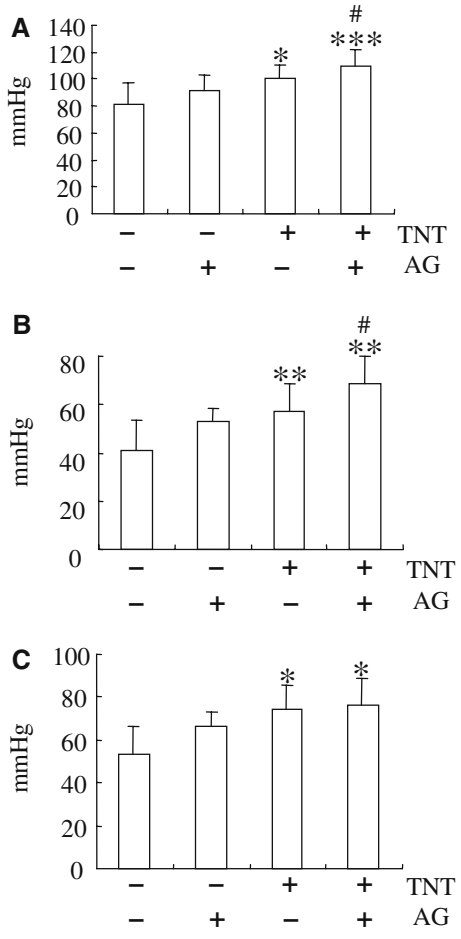

Fig. 5 Effects of TNT and/or iNOS inhibitor on blood pressure in rats. Effect of TNT and/or iNOS inhibitor on blood pressure in rats at 1 h after administration is shown in this figure. Rats were treated with TNT (30 mg/kg), aminoguanidine (AG, 100 mg/kg), TNT (30 mg/kg) and AG (100 mg/kg) together, respectively. Blood pressure was measured for 1 h after injection of TNT. (**a**) Systolic blood pressure (SBP); (**b**) Diastolic blood pressure (DBP); (**c**) mean blood pressure (MBP). Each value is the mean ± SE of six rats. $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) compared to the control; $P < 0.05$ (****) compared to AG

(Rosenblatt et al. 1991; Yinon 1990; Hathaway 1985). Vasquez-Vivar et al. showed that redox-active drugs convert the activity of eNOS from NOS to an NADPH oxygenase activity, an action referred to as "NOS uncoupling" (Vasquez-Vivar et al. 1999). We reported that TNT interacts with the P450 reductase domain of nNOS and thus suppresses L-citrulline formation by shunting electrons away form the normal catalytic pathway (Kumagai et al. 2004). In the present study, we showed that TNT is also an inhibitor for eNOS with an IC_{50} value of 49.4 μM (Fig. 2). Since it was reported that the sequence of the P450 reductase domain of nNOS is similar to that of eNOS (Bredt et al. 1991; Lamas et al. 1992), we speculate that a similar mechanism is also involved in TNT-derived inhibition of eNOS activity.

In recent years, it was shown that 9,10-phenanthraquinone has a potent inhibitory action on eNOS activity through a mechanism similar to that for nNOS (Kumagai et al. 1998), thereby causing the suppression of NO-dependent vasorelaxation and the elevation of blood pressure (Kumagai et al. 2001). In this study, we found that blood pressure was elevated in a TNT dose-dependent manner. For example, intraperitoneal administration of TNT (10 and 30 mg/kg) to rats caused an increase in the systolic blood pressure by 1.2- and 1.3-fold that of the control level, respectively (Fig. 4). These results suggest that the elevation of blood pressure caused by TNT exposure in rats may be, at least in part, attributable to a TNT uncoupling reaction similar to 9,10-phenanthraquinone for NOS isozymes.

Intermediate exposure of beagle dogs to TNT (32 mg/kg/day) for 26 weeks did not cause any changes in heart rates (Levine et al. 1990). TNT led to a vigorous hypertensive response without obvious changes in heart rate (Fig. 3), partly suggesting that it was not associated with cardiac contractility and output.

Reduction of NO formation by NOS inhibitors, or disruption of the gene encoding eNOS caused elevation of blood pressure (Huang et al. 1995, Rees et al. 1989, Sander 1999). However, NO produced by iNOS normally helps to prevent salt-sensitive hypertension in rats (Tan et al. 2000). iNOS expression at both the transcriptional and translational levels was prominently increased in portal hypertension rats, accompanied by enhanced NO production (Hiseh et al. 2003). This suggests that there is an adaptive response to increased vascular tone. Interestingly, the elevated blood pressure caused by TNT exposure (30 mg/kg) was augmented by co-administration with AG (100 mg/kg) (Fig. 5). In our preliminary study, we showed that systemic NO production as evaluated by NO-Hb formation did increase compared to the control, despite hypertension after exposure to TNT, and that under the conditions, iNOS gene expression in the spleens and blood of rats was transiently up-regulated by TNT exposure (Amamiya et al., unpublished observations). Thus, a reasonable explanation for the stimulatory effect of the iNOS inhibitor on increased blood pressure caused by TNT is that there is an initial response of NOS to the disruption of NO-forming activity following exposure to TNT. It is evident that systemic production of NO by iNOS in the vascularture is the major cause of hypotension and poor organ perfusion associated with these conditions (Titheradge 1999). In our field investigation at a TNT factory in China, we found that hypotension, but not hypertension, and increased NO metabolite levels in plasma were observed in workers who were chronicly exposed to TNT (SOT abstract). In order to explain how TNT gives factory workers hypotension, we suggest that iNOS expression be involved. iNOS expression might be induced as an adaptive response at the beginning of exposure with TNT to prevent hypertension. Consequently, hypotension will occur by excess iNOS-derived NO. Taken together, our findings suggest complex actions of TNT (e.g. inhibition of NOS activity, iNOS gene expression) on the human vascular system.

We examined acute effect of TNT on rats injected with the higher concentration. We need to investigate the blood pressure and toxicity in rats administrated with lower concentration of TNT than in this study.

Acknowledgements The experiments we performed were complied with the current Japanese laws on animal experimentation.

References

Biaglow JE, Varnes ME, Roizen-Towle L, Clark EP, Epp ER, Astor MB, Hall EJ (1986) Biochemistry of reduction of nitro heterocycles. Biochem Pharmacol 35:77–90

Bradford MM (1976) A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351:714–718

Bredt DS, Snyder SH (1990) Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 87:682–695

Gopalakrishna R, Anderson WB (1982) Ca^{2+} -induced hydrophobic site on calmodulin: application for purification of calmodulin by Phenyl-Sepharose affinity chromatography. Biochem Biophys Res Commun 104:830–836

Hathaway JA (1977) Trinitrotoluene: a review of reported dose-related effects providing documentation for a workplace standard. J Occup Med 19:341–345

Hathaway JA (1985) In: Rickert DE (eds), Toxicity of nitroaromatic compounds. Hemisphere Publishing Corporation, Washington DC, pp 255–274

Hsieh JS, Wang JY, Lin SR, Lian ST, Chen FM, Hsieh MC, Huang TJ (2003) Overexpression of inducible nitric oxide synthase in gastric mucosa of rats with portal hypertension: correlation with gastric mucosal damage. J Surg Res 115:24–32

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowits MA, Bevan JA, Fishman MC (1995) Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377:239–242

Kappus H (1986) Overview of enzyme systems involved in bioreduction of drugs and in redox cycling. Biochem Pharmacol 35:1–6

Kelly RA, Balligand JL, Smith TW (1996) Nitric oxide and cardiac function. Circ Res 79:363–380

Kumagai Y, Hayashi T, Miyauchi T, Endo A, Iguchi A, Kiriya-Sakai M, Sakai S, Yuki Y, Kikushima M, Shimojo N (2001) Phenanthraquinone inhibits eNOS activity and suppresses vasorelaxation. Am J Physiol 281:R25–R30

Kumagai Y, Kikushima M, Nakai Y, Shimojo N, Kunimoto M (2004) Neuronal nitric oxide synthase (nNOS) catalyzes one-electron reduction of 2,4,6-trinitrotoluene, resulting in decreased nitric oxide production and increased nNOS gene expression: implication for oxidative stress. Free Radical Bio Med 37:350–357

Kumagai Y, Nakajima H, Midorikawa K, Homma-Takeda S, Shimojo N (1998) Inhibition of nitric oxide formation by neuronal nitric oxide synthase by quinines: nitric oxide synthase as a quinine reductase. Chem Res Toxicol 11:608–613

Lamas S, Marden PA, Li GK, Tmepst P, Michel T (1992) Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci USA 89:6348–6352

Levine BS, Rust JH, Barkley JJ, Furedi EM, Lish PM (1990) Six month oral toxicity study of trinitrotoluene in beagle dogs. Toxicology 63:233–244

Liu YY, Suen DQ, Yang XE, He GB, Zheng Q, Zhang MY, Wu ML, Yang WY, Suen YZ (1988) Preliminary studies on the diagnosis of chronic liver damage caused by TNT. Hyg Res 17:7–11

McGookin A, Swift SR, Tittensor E (1940) Orientation problem: part III. 4:6-dinitro-*o*-toluidine. J Am Chem Soc 5:92–94

Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142

Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. FASEB J 6:3051–3064

Patel JM, Block ER (1995) Sulfhydryl-disulfide modification and the role of disulfide oxidoreductases in regulation of the catalytic activity of nitric oxide synthase in pulmonary artery endothelial cells. Am J Respir Cell Mol Biol 13:352–359

Pollock JS, Forstermann U, Mitchell JA, Warner TD, Schmidt HHHW, Nakane M, Murad F (1991) Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc Natl Acad Sci USA 88:10480–10484

Rees DD, Palmer RMJ, Moncada S (1989) Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sic USA 86:3375–3378

Rosenblatt DH, Burrows EP, Mitchell WR, Parmer DL (1991) In: Hutzinger O (eds) Handbook of environmental chemistry. Springer, Berlin Heidelberg, pp 195–234

Sander M, Chavoshan B, Victor RG (1999) A large blood pressure-raising effect of nitric oxide inhibition in humans. Hypertension 33:937–942

Schmidt HHHW, Pllock JS, Nakane N, Gorsky LD, Forstermann U, Murad F (1991) Purification of a soluble isoform of guanylyl cyclase-activating factor synthase. Proc Natl Acad Sci USA 88:365–369

Schmidt HHHW, Walter U (1994) NO at work. Cell 78:919–925

Snyder RK, Oettingen WF (1942) Clinical note suggestions and new instruments-A new test for the detection and the appraisal of exposure to trinitroluene. J Am Med Assoc 123:202–203

Stuehr DJ, Cho HJ, Kwon NS, Weise MF, Nathan CF (1991) Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. Proc Natl Acad Sci USA 88:7773–7777

Tan DY, Meng SM, Cason GW, Davis Manning Jr R (2000) Mechanims of salt-sensitive hypertension: role of inducible nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol 279:R2297-R2303

Tigheradge MA (1999) Nitric oxide in septic shock. Biochim Biophys Acta 1411:437–455

Umans JG, Levi R (1995) Nitric oxide in the regulation of blood flow and arterial pressure. Annu Rev Physiol 57:771–790

Vasqiez-Vivar J, Hogg N, Martasek P, Karoui H, Tordo P, Pritchard KA Jr, Kalyanaraman B (1999) Effect of redox-active drugs on superoxide generation from nitric oxide synthases: biological and toxicological implications. Free Radic Res 31:607–617

Voegtin C, Hooper CW, Johnson JN (1921) Trinitrotoluene poisoning-its nature, diagnosis and prevention. J Ind Hyg 3:280–292

Vychub VN (1970) Capillary resistance and permeability in workers with chronic trinitrotoluene poisoning. Gigiena Truda I Professional'Nye Zabolevaniia 14:54–56

Yinon J (1990) Toxicity and metabolism of explosives. CRC Press, Boca Raton, pp 1–67