ELSEVIER

European Journal of Pharmacology 342 (1998) 241–245

ejp

Short communication

Platelet-activating factor receptor antagonist attenuates endotoxin-induced vascular hyporeactivity in the pithed rat

Daisuke Yoshikawa *, Tatsuya Shiga, Shigeru Saito, Toshihiro Morita, Takasuke Imai, Fumio Goto

Department of Anesthesiology and Reanimatology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi 371, Japan

Received 5 September 1997; revised 6 November 1997; accepted 11 November 1997

Abstract

The role of platelet activating factor (PAF) and nitric oxide (NO) in the endotoxin-induced hyporeactivity to noradrenaline was studied in the pithed rat. Pressor dose–response curves to noradrenaline (0.01–10 μg/kg, i.v.) were made starting 1 h after the administration of endotoxin (0.5 mg/kg, i.v.) to the rats. Saline was administered to the control rats. The PAF receptor antagonist, TCV-309 (3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolylcarbonyloxy)ethyl]carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate, 100 μg/kg, i.v.), or the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 30 mg/kg, i.v.), was administered to the endotoxin-treated rats 20 or 10 min before the noradrenaline challenge. L-NMMA reversed endotoxin-induced hyporeactivity completely. TCV-309 produced a significant, but partial attenuation of the hyporeactivity to noradrenaline ($P < 0.01$). There was still significant hyporeactivity when compared with the control rats ($P < 0.01$) and the L-NMMA-treated endotoxin-administered rats ($P < 0.05$). These data suggest that endogenous PAF contributes to the vascular hyporeactivity to noradrenaline induced by endotoxin and that NO plays a major role in the endotoxin-induced hyporeactivity. © 1998 Elsevier Science B.V.

Keywords: PAF (platelet activating factor); Endotoxin; Vascular hyporeactivity; TCV-309; N^G-Monomethyl-L-arginine; Noradrenaline

1. Introduction

The loss of vascular responsiveness to a variety of vasoconstrictor agents is associated with the high mortality observed in septic shock (Groeneveld et al., 1986). In vivo and in vitro studies have shown that the vascular response to catecholamines is attenuated during endotoxemia (Fink et al., 1985; Wakabayashi et al., 1987; Gray et al., 1990). This may contribute to the unrelenting hypotension and circulatory collapse associated with septic shock (Parrillo, 1989). The mechanism underlying vascular hyporeactivity remains to be determined, but there is strong evidence that endotoxin induces NO synthase and stimulates NO production from L-arginine (Julou-Schaeffer et al., 1990; Thiemermann and Vane, 1990; Gray et al., 1991; Szabó et al., 1993b). NO formation as a consequence of activation of the constitutive NO synthase may be involved in the vascular hyporeactivity observed in the early stage of endotoxemia (Szabó et al., 1993b).

Platelet-activating factor (PAF) is a lipid mediator with a wide range of pharmacological activity (Braquet et al., 1987). Intravenous administration of PAF produces several cardiovascular features of circulatory shock, including hypotension, peripheral vasodilatation, plasma extravasation and cardiac failure (Bessin et al., 1983). Recently, Chiba et al. and Moritoki et al. reported that the vasodilator action of PAF is, at least in part, mediated by the release of NO (Chiba et al., 1990; Moritoki et al., 1992).

PAF is released during endotoxemia (Doebber et al., 1985) and there is evidence indicating that PAF has a crucial role in the development of the vascular collapse provoked by sepsis. For example, the immediate hypotension following the administration of a high dose of endotoxin can be prevented by a PAF receptor antagonist, WEB 2086 (3-[4-(2-(chlorophenyl)-9-methyl-6*H*-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone) (Casals-Stenzel, 1987) and reversed by another PAF receptor antagonist, TCV-309 (Terashita et al., 1992). Together with the vasodilator action, PAF can impair vascular reactivity to noradrenaline within 60 min (Bouvier et al., 1994; Shiga and Yoshikawa, 1995). As this

* Corresponding author. Tel.: +81-27-2208454; fax: +81-27-2208473.

PAF-induced hyporeactivity in the pithed rat is reversed by an NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA), PAF-induced hyporeactivity may also be mediated by the activation of constitutive NO synthase (Shiga and Yoshikawa, 1995). Even though these data suggest that PAF may be involved in the endotoxin-induced early phase hyporeactivity to noradrenaline, Szabó et al. and Bouvier et al. demonstrated that the PAF receptor antagonist, WEB2086, had no effect on the early phase hyporeactivity in anesthetized rats (Szabó et al., 1993a; Bouvier et al., 1994).

The purpose of the present study was to compare the effect of the specific PAF receptor antagonist, TCV-309, with that of the NO synthase inhibitor, L-NMMA, on restoration of the endotoxin-induced vascular hyporeactivity to noradrenaline in pithed rats as a means of evaluating the role of PAF and NO in endotoxin-induced vascular hyporeactivity in vivo.

2. Materials and methods

This study was performed in accordance with the ethical principles set out by the Experimental Animal Laboratory of Gunma University School of Medicine.

2.1. Surgical preparation

Male Wistar rats (260–300 g) were anesthetized with 4% isoflurane in oxygen. The animals were ventilated artificially (Harvard rodent ventilator) via a tracheal cannula at a rate of 50–60 breaths/min with a stroke volume of 1 ml per 100 g body weight to maintain arterial $PaCO_2$ at 30–35 mmHg. Arterial blood pressure was monitored using a Gould pressure transducer connected to the left carotid artery via a cannula (PE-50) containing heparinized saline (100 IU/ml). The output from the pressure transducer was displayed on a chart recorder (Nihon Koden). The vagal nerves were cut bilaterally. The rats were pithed by introduction of a blunt steel rod into the vertebral canal through the right orbit as described by Gillespie (Gillespie and Muir, 1967). Immediately after pithing, isoflurane anesthesia was discontinued. Twenty-four gauge Teflon cannulas were placed in the tail vein for the administration of drugs. Body temperature was measured using a rectal thermistor probe and was maintained at 36–37°C using a heated underblanket.

2.2. Experimental protocols

The animals were allowed to stabilize for 1 h prior to any experimental intervention. After recording of the baseline hemodynamic parameters, the animals were given either endotoxin (0.5 mg/kg i.v., in 1 ml/kg saline) or saline (1 ml/kg) as an injection over a 2 min period. Pressor dose–response curves to noradrenaline (0.01–10 μg/kg i.v.) were made starting 1 h after the administration of endotoxin to the test group or saline to the control group. The pressor responses were evaluated using cumulative administration of noradrenaline. Where the PAF receptor antagonist (or its vehicle) or NO synthase inhibitor (or its vehicle) was used, TCV-309 (100 μg/kg i.v.) or its vehicle (saline) was injected 20 min prior to the noradrenaline challenge and L-NMMA (30 mg/kg, i.v.) or its vehicle (saline) was injected 10 min prior to the noradrenaline challenge.

2.3. Drug used

Lipopolysaccharide (*E. coli* 055: B5) was obtained from Difco. Heparin sodium was obtained from Upjohn. Noradrenaline bitartrate and N^G-monomethyl-L-arginine (L-NMMA) were obtained from Sigma. TCV-309 (3-bromo-5-[*N*-phenyl-*N*-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolylcarbonyloxy)ethyl]carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate) was a generous gift of Takeda Chemical Industry (Osaka, Japan). Noradrenaline, L-NMMA and TCV-309 were dissolved in saline.

2.4. Statistical analysis

All data are expressed as the arithmetic mean ± S.E.M. The mean arterial blood pressures and the pressor responses to noradrenaline were compared by analysis of variance (ANOVA). Further analysis was performed using Scheffe's *F*-test for multiple comparisons in cases where ANOVA showed significant differences. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effects of the infusion of endotoxin and drugs on the mean arterial blood pressure

The mean arterial pressures immediately before the noradrenaline challenge are shown in Table 1. In the

Table 1
The mean arterial blood pressures (MABPs) immediately before the administration of noradrenaline

Group	MABP (mmHg)
Control (saline)	63.1 ± 1.8
TCV-309	73.7 ± 4.4[a]
Endotoxin	46.4 ± 2.6[b]
Endotoxin + TCV-309	60.0 ± 4.5
Endotoxin + L-NMMA	63.3 ± 3.7[c]

The mean arterial blood pressures 60 min after endotoxin (0.5 mg/kg, i.v.) or saline administration. TCV-309 (100 μg/kg, i.v.) or its vehicle was injected 40 min after endotoxin or saline administration. The nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 30 mg/kg, i.v.), or its vehicle was administered 50 min after endotoxin or saline. Each value is the mean ± S.E.M. of 6–9 observations.
[a] $P < 0.01$ compared with the endotoxin only group.
[b] $P < 0.01$ compared with the control group.
[c] $P < 0.05$ compared with the endotoxin only group.

endotoxin group, the mean arterial blood pressure 60 min after endotoxin administration was significantly lower than in the control, TCV-309 and the endotoxin + L-NMMA groups. In the endotoxin + TCV-309 group, the mean arterial pressure 60 min after endotoxin administration was higher than in the endotoxin group, but there was no significant difference between the groups. TCV-309 did not modify blood pressure in the control animals, which were not given endotoxin (TCV-309 group).

3.2. Effects of TCV-309 and L-NMMA on the endotoxin-induced hyporesponsiveness to noradrenaline

Endotoxin significantly impaired the pressor response to noradrenaline compared to the control (Fig. 1). Treatment with L-NMMA 10 min before the noradrenaline infusion (endotoxin + L-NMMA group) completely reversed the endotoxin-induced hyporeactivity to noradrenaline. Treatment with TCV-309 20 min prior to the infusion of noradrenaline (endotoxin + TCV-309 group) significantly attenuated the development of vascular hyporeactivity to noradrenaline ($P < 0.01$, Fig. 1), however, there was still a significant degree of impairment in reactivity compared to the control group ($P < 0.01$) and the endotoxin + L-NMMA group ($P < 0.05$). TCV-309 did not affect the reactivity to noradrenaline in the control rats.

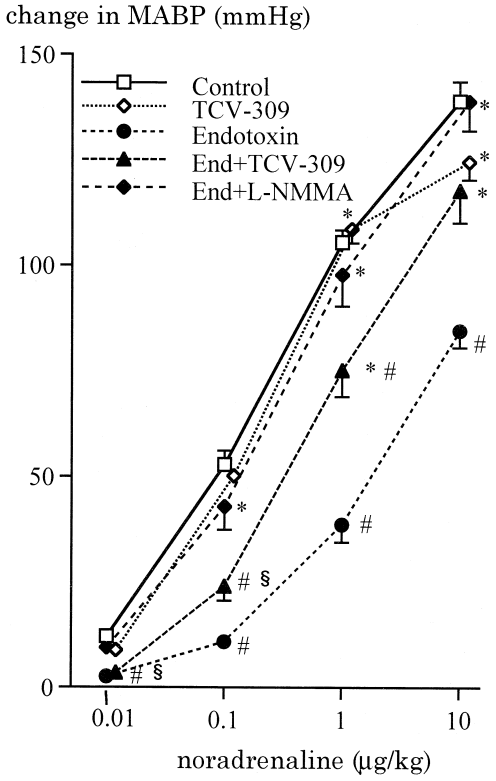

Fig. 1. The effects of TCV-309 and L-NMMA on endotoxin-induced hyporeactivity to noradrenaline. Pressor dose–response curves to noradrenaline were made starting 1 h after the administration of either endotoxin (0.5 mg/kg, i.v.) or saline (control). The PAF antagonist, TCV-309 (100 μg/kg, i.v), or its vehicle was administered 20 min prior to the noradrenaline challenge. N^G-monomethyl-L-arginine (L-NMMA, 30 mg/kg, i.v.) or its vehicle was administered 10 min prior to the noradrenaline challenge. Each point is the mean ± S.E.M. of 6–9 observations. $^{*}P < 0.01$ compared with the endotoxin only group. $^{\#}P < 0.01$ compared with the control group. $^{\S}P < 0.05$ compared with End + L-NMMA group.

4. Discussion

This study has demonstrated that endogenous PAF contributes to the vascular hyporeactivity to noradrenaline induced by endotoxin, because the specific PAF receptor antagonist TCV-309 significantly attenuated the endotoxin-induced hyporeactivity to noradrenaline. The NO synthase inhibitor, L-NMMA, however, completely reversed the endotoxin-induced vascular hyporeactivity. The extent of the restoration by L-NMMA was significantly different from that by TCV-309. These results suggest strongly that NO plays a major role in endotoxin-induced vascular hyporeactivity and that PAF also has a role in causing this hyporeactivity.

TCV-309 was administered 20 min before the noradrenaline challenge, while L-NMMA was administered 10 min prior to the noradrenaline injection. This was because the effect of TCV-309 was long-lasting, but the effect of L-NMMA was short-acting. Terashita et al. reported that TCV-309 (100 μg/kg, i.v.) completely prevented PAF-induced hypotension for 1 h after its injection to adult rats and the significant effect continued for up to 8 hr after the injection (Terashita et al., 1992). In contrast, the hypertension induced by L-NMMA was reported to be of rapid onset, reaching a plateau within 5 min. Its duration was dose-dependent and the hypertension induced by 50 mg/kg and 30 mg/kg of L-NMMA lasted 45 and 30 min, respectively, in the rat (Whittle et al., 1989; Gray et al., 1991).

In this study, we did not test the effect of L-NMMA on vascular reactivity in control animals. However, we have already reported that a 30 mg/kg dose of L-NMMA did not affect the reactivity to 0.01 and 0.1 μg/kg noradrenaline and produced a slight attenuation of the pressor response to 1 μg/kg noradrenaline in the same experimental model (Shiga and Yoshikawa, 1995). These data indicate that L-NMMA itself does not enhance the vascular reactivity in the pithed rat, and that the vascular hyporeactivity reversing action of L-NMMA, observed in endotoxin-treated animals in this experiment, should result from the antagonistic action against endotoxin or its mediators.

Szabó et al. have reported that the PAF receptor antagonist, WEB2086, has no effect on the early hyporeactivity induced by endotoxin in anesthetized rats. (Szabó et al., 1993a). Also, Bouvier et al. have reported that in anesthetized rats, neither WEB 2086 nor BN 50739 (another PAF receptor antagonist) can alter the vascular hyporeactivity to noradrenaline induced by endotoxin injection

(Bouvier et al., 1994). However, in the present study, the PAF receptor antagonist, TCV-309, significantly attenuated the early hyporeactivity to noradrenaline elicited by endotoxin in the pithed rat. We consider that these conflicting results may be explainable by the difference in experimental setting, anesthetized rats and pithed rats. The major differences between the pithed rat model and the anesthetized rat model are: (1) there is no intrinsic sympathetic nerve activity and compensatory reflex in the pithed rat (Gray et al., 1990). The true pressor response–dose relationship may be modified by compensatory reflexes in the anesthetized rat. (2) There is no anesthetic effect on pressor responsiveness in the pithed rat. Use of anesthetics may affect the reactivity, since anesthetic agents have a profound effect on NO synthase activity (Galley et al., 1995; Zuo et al., 1996) and they also affect the change in blood pressure after the administration of the NO synthase inhibitor, N^G-nitro-L-arginine (Wang et al., 1991). (3) Endotoxin infusion produces a massive release of catecholamines from the adrenal medulla and from sympathetic nerves in the conscious rat and in the anesthetized rat (Jones and Yelich, 1987), which may itself result in impaired responsiveness to noradrenaline and thus obscure the action of the other agents. In contrast, it has been reported that endotoxin does not elevate plasma noradrenaline in pithed rats (Zhou and Jones, 1990). In addition, the pithed rat is much more sensitive to endotoxin. In the study of Bouvier et al., a small dose of endotoxin was infused continuously for 1 h and the endotoxin-induced hyporeactivity was far less than that observed in our study. This difference in hyporeactivity may also explain the discrepancy in the results.

It has been reported that the immediate and delayed hypotension and vascular hyporeactivity to vasoconstrictor agents elicited by endotoxin in the rat are due to an enhanced formation of NO that is triggered by two distinct mechanisms, namely, the activation of constitutive NO synthase (early phase) and the induction of NO synthase (delayed phase) (Thiemermann and Vane, 1990; Szabó et al., 1993b). The vascular hyporeactivity to noradrenaline and the fall in blood pressure which occurs within 60 min of endotoxemia in the anesthetized rat are mediated by the enhanced formation of NO by the constitutive NO synthase (Szabó et al., 1993a). It is considered that in the present study the excessive NO production through the activation of constitutive NO synthase by endotoxin was also responsible for the vascular hyporeactivity observed in the pithed rat, because: (1) the dose–response to noradrenaline was assessed 1 h after endotoxin administration, when inducible NO synthase could not be induced, (2) L-NMMA completely reversed endotoxin-induced vascular hyporeactivity, while L-NMMA does not increase vascular reactivity in control animals (Shiga and Yoshikawa, 1995) and (3) L-NMMA was reported to inhibit both the constitutive and the inducible form of NO synthase (Knowles and Moncada, 1994).

We now observed that the PAF receptor antagonist attenuated the endotoxin-induced vascular hyporeactivity. Since PAF-induced vascular hyporeactivity is mediated by the enhanced production of NO by constitutive NO synthase (Shiga and Yoshikawa, 1995), there should be a cascade connecting endotoxin, PAF, NO and vascular hyporeactivity. PAF is first released in response to endotoxin (Klosterhalfen et al., 1992). PAF is known to cause endothelium-dependent relaxation of isolated rat blood vessels by stimulating the production of NO from constitutive NO synthase in endothelial cells (Chiba et al., 1990; Moritoki et al., 1992). Moreover, PAF releases NO from polymorphonuclear granulocytes by activating the constitutive nitric oxide synthase (Schmidt et al., 1989). It is conceivable that the release of PAF in response to endotoxin triggers the release of NO from the constitutive NO synthase, which in turn contributed to the hyporeactivity to noradrenaline in the present experiment.

The finding that TCV-309 does not completely reverse the hyporeactivity supports the view that other mechanisms, independent of PAF, also contribute to the activation of constitutive NO synthase by endotoxin. Further study concerning other chemical mediators will be needed to clarify the complete mechanisms involved in endotoxin-induced hyporeactivity.

References

Bessin, P., Bonnet, J., Apffel, D., Soulard, C., Desgroux, L., Pelas, I., Benveniste, J., 1983. Acute circulatory collapse caused by platelet-activating factor (PAF-acether) in dogs. Eur. J. Pharmacol. 86, 403–413.

Bouvier, C., Guc, M.O., Furman, B.L., Parratt, J.R., 1994. Platelet activating factor impairs pressor responses to noradrenaline in the anaesthetized rat but does not mediate endotoxin-induced hyporeactivity. Circ. Shock 42, 14–19.

Braquet, P., Touqui, L., Shen, T.Y., Vargaftig, B.B., 1987. Perspectives in platelet-activating factor research. Pharmacol. Rev. 39, 97–145.

Chiba, Y., Mikoda, N., Kawasaki, H., Ito, K., 1990. Endothelium-dependent relaxant action of platelet-activating factor in the rat mesentery. Naunyn Schmiedebergs Arch. Pharmacol. 341, 68–73.

Casals-Stenzel, J., 1987. Protective effect of WEB 2086, a novel antagonist of platelet-activating factor in endotoxin shock. Eur. J. Pharmacol. 135, 117–122.

Doebber, T.W., Wu, M.S., Robins, J.C., Ma-Choy, B., Chang, M.N., Shen, T.Y., 1985. Platelet-activating factor (PAF) involvement in endotoxin-induced hypotension in rats: Studies with PAF-receptor antagonist kadsurenone. Biochem. Biophys. Res. Commun. 127, 799–808.

Fink, M.P., Homer, L.D., Fletcher, J.R., 1985. Diminished pressor response to exogenous norepinephrine and angiotensin II in septic, unanaesthetized rats: Evidence for a prostaglandin-mediated effect. J. Surg. Res. 38, 335–342.

Galley, H.F., Nelson, L.R., Webster, N.R., 1995. Anaesthetic agents decrease the activity of nitric oxide synthase from human polymorphonuclear leucocytes. Br. J. Anaesth. 75, 326–329.

Gillespie, J.S., Muir, T.C., 1967. A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br. J. Pharmacol. Chemother. 30, 78–87.

Gray, G.A., Furman, B.L., Parratt, J.R., 1990. Endotoxin-induced impair-

ment of vascular reactivity in the pithed rat: Role of arachidonic acid metabolites. Circ. Shock 31, 395–406.

Gray, G.A., Schott, C., Julou-Schaeffer, G., Fleming, I., Parratt, J.R., Stoclet, J.-C., 1991. The effect of inhibitors of the L-arginine/nitric oxide pathway on endotoxin-induced loss of vascular responsiveness in anaesthetized rats. Br. J. Pharmacol. 103, 1218–1224.

Groeneveld, A.B.J., Bronsveld, W., Thijs, L.J., 1986. Hemodynamic determinants of mortality in human septic shock. Surgery 99, 140–152.

Jones, S.B., Yelich, M.R., 1987. Simultaneous elevation of plasma insulin and catecholamines during endotoxicosis in the conscious and anesthetized rat. Life Sci. 41, 1935–1943.

Julou-Schaeffer, G., Gray, G.A., Fleming, I., Schott, C., Parratt, J.R., Stoclet, J.-C., 1990. Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. Am. J. Physiol. 259, H1038–H1043.

Klosterhalfen, B., Horstmann-Jungemann, K., Vogel, P., Flohé, S., Offner, F., Kirkpatrik, C.J., Heinrich, P.C., 1992. Time course of various inflammatory mediators during recurrent endotoxemia. Biochem. Pharmacol. 43, 2103–2109.

Knowles, R.G., Moncada, S., 1994. Nitric oxide synthases in mammals. Biochem. J. 298, 249–258.

Moritoki, H., Hisayama, T., Takeuchi, S., Miyano, H., Kondoh, W., 1992. Involvement of nitric oxide pathway in the PAF-induced relaxation of rat thoracic aorta. Br. J. Pharmacol. 107, 196–201.

Parrillo, J.E., 1989. The cardiovascular pathology of sepsis. Annu. Rev. Med. 40, 469–485.

Schmidt, H.H.H.W., Seifert, R., Bohme, E., 1989. Formation and release of nitric oxide from human neutrophils and HL-60 cells induced by a chemotactic peptide, platelet-activating factor and leukotriene B_4. FEBS Lett. 244, 357–360.

Shiga, T., Yoshikawa, D., 1995. Platelet-activating factor-induced loss of vascular responsiveness to noradrenaline in pithed rats: Involvement of nitric oxide. Eur. J. Pharmacol. 282, 151–156.

Szabó, C., Wu, C.C., Mitchell, J.A., Gross, S.S., Thiemermann, C., Vane, J.R., 1993a. Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. Circ. Res. 73, 991–999.

Szabó, C., Mitchell, J.A., Thiemermann, C., Vane, J.R., 1993b. Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. Br. J. Pharmacol. 108, 786–792.

Terashita, Z., Kawamura, M., Takatani, M., Tsushima, S., Imura, Y., Nishikawa, K., 1992. Beneficial effect of TCV-309, a novel potent and selective platelet activating factor antagonist in endotoxin and anaphylactic shock in rodents. J. Pharmacol. Exp. Ther. 260, 748–755.

Thiemermann, C., Vane, J.R., 1990. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. Eur. J. Pharmacol. 182, 591–595.

Wakabayashi, I., Hatake, K., Kakishita, E., Nagai, K., 1987. Diminution of contractile response of the aorta from endotoxin-injected rat. Eur. J. Pharmacol. 141, 117–122.

Wang, Y.X., Zhou, T., Chua, T.C., Pang, C.C.Y., 1991. Effects of inhalation and intravenous anesthetic agents on pressor response to N^G-nitro-L-arginine. Eur. J. Pharmacol. 198, 183–188.

Whittle, B.J.R., Lopez-Belmonte, J., Rees, D.D., 1989. Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. Br. J. Pharmacol. 98, 646–652.

Zhou, Z.Z., Jones, S.B., 1990. The vascular response to adrenergic stimulation in pithed rats following endotoxin. Circ. Shock 32, 55–66.

Zuo, Z., Tichotsky, A., Johns, R.A., 1996. Halothane and isoflurane inhibit vasodilation due to constitutive but not inducible nitric oxide synthase. Implications for the site of anesthetic inhibition of the nitric oxide/guanylyl cyclase signaling pathway. Anesthesiology 84, 1156–1165.