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Bilateral kidney ligation abolishes pressor response to N^G-nitro-D-arginine

Yong-Xiang Wang, Xing Cheng, Catherine C.Y. Pang *

Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, BC, Canada V6T 1Z3

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Abstract

We have shown that N^G-nitro-D-arginine (D-NNA) is 50% as potent as N^G-nitro-L-arginine (L-NNA) in causing pressor response and 2–3% as potent as L-NNA in inhibiting endothelium-dependent relaxation in vitro. These results suggest in vivo activation of D-NNA. Furthermore, the potency of D-NNA was markedly increased after it had been incubated with homogenate of the kidney, but not plasma or homogenate of the aorta, lungs or liver. This study examined if bilateral ligation of the kidneys attenuated the biological action of D-NNA. I.v. bolus of D-NNA (16 mg/kg), L-NNA (3 mg/kg) and norepinephrine (0.25–16 μg/kg) increased arterial pressure in sham-operated rats. Bilateral ligation of the kidneys abolished pressor response to D-NNA, but not L-NNA and norepinephrine. I.v. bolus D-NNA in sham-operated rats, but not kidney-ligated rats, inhibited relaxation response to acetylcholine in pre-constricted aortic rings ex vivo. These results indicate that the kidney is the primary organ which activates D-NNA.

Keywords: N^G-Nitro-L-arginine; N^G-Nitro-D-arginine; (Ex vivo); (In vivo); Nitric oxide (NO); Chiral inversion

1. Introduction

N^G-substituted arginine (Arg) analogs such as N^G-monomethyl-L-arginine, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NNA) have been shown to inhibit endothelium-dependent relaxation of isolated arteries (Palmer et al., 1988; Rees et al., 1989b, 1990; Mülsch and Busse, 1990; Wang et al., 1993) and cause sustained pressor responses in whole animals (Rees et al., 1989a, 1990; Wang and Pang, 1990a; Wang et al., 1991). It is extensively reported that N^G-substituted Arg analogs exhibit stereospecificity such that the L but not the D enantiomers raise blood pressure and inhibit endothelium-dependent relaxation (Rees et al., 1989a, 1990; Gardiner et al., 1990a,b; Mülsch and Busse, 1990; Humphries et al., 1991; Moncada et al., 1991). D-NNA is, however, as efficacious as L-NNA in raising blood pressure in anesthetized (Wang and Pang, 1990b; Raszkiewicz et al., 1992) and conscious (Wang et al., 1991, 1992) rats. Pressor responses to both L-NNA and D-NNA are attenuated by L-Arg but not D-Arg suggesting involvement of the L-Arg pathway (Wang et al., 1991). D-NNA is also as efficacious as L-NNA in inhibiting endothelium-dependent relaxation in vitro (Wang et al., 1992, 1993). D-NNA is, however, less potent than L-NNA—the potency (ED_{50} or EC_{50}) ratio of L-NNA to D-NNA is approximately 1:2 in raising arterial pressure and 1:39 in suppressing endothelium-dependent relaxation (Wang et al., 1991, 1993). The greater discrepancy in the potency of D-NNA relative to that of L-NNA in vitro than in vivo, the longer onset of action of D-NNA, but similar efficacy and similar sensitivity to blockade by L-Arg but not D-Arg suggest that D-NNA is biologically converted to a compound that acts similar to L-NNA.

We hypothesized that chiral conversion might account for the difference in potencies between D-NNA and L-NNA (Wang et al., 1993). Metabolic chiral inversion occurred after the administration of stereospecific drugs (e.g., Hutt and Caldwell, 1983; Drummond et al., 1990; Sanins et al., 1991; King et al., 1994; Walters and Hsu, 1994). Since D-NNA is less potent and has a slower onset of action than L-NNA (Wang et al., 1991), D-NNA may be more readily converted to L-NNA in vivo than in vitro thus accounting

* Corresponding author. Tel.: +1-604-822-2039; Fax: +1-604-822-6012; E-mail: ccypang@unixg.ubc.ca

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for the difference in the potency ratios of D-NNA and L-NNA between in vivo and in vitro settings. We have found that incubation of D-NNA with homogenate of the kidneys, but not with plasma nor homogenates of the liver, lungs or blood vessels increases the inhibitory effect of D-NNA on endothelium-dependent relaxation in vitro (Cheng et al., 1997). To find out if the kidney is the primary organ responsible for the biological activation of D-NNA, we examined if bilateral ligation of the kidneys suppressed the effect of D-NNA on blood pressure and endothelium-dependent relaxation ex vivo.

2. Materials and methods

2.1. Surgical preparation

2.1.1. In vivo studies and tissue preparation

Sprague–Dawley rats (250–320 g) were anesthetized with sodium pentobarbital (65 mg/kg, i.p.). Polyethylene cannulae (PE_{50}) were inserted into the left iliac artery, for the measurement of mean arterial pressure by a P23DB pressure transducer (Gould Statham, CA, USA) and into the left iliac vein for the injection of drugs. The body temperature of the rats was maintained at 37°C with a heating lamp connected to a thermostat (73A, Yellow Springs Instruments). The rats were given either a midline incision along the abdomen followed by bilateral ligation of the renal arteries and veins or sham-operation. Drug or vehicle was injected 10 min after surgery and arterial pressure was continuously monitored.

2.1.2. Ex vivo studies

In the ex vivo studies, 10 min after sham operation or kidney ligation, the vehicle or drugs was i.v. bolus injected and arterial pressure was monitored for 1 h. The rats were then sacrificed by a blow on the head followed by exsanguination. The thoracic aorta was removed and cleared of connective tissue. Ring segments of 0.5 cm length were prepared from one aorta and suspended randomly in separate organ baths. Each ring was connected to a Grass FT-03-C force-displacement transducer for isometric recording. Before the study commenced, a preload of 1 g was applied and the rings were equilibrated for 1 h (with three washouts at intervals of 20 min) in Krebs' solution (pH 7.4) at 37°C and bubbled with a gas mixture containing 95% O_2 and 5% CO_2. The composition of the Krebs' solution was as follows (1×10^{-3} mol/l): NaCl, 118; glucose, 11; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2 6H_2O$, 1.2.

Phenylephrine (1×10^{-6} mol/l, EC_{90}) was added to the baths. At the steady state phase of contraction to phenylephrine (10–20 min after addition), a cumulative concentration-response curve of acetylcholine or sodium nitroprusside was constructed. Each concentration was left in the bath until a plateau response was obtained. The time taken to complete each concentration-response curve was approximately 15 min.

2.2. Experimental protocol

Each group of in vivo or in vitro studies consisted of 6 rats.

2.2.1. In vivo studies

Rats were randomly divided into 6 groups ($n = 6$ each). Three groups of sham-operated and three groups of kidney-ligated rats were i.v. bolus injected with either the vehicle (0.9% NaCl, 4 ml/kg), D-NNA (16 mg/kg, ED_{75} dose) or L-NNA (4 mg/kg, ED_{50} dose). A higher dose of D-NNA than L-NNA was used because of its lower potency and longer onset of action (Wang et al., 1991). Mean arterial pressure was followed for 1 h. At 1 h after injection of D-NNA in a group of sham-operated rats, kidney ligation was performed and arterial pressure was continuously recorded for another h. Another group of sham-operated rats and one other group of kidney-ligated rats were i.v. bolus injected with norepinephrine (0.25–16 μg/kg) at dose-intervals of 5 min which was the time required for the recovery of arterial pressure response.

2.2.2. Ex vivo studies

Sham-operated rats and bilateral kidney-ligated rats were i.v. bolus injected with D-NNA (16 mg/kg). Another group of bilateral kidney-ligated rats was injected with the vehicle. After 1 h, the rats were sacrificed and ring segments were prepared from each aorta for ex vivo study. The rings were preconstricted with phenylephrine then relaxed with acetylcholine (3×10^{-8} to 1×10^{-5} mol/l) or sodium nitroprusside (3×10^{-10} to 1×10^{-6} mol/l). In a second batch of aortic rings derived from sham-operated rats treated with D-NNA, L-Arg (1×10^{-3} mol/l) was added 10 min prior to preconstriction with phenylephrine.

2.3. Drugs

L-Arg hydrochloride, L-NNA, phenylephrine hydrochloride and acetylcholine chloride were obtained from Sigma (MO, USA). D-NNA was from Bachem Bioscience (PA, USA) while sodium nitroprusside was from Fisher Scientific (NJ, USA). L-Arg was dissolved in distilled water and the pH of the solution was adjusted to 7.0 with NaOH solution. The remaining drugs were dissolved in normal saline and the dissolution of L-NNA and D-NNA required 20 min of sonication.

2.4. Calculations and statistics

Changes in mean arterial pressure in response to the vehicle in the kidney-ligated rats and to D-NNA in the kidney-ligated rats as well as sham-operated rats in the in

vivo and ex vivo studies were pooled (i.e., $n = 12$) prior to statistical analysis. Relaxation responses to acetylcholine and sodium nitroprusside were calculated as percent of contraction elicited by phenylephrine. ED_{50} and E_{max} of the pressor response to norepinephrine were best-fitted by a computer program (Wang and Pang, 1993). All results were analyzed by the analysis of variance/co-variance and expressed as mean ± S.E.M. Duncan's multiple range test was used to compare group means, with $P < 0.05$ selected as the criterion for statistical significance.

3. Results

3.1. In vivo studies

Baseline mean arterial pressure was similar in the kidney-ligated rats given the vehicle (104 ± 4 mm Hg, $n = 12$) and the sham-operated rats (104 ± 5 mm Hg, $n = 6$). Bilateral kidney ligation significantly reduced mean arterial pressure (by −24 ± 5 mm Hg) at 10 min after surgery. Mean arterial pressure in the kidney-ligated rats gradually recovered toward the baseline level; mean arterial pressure was −18 ± 5, −15 ± 6, −14 ± 6, −11 ± 7, −9 ± 7 and −6 ± 7 mm Hg below baseline level at 20, 30, 40, 50, 60 and 70 min after surgery; the last four readings were insignificantly different from baseline mean arterial pressure. Sham-operation insignificantly reduced mean arterial pressure at all times and this ranged from −5 ± 7 to mm Hg to −3 ± 3 mm Hg between 10 and 70 min after sham-operation.

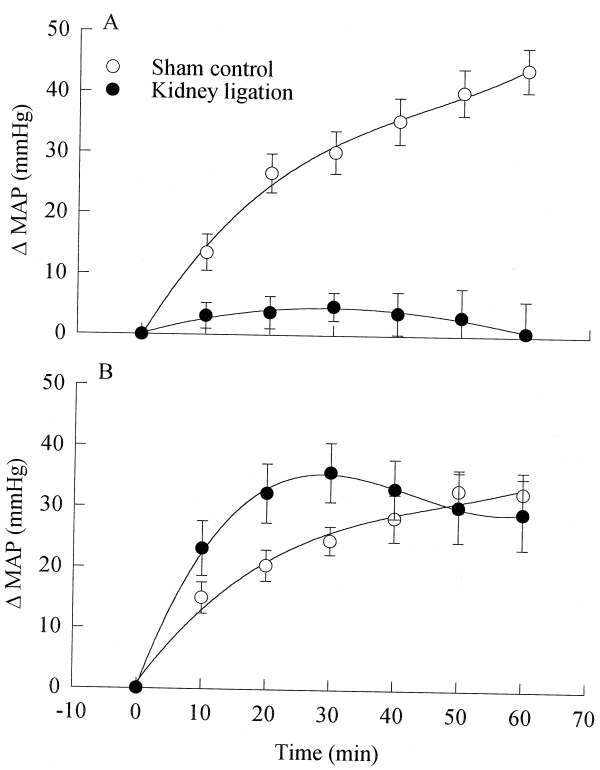

Fig. 1. Increments of mean arterial pressure (MAP) in response to i.v. injection of N^G-nitro-D-arginine (D-NNA, 16 mg/kg, $n = 12$, A) or N^G-nitro-L-arginine (L-NNA, 4 mg/kg, $n = 6$, B) in sham-operated or bilateral kidney-ligated rats anesthetized with pentobarbital. These values are normalized as increments of MAP from the corresponding changes in the time-control rats subjected to sham-operation or kidney-ligation. All readings are mean ± S.E.M.

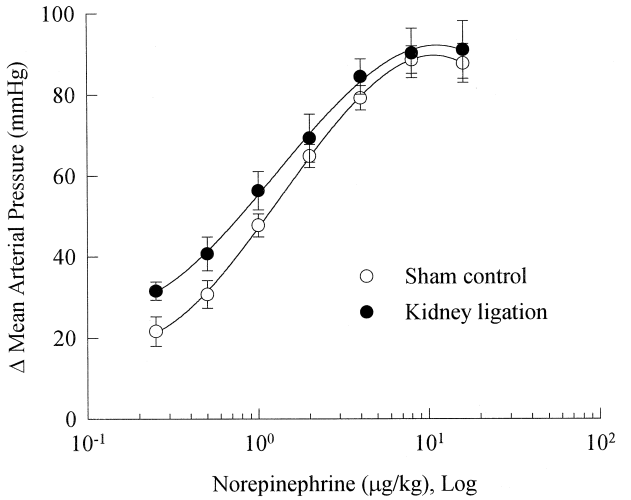

Fig. 2. Increments of mean arterial pressure (MAP) in response to i.v. bolus injection of norepinephrine in sham-operated or bilateral kidney-ligated rats anesthetized with pentobarbital ($N = 6$ each group). The data are mean ± S.E.M.

Fig. 1 shows changes of mean arterial pressure in response to D-NNA and L-NNA in kidney-ligated and sham-operated rats; these values were normalized as increments in mean arterial pressure from the corresponding changes at the exact time in kidney-ligated rats or sham-operated rats given the vehicle. In the sham-operated rats, i.v. bolus injection of D-NNA (16 mg/kg) caused a slow rise in mean arterial pressure (+43 ± 3 mm Hg at 60 min post-injection) ($n = 12$; Fig. 1A). At 60 min post-injection, kidney ligation did not affect arterial pressure and mean arterial pressure remained elevated (+43 ± 5 mm Hg at 120 min after injection of D-NNA, $n = 6$). In the kidney-ligated rats however, pressor response to the same dose of D-NNA was abolished (Fig. 1A, $n = 12$). I.v. bolus injection of L-NNA (4 mg/kg) raised mean arterial pressure in sham-operated rats (+33 ± 3 mm Hg, $n = 6$) and kidney-ligated rats (+30 ± 5 mm Hg, $n = 6$) at 60 min post-injection (Fig. 1B). Although similar mean arterial pressure was reached at 60 min following injection of L-NNA, the rise in mean arterial pressure was faster in kidney-ligated than sham-control rats; the reason of the discrepancy in the rise rate of L-NNA is unclear.

I.v. bolus injections of norepinephrine (0.25–16 μg/kg) caused similar dose-dependent increments in mean arterial pressure in sham-operated rats (ED_{50} of 0.95 ± 0.08 μg/kg, E_{max} of 95 ± 5 mm Hg) and bilateral kidney-ligated rats (ED_{50} of 0.74 ± 0.17 μg/kg, E_{max} of 97 ± 7 mm Hg) (Fig. 2).

3.2. Ex vivo studies

Phenylephrine (1×10^{-5} mol/l) constricted aortae with peak responses of 0.98 ± 0.12, 1.45 ± 0.09 and 1.32 ± 0.13 g in the kidney-ligated rats treated with the vehicle, sham-operated rats treated with D-NNA and kidney-ligated rats treated with D-NNA, respectively. Contraction to phenylephrine in the sham-operated rats given D-NNA, but not the kidney-ligated rats given D-NNA, was significantly ($P < 0.05$) greater than that in the kidney-ligated rats treated with the vehicle. Acetylcholine caused concentration-dependent relaxation of aortae from the vehicle-treated, kidney-ligated rats (Fig. 3). Curve analysis show that ex vivo relaxation response to acetylcholine was significantly reduced after treatment of sham-operated rats with D-NNA; the inhibitory effect of D-NNA on acetylcholine-induced relaxation in these rats was attenuated by pre-treatment with L-Arg (Fig. 3A). The injection of kidney-ligated rats with D-NNA, on the other hand, did not significantly alter ex vivo relaxation response to acetylcholine (Fig. 3A). Sodium nitroprusside caused similar ex vivo concentration-dependent relaxation of aortae from vehicle-treated, kidney-ligated rats as in D-NNA-treated, sham-operated rats or D-NNA-treated, kidney-ligated rats (Fig. 3B).

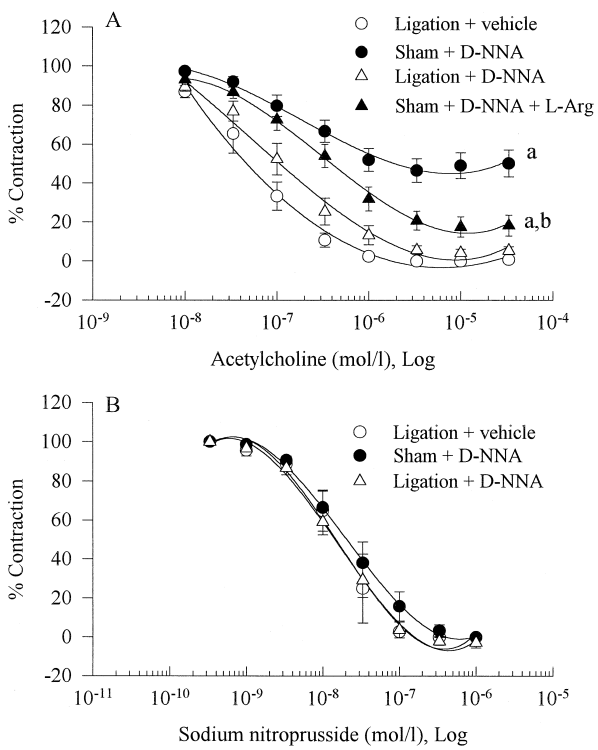

Fig. 3. Relaxant effects of acetylcholine (A) and sodium nitroprusside (B) in phenylephrine (1×10^{-6} mol/l)-preconstricted aortae from bilateral kidney-ligated rats i.v. bolus injected with normal saline or N^G-nitro-D-arginine (D-NNA) and sham-operated rats treated with D-NNA. One group of aortae from sham-operated rats treated with D-NNA was pre-treated with L-arginine (L-Arg, 1×10^{-3} mol/l, A) prior to constriction with phenylephrine. The data represent mean ± S.E.M. ($N = 6$ rats each group). [a]Denotes significant ($P < 0.05$) difference from readings in vehicle-treated, kidney ligated rats. [b]Denotes significant difference from readings in D-NNA-treated, sham-operated rats.

4. Discussion

Similar to previous observations (Wang and Pang, 1990b; Wang et al., 1991), i.v. bolus injection of D-NNA caused a slow-onset and sustained pressor response in sham-operated rats. Pressor response to D-NNA was abolished by bilateral kidney ligation suggesting that D-NNA has minimal direct constrictor action. We have previously shown that D-NNA suppresses acetylcholine-induced relaxation of the isolated aorta in vitro, although its potency ($IC_{50} = 3.9 \times 10^{-5}$ M) is only 1/39 that of L-NNA ($IC_{50} = 10^{-6}$ M) (Wang et al., 1993). It is unclear why D-NNA lacks direct pressor action in vivo but inhibits acetylcholine-induced relaxation in vitro. Kidney ligation did not attenuate pressor response to i.v. bolus L-NNA or norepinephrine. Furthermore, after the development of hypertensive response to D-NNA, kidney ligation did not alter mean arterial pressure. These results are supportive of the conversion of D-NNA by the kidney to an active form. There was no appreciable conversion of L-NNA to D-NNA as ligation of the kidneys did not significantly affect pressor response to L-NNA. Pressor response to norepinephrine was also unaffected by bilateral ligation of the kidneys.

Similar to our previous observations of D-NNA (32 mg/kg) in intact conscious rats (Wang et al., 1993), i.v. injection of D-NNA (16 mg) in sham-operated, anesthetized rats caused ex vivo inhibition of acetylcholine- but not sodium nitroprusside-induced relaxation, and the inhibitory effect on acetylcholine-induced, or endothelium-dependent, relaxation was antagonized by L-Arg. In contrast, similar ex vivo response to acetylcholine was found in kidney-ligated rats given D-NNA as in kidney-ligated rats given the vehicle. Therefore, i.v. injection of D-NNA in kidney-ligated rats did not suppress acetylcholine-induced relaxation ex vivo. These results are again supportive of the conversion of D-NNA to an active form by the kidney.

We reported that D-NNA was slightly less potent than L-NNA in raising mean arterial pressure in conscious rats but substantially less potent than L-NNA in suppressing endothelium-dependent relaxation in vitro (Wang et al., 1991, 1993). Furthermore, both compounds were equally efficacious and their in vivo and in vitro effects were antagonized by L-Arg but not D-Arg. The essential role of kidneys in the in vivo conversion of D-NNA to a compound which acts similar to L-NNA readily explains the similar efficacy, sensitivity to antagonism by L-Arg, the longer onset of action of D-NNA as well as the potency difference of D-NNA versus L-NNA between in vivo and

in vitro settings. We have found that the biological activity of D-NNA but not that of L-NNA is increased (by four-fold) following incubation of the compound with homogenate of the kidney, but not with plasma samples nor homogenates of the liver, lungs or aorta (Cheng et al., 1997); these findings also demonstrate a role of the kidney in the activation of D-NNA.

We do not know if the D-enantiomers of other N^G-nitro-arginine analogues are also biologically converted to the L-enantiomers. We have reported that potency ratios of D-NAME to L-NAME is 1/55 in vivo and 1/359 in vitro. The large potency ratio (1:55) of D-NAME to L-NAME in vivo suggest that there is limited, if at all, conversion of D-NAME into L-NAME.

Our findings caution against the use of D-NNA as a negative control for L-NNA in biological studies, particularly in vivo. D-NNA can be used in place of L-NNA in conditions where a longer duration of action is desired.

Acknowledgements

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References

Cheng, X., Wang, Y.X., Pang, C.C.Y., 1997. Biological activation of N^G-nitro-D-arginine by kidney homogenate. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 495–499.

Drummond, L., Caldwell, J., Wilson, H.K., 1990. The stereoselectivity of 1,2-phenylethanediol and mandelic acid metabolism and disposition in the rat. Xenobiotica 20, 159–168.

Gardiner, S.M., Compton, A.M., Bennett, T., Palmer, R.M.J., Moncada, S., 1990a. Control of regional blood flow by endothelium-derived nitric oxide. Hypertension 15, 486–492.

Gardiner, S.M., Compton, A.M., Kemp, P.A., Bennett, T., 1990b. Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious Long Evans rats. Br. J. Pharmacol. 101, 625–631.

Humphries, R.G., Carr, R.D., Nicol, A.K., Tomlinson, W., O'Connor, S.E., 1991. Coronary vasoconstriction in the conscious rabbit following intravenous infusion of L-N^G-nitro-arginine. Br. J. Pharmacol. 102, 565–566.

Hutt, A.J., Caldwell, J., 1983. The metabolic chiral inversion of 2-arylpropionic acids—a novel route with pharmacological consequences. J. Pharm. Pharmacol. 35, 693–704.

King, J.N., Mauron, C., LeGoff, C., Hauffe, S., 1994. Bidirectional chiral inversion of the enantiomers of the nonsteroidal antiinflammatory drug oxindanac in dogs. Chirality 6, 460–466.

Moncada, S., Palmer, R.M., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Mülsch, A., Busse, R., 1990. N^G-nitro-L-arginine (N5-[imino (nitroamino) methyl]-L-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine. Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 143–147.

Palmer, R.M.J., Rees, D.D., Ashton, D.S., Moncada, S., 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Rees, D.D., Palmer, R.M.J., Moncada, S., 1989a. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc. Natl. Acad. Sci., USA 86, 3375–3378.

Rees, D.D., Palmer, R.M.J., Hodson, H.F., Moncada, S., 1989b. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br. J. Pharmacol. 96, 418–424.

Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F., Moncada, S., 1990. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.

Raszkiewicz, J.L., Linville, D.G., Kerwin, J.F. Jr., Wagenaar, F., Arneric, S.P., 1992. Nitric oxide synthase is critical in mediating basal forebrain regulation of cortical cerebral circulation. J. Neurosci. Res. 33, 129–135.

Sanins, S.M., Adams, W.J., Kaiser, D.G., Halstead, G.W., Hosley, J., Barnes, H., Baillie, T.A., 1991. Mechanistic studies on the metabolic chiral inversion of R-ibuprofen in the rat. Drug Metab. Dispos. 19, 405–410.

Walters, R.R., Hsu, C.Y., 1994. Chiral assay methods for lifibrol and metabolites in plasma and the observation of unidirectional chiral inversion following administration of the enantiomers to dogs. Chirality 6, 105–115.

Wang, Y.X., Pang, C.C.Y., 1990a. Pressor effect of N^G-nitro-L-arginine in pentobarbital-anesthetized rats. Life Sci. 47, 2217–2224.

Wang, Y.X., Pang, C.C.Y., 1990b. Effect of L and D enantiomers of N^G-nitro-arginine on blood pressure in pentobarbital-anaesthetized rats. Pharmacology (Life Sci. Adv.) 9, 723–728.

Wang, Y.X., Pang, C.C.Y., 1993. Functional integrity of the central and sympathetic nervous systems is a prerequisite for pressor and tachycardic effects of diphenyleneiodonium, a novel inhibitor of nitric oxide synthase. J. Pharmacol. Exp. Ther. 265, 263–272.

Wang, Y.X., Zhou, T., Pang, C.C.Y., 1991. Pressor effects of L and D enantiomers of N^G-nitro-arginine in conscious rats are antagonized by L- but not D-arginine. Eur. J. Pharmacol. 200, 77–81.

Wang, Y.X., Poon, C.I., Yau, L.C.Y., Pang, C.C.Y., 1992. The in vivo and in vitro vasoconstrictor effects of L and D enantiomers of N^G-nitro-arginine methyl ester (NAME). Can. Fed. Biol. Soc. 35, 80.

Wang, Y.X., Poon, C.I., Pang, C.C.Y., 1993. In vitro and ex vivo inhibitory effects of L- and D-enantiomers of N^G-nitro-arginine on endothelium-dependent relaxation of rat aorta. J. Pharmacol. Exp. Ther. 265, 112–119.