

## Influence of Pertussis Toxin Pretreatment on the Development of L-NAME-Induced Hypertension

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### Summary

High blood pressure (BP) of L-NAME hypertensive rats is maintained not only by the absence of nitric oxide (NO)-dependent vasodilatation but also by the enhancement of both sympathetic and angiotensin II-dependent vasoconstriction. The aim of the present study was to evaluate the role of inhibitory G (G<sub>i</sub>) proteins, which are involved in tonic sympathetic vasoconstriction, in the pathogenesis of NO-deficient hypertension. We therefore studied BP response to chronic L-NAME administration (60 mg/kg/day for 4 weeks) in rats in which the *in vivo* inactivation of G<sub>i</sub> proteins was induced by injection of pertussis toxin (PTX, 10 µg/kg i.v.). The impairment of sympathetic vasoconstriction due to PTX-induced G<sub>i</sub> protein inactivation prevents the full development of NO-deficient hypertension because BP of PTX-treated rats subjected to chronic L-NAME administration did not reach hypertensive values. Nevertheless, chronic NO synthase inhibition *per se* is capable to increase moderately BP even in PTX-treated rats. Our data suggest that the sympathetic vasoconstriction is essential for the development of established NO-deficient hypertension.

### Key words

Sympathetic nervous system • Nitric oxide • Inhibitory G proteins  
• Blood pressure

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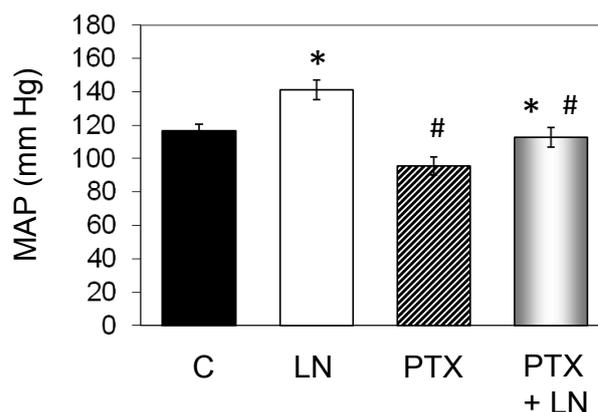
Although the inhibition of NO synthesis is the primary stimulus for the development of hypertension elicited by chronic administration of NO synthase inhibitor L-NAME, the enhanced activity of sympathetic nervous system is a necessary prerequisite for establishing the chronic phase of this form of experimental hypertension (Sander and Victor 1999). Our previous study (Pecháňová *et al.* 2004) documented the enhanced pressor contribution of both renin-angiotensin and sympathetic nervous systems to blood pressure (BP) maintenance in L-NAME hypertensive rats. Several earlier papers (Pecháňová *et al.* 1997, Bernátová *et al.* 1999) reported the prevention of the development of NO-deficient hypertension in rats subjected to simultaneous administration of captopril or other angiotensin converting enzyme inhibitors. It should, however, be noted that the main mechanism through which chronic captopril treatment diminished BP rise in L-NAME-treated rats, is the attenuation of sympathetic vasoconstriction (Zicha *et al.* 2006).

To examine the role of sympathetic nervous system in the development of NO-deficient hypertension, we decided to impair the efficiency of sympathetic vasoconstriction by the *in vivo* pretreatment of rats with pertussis toxin (PTX) which inactivates inhibitory G (G<sub>i</sub>) proteins participating in  $\alpha$ -adrenergic contraction (Li and Triggle 1993). The inactivation of G<sub>i</sub> proteins leads to augmented formation of cyclic AMP and consequent vasodilatation due to attenuated calcium influx (Orlov *et al.* 1996). The up-regulation of G<sub>i</sub> protein pathway is

involved in the pathogenetic mechanisms of various forms of experimental hypertension (Anand-Srivastava 1997, Bassil and Anand-Srivastava 2006) including genetic (Marcil *et al.* 1997, Li and Anand-Srivastava 2002) and NO-deficient hypertension (Di Fusco and Anand-Srivastava 1997, 2000). PTX pretreatment of Wistar rats causes a major attenuation of norepinephrine-induced contraction of isolated arteries *in vitro* (Lišková *et al.* 2007a) and a substantial reduction of pressor contribution of sympathetic nervous system to BP maintenance which is only partially compensated by considerable augmentation of angiotensin II-dependent vasoconstriction (Pintérová *et al.* 2007). The aim of the present study was to evaluate BP response to chronic L-NAME administration under the conditions of impaired sympathetic nervous system activity and augmented renin-angiotensin system activity.

Twenty-four 12-week-old male Wistar rats (bred in the Institute of Physiology AS CR, Prague) were used in this study. All animals were kept under standard laboratory conditions (12 h light, 12 h darkness,  $23 \pm 1$  °C, pelleted ST-1 diet, drinking *ad libitum*). All procedures and experimental protocols were approved by the *Animal Care Ethical Committee* of the Institute of Physiology AS CR and conformed to the *European Convention on Animal Protection and Guidelines on Research Animal Use*. Twelve rats were injected 10 µg PTX/kg b.w. into jugular vein under ether anesthesia. Half of PTX-treated rats and six untreated animals were subjected to chronic L-NAME administration (60 mg/kg/day) for three weeks, whereas equal number of PTX-treated and untreated rats drank tap water only. At the end of the experiment blood pressure was measured by a direct puncture of carotid artery under light ether anesthesia. Heart, aorta, kidney, liver, cerebellum and brain cortex were dissected and homogenized. Total NO synthase (NOS) activity and neuronal NOS (nNOS) activity (susceptible to the inhibition by  $10^{-4}$  M S-methyl-L-thiocitrulline, SMTC) were determined in crude homogenates of heart, aorta, kidney and cerebellum by measuring L-[ $^3$ H]citrulline from L-[ $^3$ H]arginine (Amersham, UK) as previously described by Bredt and Snyder (1990) with minor modifications (Pecháňová *et al.* 1997).

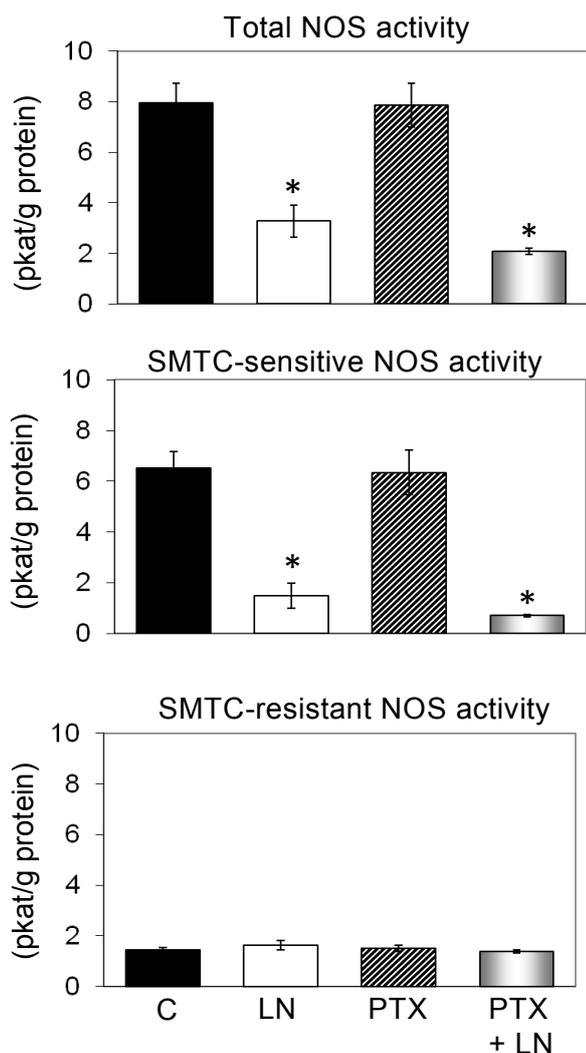
The concentration of conjugated dienes was measured in lipid extracts of heart, kidney and brain cortex homogenates by the method of Kogure *et al.* (1982). After chloroform evaporation under the inert atmosphere and addition of cyclohexane, conjugated diene concentrations were determined



**Fig. 1.** Mean arterial pressure in L-NAME-treated rats and their controls which were either left intact or pretreated with pertussis toxin (PTX). Data are means  $\pm$  S.E.M. ( $n=6$ ). Asterisks indicate significant ( $p<0.001$ ) effects of chronic L-NAME treatment, whereas crosses indicate significant ( $p<0.001$ ) effects of PTX treatment.

spectrophotometrically ( $\lambda = 233$  nm, GBC 911A, Bio-Rad Laboratories). Reduced glutathione (GSH) levels were measured in heart, kidney, liver and brain cortex by the method of Ellman (1959). Samples of tissue were homogenized in 0.5 vol of ice-cold 5 % sulphosalicylic acid and, after centrifugation at  $12,000 \times$  for 15 min, GSH concentration was determined spectrophotometrically in the acid-soluble fractions ( $\lambda = 412$  nm, GBC 911A, Bio-Rad Laboratories). Results are expressed as means  $\pm$  S.E.M. One-way ANOVA and Bonferroni test were used for the statistical analysis.  $P<0.05$  value was considered as statistically significant.

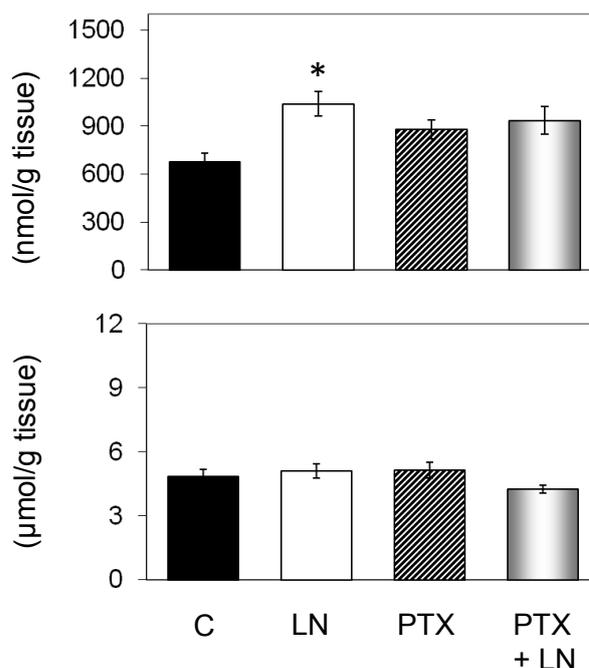
Three weeks of L-NAME treatment increased blood pressure by about 25 mm Hg. PTX pretreatment prevented the development of L-NAME-induced hypertension because BP of these animals did not surpass BP values seen in intact controls. However, we can still see a significant L-NAME-induced BP rise by 17 mm Hg if we compare these rats with PTX-pretreated controls (Fig. 1). The inactivation of inhibitory G protein by PTX administration did not influence total NOS activity in either organ examined (heart, aorta or kidney) (data not shown). Figure 2 shows that neither total NOS activity nor its SMTC-sensitive fraction (corresponding to nNOS activity) in the cerebellum were affected by PTX pretreatment. It also demonstrates that the reduction of total NOS activity in the cerebellum of L-NAME-treated rats was entirely at the expense of nNOS inhibition (Fig. 2). The concentration of conjugated dienes was increased in the brain cortex (Fig. 3) but not in the heart or kidney of L-NAME hypertensive rats (data not



**Fig. 2.** Total NOS activity (upper panel), NOS activity susceptible (middle panel) or resistant (lower panel) to the inhibition by  $10^{-4}$  M SMTC in cerebellum of L-NAME-treated rats and their controls which were either left intact or pretreated with pertussis toxin (PTX). Data are means  $\pm$  S.E.M. (n=6). Asterisks indicate significant ( $p < 0.001$ ) effects of chronic L-NAME treatment.

shown). The same tendency was also observed in PTX-pretreated rats. Thiol levels in brain cortex were not affected by chronic L-NAME treatment of either intact or PTX-pretreated rats (Fig. 3). No important changes of thiol levels were seen in the heart, kidney and liver of NO-deficient animals (data not shown).

Our present results confirm the importance of sympathetic nervous system for the pathogenesis of NO-deficient hypertension as it was suggested by Sander and Victor (1999). Furthermore, these data indicate that our previous observation (Zicha *et al.* 2006) on the antihypertensive action of captopril in L-NAME-treated rats through the attenuation of sympathetic vasoconstriction was correct. The fact that blood pressure



**Fig. 3.** Conjugated diene concentrations (upper panel) and thiol levels (lower panel) in brain cortex of L-NAME-treated rats and their controls which were either left intact or pretreated with pertussis toxin (PTX). Data are means  $\pm$  S.E.M. (n=6). Asterisks indicate significant ( $p < 0.05$ ) effects of chronic L-NAME treatment.

of PTX-pretreated rats rose following L-NAME administration, but did not reach hypertensive values, resembles our recent findings in PTX-treated spontaneously hypertensive rats in which blood pressure decreased to the level of normotensive controls, but it was still elevated compared to PTX-treated Wistar-Kyoto rats (Líšková *et al.* 2007b). It is important to note that pressor action of angiotensin II, which is several fold augmented in PTX-treated rats, is insufficient *per se* to maintain hypertensive BP values in either NO-deficient (this study) or genetic hypertension (Pintérová *et al.* 2009).

Since optimal nitric oxide/ROS balance in the brain seems to be an important parameter in the prevention of hypertension, concentrations of both thiols and CD – marker of membrane oxidative damage – were determined. While thiol levels in the brain cortex were affected neither after the L-NAME nor after PTX treatment, concentration of CD was increased after the L-NAME treatment significantly. The same tendency was observed in PTX-pretreated rats. The brain is especially susceptible to the oxidative damage because it is not particularly endowed with an antioxidant defense. It has only low catalase activity and moderate levels of the antioxidant enzymes like superoxide dismutase and

glutathione peroxidase. The high levels of iron and ascorbate in the brain may participate significantly at the catalysis of lipid peroxidation (for review see Lau *et al.* 2005). Since L-NAME administration may lead to down-regulation of superoxide dismutase (Husain, 2003) and attenuation of glutathione peroxidase activity in the brain (Barthwal *et al.* 2000), it is quite plausible that decreased antioxidant defense is accounted for increased CD concentration in the brain cortex. Similarly, increased CD concentration and up-regulation of redox sensitive factor NF- $\kappa$ B were documented in different brain regions after long-term L-NAME treatment (Pechánová *et al.* 2006).

In conclusion, the presence of functional sympathetic nervous system is critical for the full development of NO-deficient hypertension and its

decisive role cannot be replaced by enhanced activity of renin-angiotensin system. Further studies should be focused on the moderate BP difference persisting in NO-deficient rats treated with either pertussis toxin or captopril when compared to similarly treated controls.

### Conflict of Interest

There is no conflict of interest.

### Acknowledgements

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