ELSEVIER

European Journal of Pharmacology 275 (1995) R1–R3

ejp

Rapid communcation

Increase in serum N^G-hydroxy-L-arginine in rats treated with bacterial lipopolysaccharide

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Received 18 January 1995; accepted 20 January 1995

Abstract

Aortic rings isolated from rats 4 h after an injection i.p. of 30 mg/kg *Escherichia coli* lipopolysaccharide showed a marked hyporeactivity to noradrenaline. This effect was paralleled by an increase in the level of nitrite/nitrate in the serum of lipopolysaccharide-treated rats, indicative of an enhanced nitric oxide (NO) synthase activity. Most important, however, the serum concentration of the NO synthase intermediate, N^G-hydroxy-L-arginine, was also markedly elevated from 3.7 to 15.8 μM. Circulating N^G-hydroxy-L-arginine may thus represent a sensitive and specific marker of NO synthase activity in vivo.

Keywords: Bacterial lipopolysaccharide; Nitric oxide synthase, inducible; N^G-Hydroxy-L-arginine

In addition to nitric oxide (NO) and L-citrulline the stable intermediate, N^G-hydroxy-L-arginine (Stuehr et al., 1991), can be liberated from the active site of NO synthase upon reaction with L-arginine (Klatt et al., 1993). Moreover, cells expressing the inducible Ca^{2+}-independent isoform of NO synthase, such as EMT-6 mammary adenocarcinoma cells stimulated with bacterial lipopolysaccharide/interferon-γ (Chénais et al., 1993), lipopolysaccharide-stimulated RAW 264.7 macrophages or interleukin-1β-stimulated vascular smooth muscle cells (Hecker et al., unpublished observations), release substantial amounts of N^G-hydroxy-L-arginine into the extracellular space. Since N^G-hydroxy-L-arginine appears to compete with L-arginine for the same amino acid carrier (system y^+; Schott et al., 1994), and because NO synthase exhibits a 2-fold higher K_m value for N^G-hydroxy-L-arginine than L-arginine (Klatt et al., 1993; Stuehr et al., 1991), N^G-hydroxy-L-arginine re-uptake and metabolism by these cells may only be facilitated when both the extracellular and intracellular concentrations of L-arginine fall below a critical threshold. We have hypothesized therefore that, as a consequence, N^G-hydroxy-L-arginine released from vascular cells exposed to bacterial lipopolysaccharide or cytokines in vivo may circulate in the blood.

Male Wistar rats (300–350 g) were injected i.p. with either saline or 30 mg/kg *Escherichia coli* lipopolysaccharide (serotype 055:B5). After 4 h, the rats were killed by decapitation and exsanguinated. The blood was centrifuged and the serum was extracted with methanol (4:1, v/v) followed by high-performance liquid chromatography (HPLC) analysis with pre-column *o*-phthalaldehyde derivatization as previously described (Hecker et al., 1990). The column (250 × 4.6 mm (i.d.) UltraTechsphere 5-ODS, HPLC Technology) was isocratically eluted with 10 mM KH_2PO_4, pH 5.85/acetonitrile/methanol/tetrahydro-furan 79 : 10 : 10 : 1 (v/v/v/v) at a flow rate of 1 ml/min. L-Arginine, L-citrulline and N^G-hydroxy-L-arginine were eluted from the column with retention times of 20.5, 14.1 and 18.5 min respectively.

When compared to serum of control rats, there was a 35% decrease in the concentration of L-arginine in the serum of lipopolysaccharide-treated rats which was accompanied by a marked rise ($\geq$ 4-fold) in the level of N^G-hydroxy-L-arginine (Fig. 1a). The concentration of L-citrulline also increased by 11%, but this effect was too small to reach statistical significance. Since there is

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SSDI 0014-2999(95)00046-1

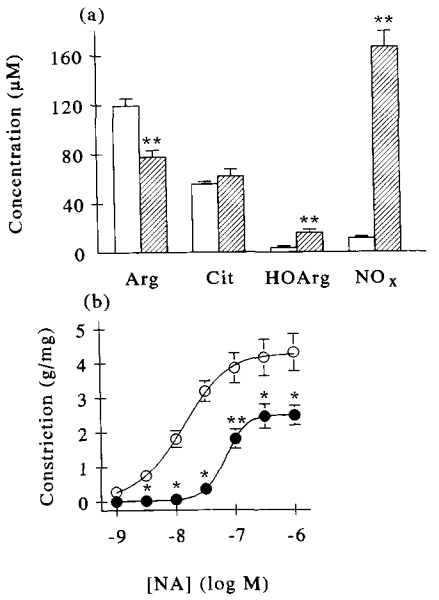

Fig. 1. (a) Concentration of L-arginine (Arg), L-citrulline (Cit), N^G-hydroxy-L-arginine (HOArg) and nitrite/nitrate (NO_x) in the serum of lipopolysaccharide-treated rats (hatched columns) and control rats (open columns; means ± S.E.M., $n = 6$, $^{**}P < 0.01$ vs. control by two-sided Student's t-test for unpaired data). (b) Concentration-dependent constriction (expressed in g/mg dry weight) induced by noradrenaline (NA) in endothelium-denuded aortic rings (4 mm width) from control rats (○, $n = 3$) and lipopolysaccharide-treated rats (●, $n = 6$). The rings were suspended in organ chambers filled with 10 ml oxygenated Krebs-Henseleit solution under a resting tension of 2 g. The experiments were performed 4 h after removal of the aortae from the animals ($^{*}P < 0.05$, $^{**}P < 0.01$).

no other known source of N^G-hydroxy-L-arginine in the body, these findings point to an enhanced expression of NO synthase by the lipopolysaccharide-treated rats, which may have occurred preferentially at the level of the vascular smooth muscle cells. This notion was supported by the finding that the vasoconstrictor effect of noradrenaline in aortic rings from lipopolysaccharide-treated rats was strongly impaired (Fig. 1b). As reported previously (Julou-Schaeffer et al., 1990), this hyporeactivity could be reversed by the addition of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (1 mM), to the organ bath (not shown). Moreover, the enhanced systemic NO synthase activity following lipopolysaccharide treatment was also reflected by a ≥ 14-fold rise in the serum concentration of nitrite/nitrate (Fig. 1a) which was determined essentially as previously described (Green et al., 1982). Interestingly, the serum level of L-citrulline was only marginally elevated in lipopolysaccharide-treated rats, presumably due to the recycling of L-citrulline to L-arginine by the NO-producing cells (Hecker et al., 1990).

The increase in the concentration of N^G-hydroxy-L-arginine in the serum of lipopolysaccharide-treated rats may have additional implications relating to the inhibition by N^G-hydroxy-L-arginine of arginase (Boucher et al., 1994) and, as a consequence, the synthesis of polyamines as well as the metabolism of N^G-hydroxy-L-arginine to NO and L-citrulline by enzymes other than NO synthase, such as cytochrome $P450$ monooxygenases (Schott et al., 1994). Most important, however, the fact that N^G-hydroxy-L-arginine can be readily detected in the blood of control rats and the extent to which these levels were elevated during endotoxaemia suggest that the serum level of N^G-hydroxy-L-arginine may represent a useful diagnostic parameter for the monitoring of patients with septic shock. In this context, it should be mentioned that simply monitoring the plasma levels of nitrite/nitrate is much less reliable due to the fact that the majority of these nitrogen oxides may be derived from sources other than NO synthase. Preliminary HPLC analyses indicate that the basal level of N^G-hydroxy-L-arginine in the serum of healthy volunteers is indeed comparable to that of the serum of control rats used in this study, but may rise in patients with endotoxaemia.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (He 1587/5-1) and the Commission of the European Communities (BMH1-CT92-1893). We are indebted to Drs. U. Albus (Hoechst AG, Frankfurt/M.) and I. Wessler (Department of Pharmacology, University of Mainz) for kindly providing the HPLC equipment, to Drs. P. Mordvintcev and A. Mülsch for setting up the nitrite/nitrate determination, and to Gabriele Hoch and Annette Kirsch for expert technical assistance.

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