JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1997, 48, 4, 731—735

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THE ROLE OF NITRIC OXIDE IN REGULATION OF DEFORMABILITY OF RED BLOOD CELLS IN ACUTE PHASE OF ENDOTOXAEMIA IN RATS

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Using the shear stress laser diffractometer (Rheodyn) we have studied the role of nitric oxide on erythrocyte deformability during the initial 10 min after the i.v. administration of LPS at a dose of 5 mg/kg. At the stress shear force of 30 Pa the control erythrocytes elongation index (Ei) of untreated animals was $38\% \pm 1.5$ (mean $\pm$ SD, $n = 6$) while in LPS treated animals it was decreased to $33\% \pm 1.8$ ($n = 6$) indicating significant ($p < 0.01$) loos of red blood cell deformability. The loss of deformability was accompanied by increased fragility of erythrocyte membranes as measured by enhanced release of free hemoglobin ($E_{\lambda 420} = 0.43 \pm 0.05$ in control vs. $E_{\lambda 420} = 0.65 \pm 0.07$ in LPS group) from isolated erythrocytes exposed to centrifuging at a speed of 3000 rpm for 10 min. Inhibitor of NO-synthase, N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg i.v.), significantly decreased deformability ($Ei = 33.5-\pm 4.6$, $n = 6$, $p < 0.01$) but did not influence fragility ($E_{\lambda 420} = 0.36 \pm 0.14$, $n = 6$) of erythrocytes. However, when L-NAME was administered 10 min. prior to LPS it significantly improved the LPS-impaired fragility ($E_{\lambda 420} = 0.38 \pm 0.1$, $n = 6$, $p < 0.01$) as compared to rats treated with LPS-alone ($E_{\lambda 420} = 0.65 \pm 0.07$, $n = 6$). A similar protective effect of L-NAME was observed for LPS-induced impairment of erythrocyte deformability. It is concluded that NO seems to influence deformability and fragility of erythrocytes at the first stage of sepsis. During an acute phase of LPS action, possibly reflected by stimulation of endothelial constitutive (ecNOS) but not inducible NO-synthase (iNOS), the excessive amount of NO leads to a damage of erythrocyte plasticity and then the pretreatment with L-NAME exerts a protective action on LPS-impaired deformability and fragility of erythrocytes. On the other hand, basal release of NO maintains erythrocyte deformability at the physiological range and lowering of the basal level of NO by NOS-inhibitors leads to impairment or erythrocyte deformability.

Key words: *red blood cell deformability, septic shock, lipopolysaccharide (LPS), erythrocyte fragility, nitric oxide, nitric oxide synthase inhibitors.*

INTRODUCTION

We have found that the late phase (6 hours) of LPS-evoked lethal arterial hypotension is associated with decline in red blood cell deformability which can be reversed by nitric oxide (NO) synthase inhibitors (1). These alterations

are hypothesized to be not a direct effect of endotoxin, but plausibly require the presence and participation of white blood cells and/or their mediators. Our previous *in vitro* studies (2) showed that in non-septicaemic red blood cells, the deformability was modulated mainly by prostacyclin and by polymorphonuclear leukocytes *via* the release of nitric oxide. While the contribution of NO from inducible form of NO-synthase to the symptoms of endotoxaemia is generally accepted at the late stage of septic shock (3), the first stage of sepsis, starting with an initial fall in blood pressure that occurs minutes after the administration of LPS is of an unknown origin and it is unclear whether it may be associated with changes in red blood cell deformability.

Here, using the shear stress laser diffractometer (Rheodyn) we have studied the role of nitric oxide on erythrocyte deformability during the initial 10 min after the intravenous administration of LPS at a dose of 5 mg/kg.

MATERIALS AND METHODS

Experimental procedure

Male Wistar rats (250—350 g) were anaesthetized with pentobarbital (90 mg/kg i.p.) and heparinized (800 U/kg i.v.). N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) was injected into *femoral vein* 10 min before the administration of vehicle (saline) or *E. coli endotoxin* (LPS, 5 mg/kg, serotype 0127: B8, Sigma). Blood samples (1.5 ml) were taken from *carotid artery* 10 min after administration of LPS or vehicle.

Red blood cell deformability

Erythrocyte deformability was measured using the shear stress laser diffractometer (Rheodyn SSD). Such instrument measures ellipsoidal elongation of red blood cells in response to defined shear stresses, determinated by the rotation rate (4, 5). The samples of blood (45 µl) were suspended in 3 ml of dextran solution (MW 60 000, osmolarity 300 mOsm, pH = 7.4, viscosity 24 mPA). Red blood cell elongation index (%) was defined as $E_i = 100\ (L\text{-}W)/(L+W)$, where L and W where the means of length and width of elongated red blood cells, respectively, which had been electronically calculated from the diode output of the sensor head of the instrument.

Red blood cell fragility

The blood samples were centrifuged at 1400 rpm for 10 min. The supernatant and buffy coat were discarded. Erythrocyte suspension (0.5 ml) was taken carefully from each sample, resuspended in Tris buffer (1 ml) and then centrifuged at a speed of 3000 rpm for 10 min. The concentration of free haemoglobin in supernatant was measured spectrophotometrically ($\lambda = 420$ nm) and expressed in extinction units.

Statistical analysis

The results were expressed as means $\pm$ SD of n experiments, and analyzed by *Student's* test to determine the nature of the response. A p value of les than 0.05 was considered statistically significant.

RESULTS

At the stress shear force of 30 Pa the control erythrocytes elongation index (Ei) of untreated animals was 38% ± 1.5 (mean ± SD, n = 6) while in LPS animals it was decreased to 33% ± 1.8 (n = 6) indicating significant ($p < 0.01$) loss of red blood cell deformability (*Fig. 1*). The loss of deformability was accompanied by increased fragility of erythrocyte membranes as measured by enhanced release of free hemoglobin ($E_{\lambda 420} = 0.43 \pm 0.05$ in control vs. $E_{\lambda 420} = 0.65 \pm 0.07$ in LPS group) from isolated erythrocytes exposed to centrifuging at a speed of 3000 rpm for 10 min (Fig. 2). Inhibitor of NO-synthase, N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg i.v.), significantly decreased deformability (Ei = 33.5% ± 4.6, n = 6, $p < 0.01$) but did not influence fragility ($E_{\lambda 420} = 0.36 \pm 0.14$, n = 6) of erythrocytes (*Fig. 1 and Fig. 2*). However, when L-NAME was administered 10 min. prior to LPS it significantly improved the LPS-impaired fragility ($E_{\lambda 420} = 0.38 \pm 0.1$, n = 6, $p < 0.01$) as compared to rats treated with LPS-alone ($E_{\lambda 420} = 0.65 \pm 0.07$, n = 6) (*Fig. 2*). A similar protective effect of L-NAME was observed for LPS-induced impairment of erythrocyte deformability.

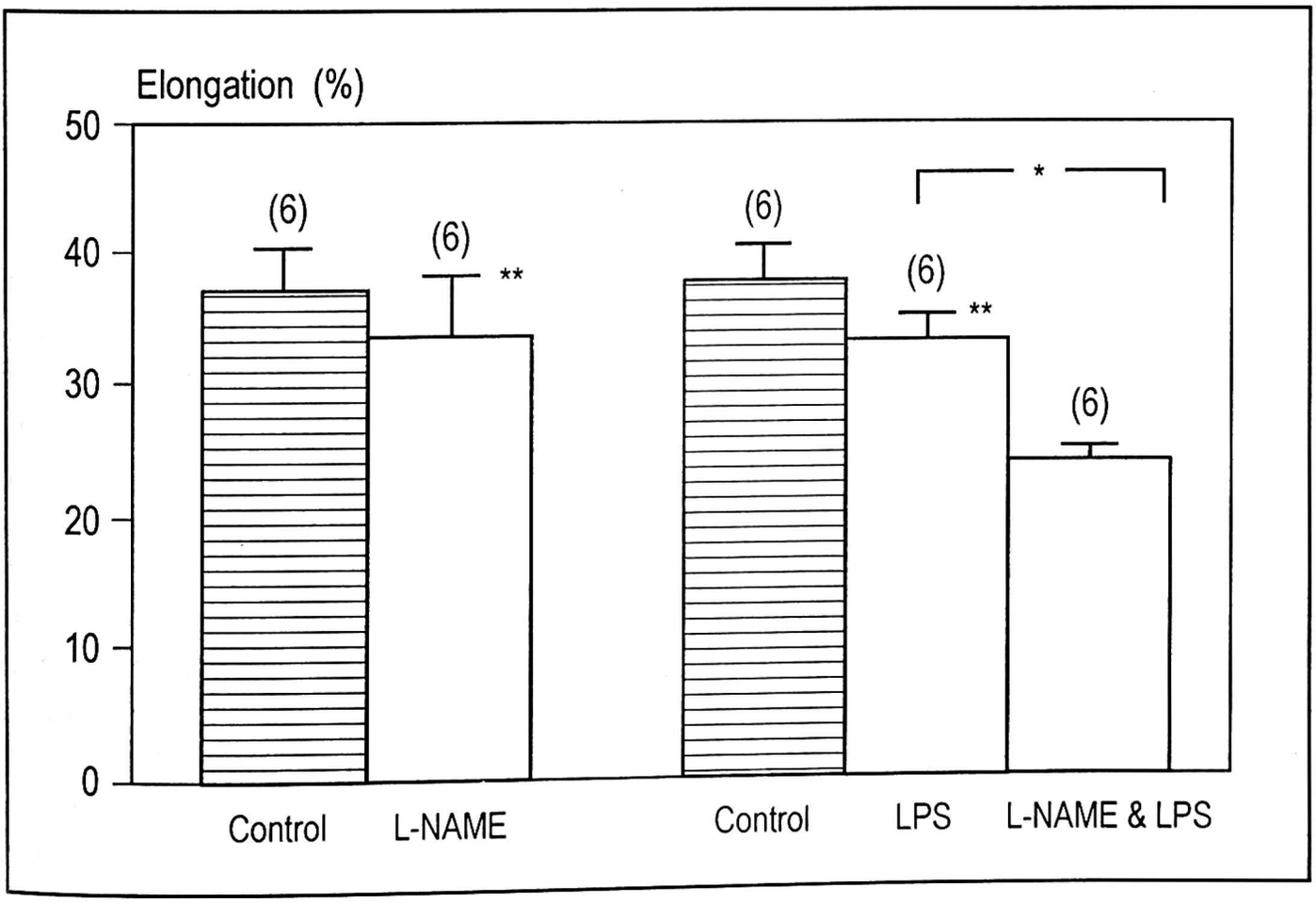

Fig. 1. The effect of L-NAME (10 mg/kg i.v.) on LPS-induced (5 mg/kg i.v.) impairment of red blood cell deformability (* $p < 0.05$, ** $p < 0.01$ as compared to control or as indicated, number of experiments in brackets)

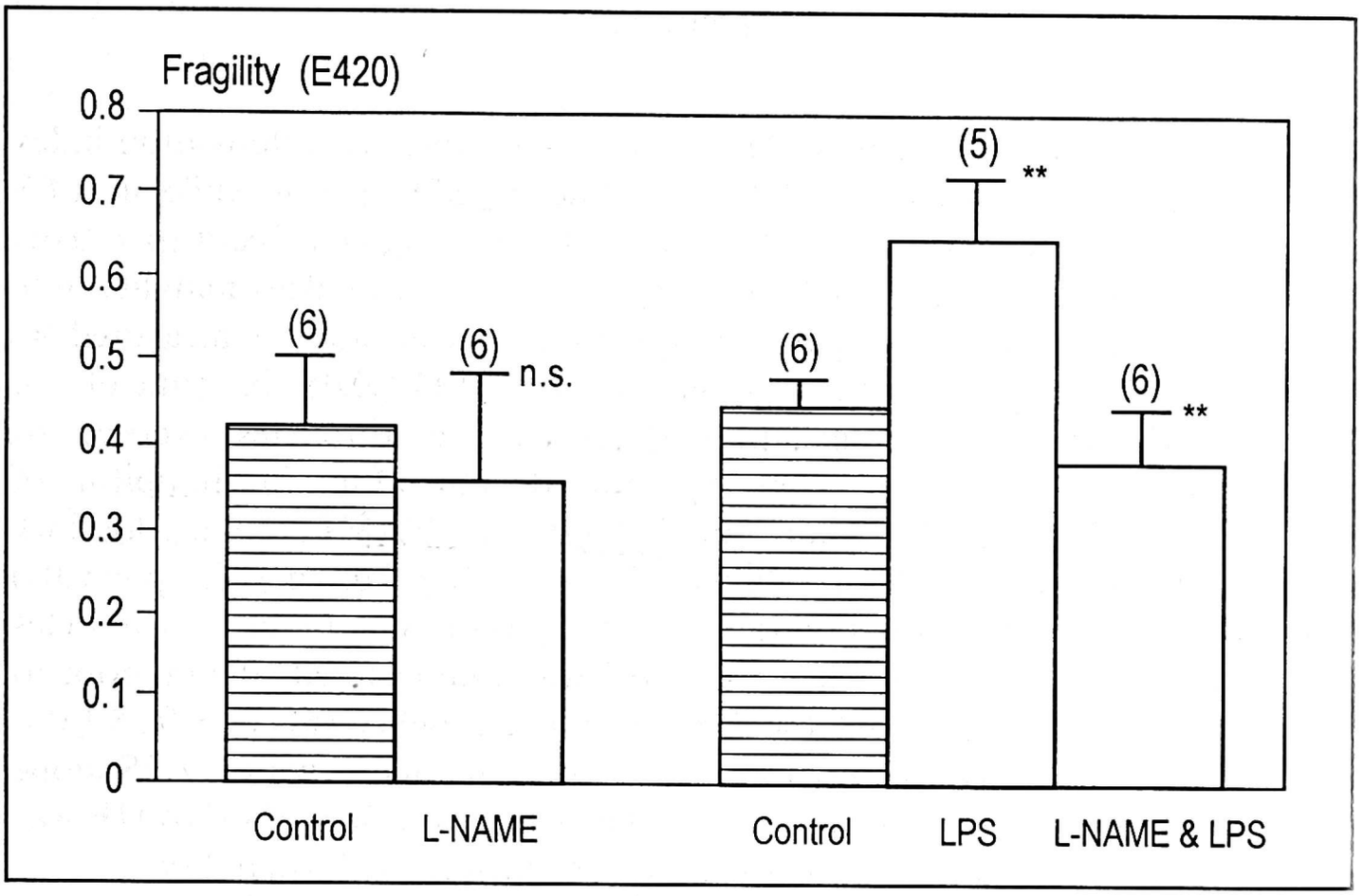

Fig. 2. The effect of L-NAME (10 mg/kg i.v.) on LPS-induced (5 mg/kg i.v.) impairment of red blood cell fragility (** $p < 0.01$ as compared to control, number of experiments in brackets).

DISCUSSION

Failure of red cell membranes to maintain the capacity of erythrocyte to traverse the microcirculation has been amply demonstrated both in clinical and experimental sepsis indicating that sepsis-associated changes in plasticity of erythrocytes may play a role in the pathophysiology of multiogran system failure (6). However, the mechanisms responsible for LPS-induced changes in red blood cell deformability remain a matter of considerable debate. So far, it has been accepted that the sepsis-evoked changes in the erythrocyte membrane are secondary to neutrophil-derived, oxidant-induced membrane damage (3). Recently, it has been shown that the effect of LPS on erythrocyte deformability depends on the exposure of lipid A of LPS to binding to erythrocyte membrane (7). Some studies, including ours (1, 8) attempted to modify protracted phase of endotoxin-induced alterations in red blood cell deformability with such agents as pentoxifylline, free radical scavengers, prostacyclin analogue, thromboxane A_2-synthase inhibitor, as well as an inhibitor of NO-synthase, all of which known to modulate the activation of leukocytes and their products.

Here, we have observed that NO influences deformability and fragility of erythrocytes also at the first stage of sepsis. Judging from the effects of

L-NAME, a non-selective NOS inhibitor, basal release of NO maintains erythrocyte deformability at the physiological range. Lowering of basal levels of NO leads to impairment of erythrocyte deformability. However, during an acute phase of LPS action, possibly reflected by stimulated of ecNOS, the excessive amount of NO leads to a damage of erythrocyte plasticity and then the pretreatment with L-NAME exerts a protective action on LPS-impaired deformability and fragility of erythrocytes.

The above results promt us to our previous hypothesis (1) that the mechanism of erythrocyte protection by NOS inhibitors, both in acute and protracted phase of sepsis, may be a complex, indirect phenomenon, related to the specific effects of NO on biochemical function of all blood elements. Such statement, however, still remains rather speculative and thus the role of NO in regulation of red blood cell deformability requires further experimental investigations.

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R e e i v e d: July 3, 1997
A c c e p t e d: September 9, 1997

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