MECHANISMS OF LPS-INDUCED CHANGES IN VASCULAR REACTIVITY TO ENDOTHELIN-1 IN RAT AORTIC AND PULMONARY VESSELS

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Lipopolysaccharide (LPS) is a powerful stimulator of inflammatory pathways and acts as a key molecule involved in the initiation of sepsis syndrome. Exposure to LPS is associated with changes in vascular reactivity and elevated plasma levels of both endothelin-1 (ET-1) and nitric oxide (NO), but the relationship between these factors and changed vascular reactivity remains unclear. The aim of this study is to examine the molecular mechanisms involved in LPS-induced changes in vascular reactivity to ET-1 in isolated rat vessels.

Male Wistar rats weighing 250-300 g were humanely killed by cervical dislocation and the descending thoracic aorta and the side branches of the pulmonary artery were separated. Aortic and pulmonary rings were incubated for 20h in culture medium (DMEM+10% FBS) either alone or supplemented with LPS (*E. coli* O55:B5, 10 $\mu g.ml^{-1}$). Vascular reactivity to ET-1 (0.3 to 100 nM) was measured in the absence and presence of L-NAME (100 μM) and also in arteries with intact or denuded endothelium. The effect of the calcium channel blocker nifedipine was examined after obtaining a maximal response to ET-1. Quantitative RT-PCR was performed to measure changes in the levels of expression of mRNA of ET-1, ET_A, ET_B, eNOS and iNOS. Non-linear regression analysis (4-parameter fit) was carried out using Graphpad Prism and significant differences between treatment groups determined with paired t-tests or ANOVA with Dunnett's test as appropriate.

Incubation with LPS significantly decreased rat aortic responses to ET-1 (table 1), while not significantly affecting the pulmonary artery. Inhibition of NOS or removal of endothelium resulted in increased contractile responses to ET-1 in both vessels, but the significant difference between medium- and LPS-treated aortic vessels was maintained.

Table 1: pEC_{50} for ET-1 (mean ± s.e.mean).

Treatment	Pulmonary artery		Aorta	
	Medium	LPS	Medium	LPS
Control (n=23)	8.61±0.04	8.51±0.04	8.05±0.05	7.87±0.03**
L-NAME (n=6)	9.03±0.02**	$8.95\pm0.02^{\#\#,\dagger\dagger}$	8.57±0.08**	$8.17\pm0.05^{\#\#,\dagger\dagger}$
Denuded (n=6)	8.98±0.04**	$8.97\pm0.05^{\#\#}$	8.39±0.04**	$8.08\pm0.04^{\#\#,\S\S}$

**P<0.01, significantly different compared with Medium - Control group; $^{\#\#}$P<0.01, compared with LPS - Control group; ††P<0.01, compared with Medium -L-NAME group; §§P<0.01, compared with Medium - Denuded aortic group.

LPS increased the relaxation response to nifedipine (10 μM) in the aorta (29.6±6.8 compared with 8.7±2.6 % for control, n=9, p<0.05) while not significantly affecting the pulmonary responses (67.7±3.7 compared with 56.9±5.2 % for control, n=9). LPS induced iNOS gene expression by a similar amount in the aorta and the pulmonary artery, while not significantly affecting ET-1, ET_A, ET_B or eNOS mRNA levels between 4 and 20 h in either vessel.

In conclusion, LPS-induced changes in vascular reactivity to ET-1 differ between different vascular beds. These changes are unlikely to be dependent on the presence of endothelium or changes in the gene expression of ET or NO systems but may be related to changes in downstream signaling such as calcium homeostasis.