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Hemorrhagic Shock-Induced Vascular Hyporeactivity in the Rat: Relationship to Gene Expression of Nitric Oxide Synthase, Endothelin-1, and Select Cytokines in Corresponding Organs

Liang-ming Liu, M.D.,*,† and Michael A. Dubick, Ph.D.*,[1]

*U.S. Army Institute of Surgical Research, San Antonio, Texas, USA; and †Research Institute of Surgery, Daping Hospital, The Third Military Medical University, Daping, Chongqing, P.R. China

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Background. **Our previous work observed that vascular hyporeactivity to norepinephrine (NE) developed after hemorrhage and the response was not the same in the 4 arteries examined. To evaluate possible mechanisms involved, the present study investigated the gene expression of iNOS, eNOS, IL-1β, IL-6, TNF-α, and endothelin-1 in the corresponding organs, and the roles of nitric oxide (NO) and endothelin (ET).**

Materials and methods. **LAnesthetized rats (n = 7/time point/group) were hemorrhaged to a mean arterial pressure of 50 mmHg for 60 min. The vascular reactivity of the superior mesenteric (SMA), celiac (CA), left renal (LRA), and left femoral arteries (LFA) to NE was measured at baseline, at the end of the hypotensive period (E), and at 1, 2, and 4 h later in the three groups (hemorrhage, hemorrhage+NG-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, or hemorrhage+PD142893, an ET receptor antagonist). Gene expression in ileum, left kidney, liver, and skeletal muscle was determined by quantitative RT-PCR at these times.**

Results. **Vascular reactivity of SMA, CA, LRA, and LFA to NE decreased as much as 98% over 4 h compared with baseline. This loss of responsiveness in CA and LFA was more severe than in SMA and LRA. Gene expression of iNOS, eNOS, IL-1β, IL-6, TNF-α, and endothelin-1 in the corresponding organs of select vasculatures increased markedly over baseline levels and the fold increase in mRNA levels of these enzymes and mediators in liver and skeletal muscle was higher than in ileum and left kidney. For example, at 4 h, iNOS expression was over 16-fold higher than baseline in liver and skeletal muscle, but 5- and 7-fold higher in ileum and kidney, respectively. L-NAME or PD142893 partially attenuated the decreased vascular reactivity induced by hemorrhagic shock and attenuated the changes in gene expression observed.**

Conclusion. **These findings suggest that the differential expression of NOS, cytokines, and endothelin-1 in different organs are associated with the development of vascular hyporeactivity after hemorrhagic shock and may account, at least in part, for the vascular bed diversity observed.**

Key Words: **hemorrhage; vascular reactivity; cytokines; nitric oxide synthase; endothelin.**

INTRODUCTION

Studies have shown that the vascular reactivity to vasoconstrictors and vasodilators can be reduced greatly after severe trauma or shock [1–7]. Many factors, including desensitized adrenoceptors [1, 8], nitric oxide (NO) [3, 7, 9–13], endogenous opioid peptides [4, 14], inflammatory cytokines such as TNFα [15, 16] and IL-1 [17] have been proposed to be involved in the development of vascular hyporeactivity during shock. This vascular hyporeactivity may also play an important role in the development and the outcome of the shock state, and can interfere with the therapy of shock by reducing the effectiveness of vasoactive agents [1]. It has been suggested that the low- or non-response of many patients to some vasoactive agents in the late stage of critical disease may be related to vascular hyporeactivity [1]. Consequently, it is very important to elucidate the mechanism responsible for this vascular hyporeactivity and the role of modulating factors.

[1] To whom correspondence and reprint requests should be addressed at U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers Ave., Fort Sam, Houston, TX 78234-6315. E-mail: Michael.Dubick@amedd.army.mil.

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TABLE 1

Aspects of the RT-PCR Reaction

Primer Pairs for the Selected Genes

	Sense	Antisense	Product length (bp)
G3PDH	5′-TCCTGCACCACCAACTGCTTAG-3′	5′-TGCTTCACCACCTTCTTGATGTC-3′	341
iNOS	5′-TAGAAACAACAGGAACCTACCA-3′	5′-ACAGGGGTGATGCTCCCGGACA-3′	907
eNOS	5′-CTGCTGCCCGAGATATCTTC-3′	5′-CAGGTACTGCAGTCCCTCCT-3′	228
ET-1	5′-TCTTCTCTCTGCTGTTTGTGGCTT-3′	5′-TCTTTTACGGCTTTCTGCATGGAT-3′	407
IL-1β	5′-GAAGCTGTGGCAGCTACCTATGTCT-3′	5′-CTCTGTTGAGAGGTGCTGATGTAC-3′	520
IL-6	5′-GATGTTGTTGACAGCCACTGC-3′	5′-CACTCCTTCTGTGACTCTAAC-3′	501
TNF-α	5′-TACTGAACTTCGGGGTGATTGTCC-3′	5′-CGTAGGACCCGATGTGACTC-3′	295

PCR condition

	GAPDH	iNOS	eNOS	ET-1	IL-1β	IL-6	TNF-α
Annealing temperature (°C)	57	58	58	55	56	56	57
Elongation temperature (°C)	72	72	72	72	72	74	72
Elongation time (s)	14	30	10	16	20	20	12

Our previous work has shown that the degree of vascular hyporeactivity after hemorrhagic shock was not the same in the 4 vascular beds examined. The loss of vascular reactivity to NE in the celiac (CA) and left femoral arteries (LFA) was more severe than in the superior mesenteric artery (SMA) and left renal artery (LRA), and NO and ET-1 inhibition improved the response to NE [3]. However, the mechanism(s) involved remain to be elucidated. The present study tested the hypotheses that the vascular bed diversity in vascular hyporeactivity to NE induced by hemorrhagic shock was associated with differential gene expression of iNOS, eNOS, IL-1β, IL-6, TNF-α, and ET-1 in the corresponding organs, and that NO or ET inhibition would improve vascular reactivity by down-regulating the gene expression of these factors.

TABLE 2

Changes in Vascular Reactivity in Superior Mesenteric Artery (SMA), Left Renal Artery (LRA), Celiac Artery (CA) and Left Femoral Artery (LFA) to Norepinephrine (NE, 3 μg/kg, iv) following Hemorrhagic Shock and the Effect of L-NAME and PD142893

	Baseline	End of hemorrhage	Post-shock 1h	Post-shock 2h	Post-shock 4h
SMA					
Hemorrhage	100	59.1 ± 12.5	44.2 ± 16.1	30.1 ± 13.5	13.9 ± 14.7
L-NAME group	100	82.5 ± 8.1	89.8 ± 9.3	72.3 ± 8.4	64.4 ± 13.1
PD142893 group	100	70.3 ± 20.8	80.3 ± 14.1	79.3 ± 16.8	65.5 ± 11.3
LRA					
Hemorrhage	100	56.7 ± 7.59	35.1 ± 9.28	16.6 ± 5.89	8.60 ± 5.84
L-NAME group	100	83.4 ± 6.18	88.5 ± 8.94	71.9 ± 17.8	69.4 ± 12.8
PD142893 group	100	76.6 ± 6.03	80.5 ± 12.0	76.1 ± 9.54	72.9 ± 16.6
CA					
Hemorrhage	100	43.2 ± 12.6†‡	31.6 ± 13.7	12.0 ± 8.05	2.98 ± 13.47
L-NAME group	100	70.1 ± 25.2	71.4 ± 21.2	58.7 ± 12.6	42.9 ± 6.64†‡
PD142893 group	100	67.9 ± 5.63‡	68.7 ± 7.79‡	70.8 ± 9.21	57.1 ± 18.5
LFA					
Hemorrhage	100	30.1 ± 11.9†‡	23.4 ± 7.26†	10.8 ± 9.03†	1.42 ± 3.47†‡
L-NAME group	100	67.4 ± 7.16†‡	60.4 ± 12.0†‡	51.3 ± 7.09†‡	38.4 ± 14.1†‡
PD142893 group	100	68.3 ± 6.19‡	62.5 ± 13.5†‡	68.3 ± 4.77*	54.0 ± 11.2*‡

Note. The vascular reactivity to NE at baseline was taken as the 100% response. n = 7 at each timepoint. * $P < 0.05$, as compared to L-NAME group; † $P < 0.05$, as compared to SMA; ‡ $P < 0.05$, as compared to LRA. All values in hemorrhage group were significantly less than baseline ($P < 0.05$). Except for the end of hemorrhage values in the PD142893 group for the SMA, all values in the L-NAME and PD142893 groups are significantly greater than corresponding values in the hemorrhage group ($P < 0.05$).