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EXPRESSION OF DIFFERENT PDH KINASE ISOENZYMES IN SKELETAL MUSCLE DURING SEPSIS. T. C. Vary, and G. Deiter*, Dept. Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA. 17033 USA

Hyperlactatemia is a frequent complication of sepsis. Increased lactate production by skeletal muscle elevates plasma lactate concentrations. Inhibition of the PDH complex contributes to the accelerated release of lactate from skeletal muscle. The inhibition of PDH activity resides in a stimulation of PDH kinase (PDHK) during sepsis. Recent evidence indicates the existence of multiple isoenzymes of PDK. In skeletal muscle, the PDK2 and PDK4 isoenzymes predominate. We investigated whether sepsis would have specific effects on the expression of PDK isoenzymes mRNA in skeletal muscle by Northern blot analysis. We also examined the time course of changes in the expression of the isozymes following induction of sepsis. PDK2 and PDK4 Northern blots were normalized by GPDH mRNA. The relative amount of PDK2 and PDK4 mRNA are shown in the Table below:

	3 DAYS		5 DAYS	
	Control	Sepsis	Control	Sepsis
PDK2	98±2	113±21	88±5	**171±24***
PDK4	12±4	**50±9***	60±10	45±12

Values shown are arbitrary units of means ±SE for 3-7 animals in each group. *P<0.05 vs Control at same time.

The results indicate that isoenzymes of PDK are differentially expressed during the course of the septic episode. (Supported by GM-50919)

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CARDIAC RESPONSE TO NITRIC OXIDE SYNTHASE INHIBITION USING AMINOGUANIDINE IN A RAT MODEL OF ENDOTOXEMIA.
L. Vona-Davis*, P. Wearden*, L. Millecchia*, J. Hill*, G. Timberlake and R. Hill*, Dept. of Surgery, West Virginia University and NIOSH, Morgantown, WV 26506.

This study evaluates the effect of aminoguanidine, a preferential inhibitor of inducible nitric oxide synthase (iNOS), on the prevention of cardiac depression in acute endotoxemia. Cardiac performance was evaluated after 4 hr of exposure to endotoxin. Animals were randomly selected to receive by i.p. injection one of 4 treatments (n=5 per treatment); Saline, LPS (lipopolysaccharide, E.Coli, 4mg/kg), AG (aminoguanidine 100 mg/kg), and LPS + AG at various times. AG and saline treatments were administered 30 min before LPS and at 1 and 3 hr after LPS injection. Hearts were perfused using the Langendorff preparation and a balloon tipped catheter was placed in the left ventricle to measure left ventricular developed pressure (LVDP). Myocyte contractile function was assessed with electrical field stimulation and video microscopy. Tissue was immunostained for the expression of iNOS and for nitrotyrosine, a byproduct of protein nitration by peroxynitrite. Perfused hearts from LPS-treated rats exhibited a 57% decrease (P=.0001) in LVDP compared to saline-treated animals. No improvement in ventricular function was observed with the administration of AG. Similarly, cardiac myocytes prepared from LPS-treated animals demonstrated a significant (P<.05) reduction in percent and velocity of shortening and this effect was unaltered with the same dose of AG. Aminoguanidine administration significantly (P=.0003) reduced serum nitrite/nitrate levels in endotoxemic rats to control levels. Localized expression of iNOS in the myocardium was lessened with AG treatment and was not associated with peroxynitrite formation in this model of endotoxemia.The results indicate that AG given i.p. before and after endotoxin (at a concentration sufficient to decrease nitric oxide production) did not reduce cardiac depression. We conclude that selective inhibition of iNOS and reduction of nitric oxide production do not prevent cardiac dysfunction in an acute model of endotoxic shock.

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HIGH MOBILITY GROUP-1 (HMG-1) PROTEIN IS A MEDIATOR OF LETHAL ENDOTOXEMIA.
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Lethal endotoxemia stimulates the release of macrophage-derived cytokines (e.g. TNF-α and IL-1β) that mediate shock. Death, however, frequently occurs several days later when serum TNF-α and IL-1β levels have returned to basal values. To discover previously unrecognized macrophage-derived mediators of endotoxin toxicity released late in endotoxemia, conditioned medium of LPS-stimulated macrophage (RAW 264.7) cell cultures was screened by SDS-PAGE for proteins appearing 8 hours or more after LPS stimulation. An LPS-induced 30 kDa protein was identified by N-terminal amino acid sequence as HMG-1 protein. HMG-1 was released from murine macrophage and human peripheral blood mononuclear cell cultures following stimulation with LPS, TNF-α, or IL-1β. Although undetectable in serum of normal mice, HMG-1 levels increased significantly at least 8 hours after the onset of endotoxemia, and remained at plateau levels (up to 350 ng/ml) for 16-32 hours. Passive immunization of mice with anti-rHMG-1 antibodies decreased the lethality from 100% in control group (treated with LD_{100} dose of LPS and pre-immune serum), to less than 30% in experimental group (treated with LD_{100} dose LPS and anti-rHMG-1 serum). Co-administration of purified rHMG-1 protein synergistically increased LPS lethality from 0% in group treated with LPS alone (3.25 mg/kg), to more than 80% in group treated with LPS (3.25 mg/kg) plus rHMG-1 (50 μg/mouse). Serum HMG-1 levels in patients with lethal septicemia were significantly increased (83.7 ± 27.5 ng/ml) compared to either normal subjects (non-detectable) or patients with non-lethal sepsis (25.2 ± 17.8 ng/ml). Thus, HMG-1 is a previously unrecognized mediator of endotoxin lethality, which can be targeted as future therapeutics.

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ADRENAL INSUFFICIENCY DURING THE LATE STAGE OF POLYMICROBIAL SEPSIS. P. Wang, D.J. Koo*, J.H. Chaudry. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Although studies have indicated that adrenal insufficiency occurs following severe hemorrhagic shock, it remains controversial whether adrenal function is depressed during polymicrobial sepsis. To study this, male rats (~300g) were subjected to sepsis by cecal ligation and puncture (CLP) or sham operation followed by the administration of normal saline solution. Systemic blood samples were taken at 20 h after CLP or sham operation to measure plasma levels of corticosterone (ng/mL) and ACTH (pg/mL) as well as adrenal contents of corticosterone (ng/mg tissue). Additional groups of animals

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