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Nitric Oxide and Skeletal Muscle Reperfusion Injury: Current Controversies (Research Review)

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Nitric oxide (NO) has been implicated in a large number of disease processes, including ischemia-reperfusion injury following the restoration of oxygenated blood to previously ischemic muscle, which is a recognized significant complication of vascular surgery. Altered metabolism of NO is implicated in the endothelial dysfunction that forms part of the pathophysiology of ischemia-reperfusion injury. However, NO can demonstrate either protective or cytotoxic effects during reperfusion injury. The use of transgenic mice, either NO synthase (NOS) gene knockout animals, or animals that over-express NOS isoforms, along with direct NO measurements and NO donor or inhibitor studies, have all demonstrated a role for NO in skeletal muscle reperfusion injury. There appears to be an initial stimulation of NO production in the first 20-min of ischemia, with a gradual decline through early reperfusion and a second higher peak of NO commencing in the later stages of reperfusion. The absolute levels of NO in the reperfused tissue and its regulation by the subtle interplay with superoxide and the subsequent production of the highly toxic peroxynitrite anion, are important factors in determining whether NO, in the context of ischemia-reperfusion injury, has damaging or protective effects in the body.

Key Words: **ischemia; reperfusion; skeletal muscle; nitric oxide; endothelium; vascular surgery.**

INTRODUCTION

Ischemia-reperfusion injury (IRI) is defined as the paradoxical exacerbation of cellular dysfunction and death following the restoration of blood flow to previously ischemic tissues. Reestablishment of blood flow is essential to salvage ischemic tissues, however reperfusion itself paradoxically causes further damage to the ischemic tissue, threatening function and viability of the organ. IRI occurs in a wide range of organs including the heart, lung, brain, kidney, gut, and skeletal muscle. Skeletal muscle IRI can arise as a consequence of a range of vascular events including thrombolytic therapy, organ transplantation, limb trauma, and aortic cross-clamping during repair of abdominal aortic aneurysms. If the initial ischemia is profound and a sufficiently large volume of tissue is rendered ischemic, subsequent revascularization and hence reperfusion of the ischemic tissue will induce systemic effects on distant organs, leading to multi-system organ failure.

Following the reperfusion of ischemic muscle, both local and systemic injuries may evolve. Like other acute inflammatory responses, the molecular interactions that occur in reperfusion injury are complex and dependent upon multiple factors. These pathways have been extensively studied over the last 20 years and are now known to involve the formation of reactive oxygen species, lipid peroxidation, eicosanoid generation, neutrophil activation and infiltration, complement activation and cytokine generation, ultimately resulting in necrotic or apoptotic cell death in the affected tissues (Fig. 1). The pathophysiological events that underlie this injury have been extensively reviewed [1–4].

Since its discovery in 1987, nitric oxide (NO) has become the subject of intense research and debate. This molecule has been implicated in a variety of biological and pathophysiological processes that include the development of atherosclerotic lesions and aneurysm formation [5]. NO is also a major mediator of tissue damage during ischemia reperfusion injury. NO displays biphasic actions of cytoprotection and cytotoxicity, which makes elucidation of its exact role in bio-

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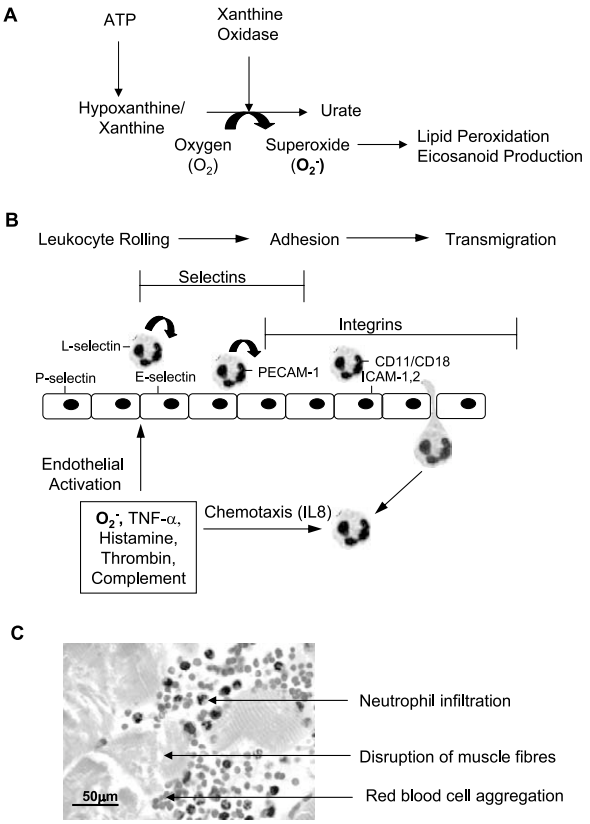

FIG. 1. Generation of superoxide anions following reperfusion of ischemic tissues initiates a cascade of events resulting in muscle damage. (A) During prolonged ischemia, ATP is broken down into hypoxanthine and xanthine. During subsequent reperfusion, an influx of fresh molecular oxygen allows xanthine oxidase-mediated breakdown of hypoxanthine and xanthine, producing uric acid and liberating the highly reactive superoxide anion (O_2^-), which then initiates lipid peroxidation, release of arachidonic acid and generation of eicosanoids. (B) Endothelial activation during reperfusion is initiated in part by O_2^-. Leukocyte rolling along the activated endothelium is then initiated by expression of P- and E-selectin on the endothelium and L-selectin constitutively expressed on neutrophils. Permanent adhesion of neutrophils to the endothelium is mediated by the interaction between leukocyte integrins and adhesion molecules, followed by transmigration through the endothelium under the action of chemotactic agents including interleukin 8. (C) Skeletal muscle from a rat subjected to 4 h hindlimb ischemia with a tourniquet, followed by 24 h reperfusion [66]. Sections were stained with hematoxylin and eosin and images captured with a Spot digital camera. Muscle fibers show considerable edema and disruption, along with neutrophil infiltration and RBC aggregation in the tissues.

logical systems difficult. Paradoxical results gained from differing experimental protocols in different animal models have also clouded the issue. The purpose of this review is to summarize the current knowledge of this paracrine and autocrine mediator of homeostasis, with specific attention to its role in skeletal muscle IRI.

BIOLOGY OF NO

The presence of an unpaired electron in the NO radical makes it highly reactive, with a half-life of only a few seconds and thus readily capable of reacting with other species such as superoxide. NO can diffuse through cell membranes and thus induce non-receptor mediated signaling events in neighboring cells. NO is formed as a metabolic product of the stepwise conversion of L-arginine to citrulline by hydroxylation of one of the L-arginine guanidino nitrogen atoms (Fig. 2). The reaction is catalyzed by the NO synthase (NOS) enzyme, of which three isoforms have been identified, each having specific localization and functions.

The inducible isoform of NOS (iNOS) becomes up regulated in response to inflammatory stimuli such as endotoxins, cytokines and lipid mediators (reviewed in [6]). Surges in NO production mediated by iNOS are cytotoxic [6] and have been implicated in the inflammatory destruction of the target tissues in infection, autoimmunity and transplant rejection [7]. iNOS is

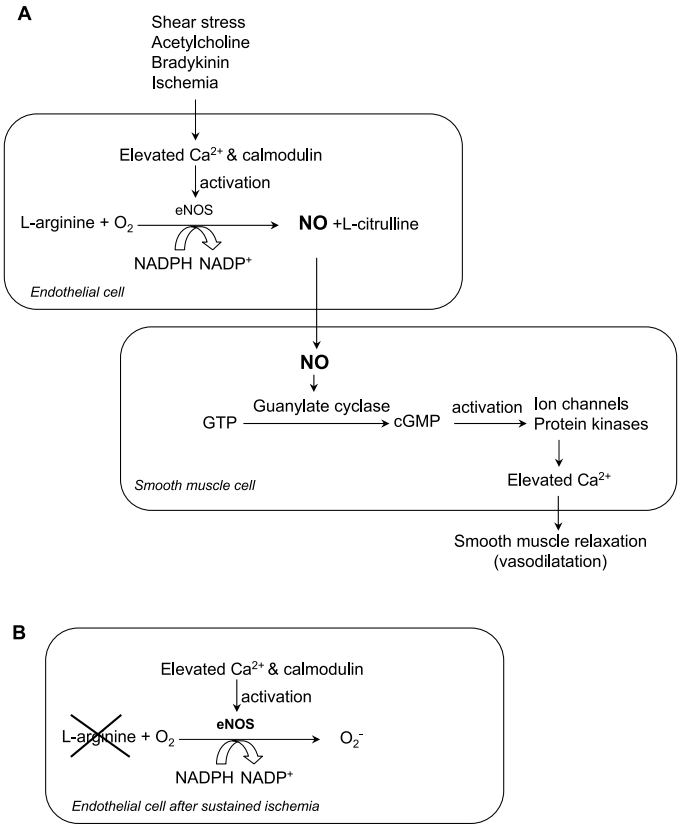

FIG. 2. Production of NO by the endothelial NOS isoform (eNOS) from the conversion of L-arginine to L-citrulline. (A) Intracellular calcium levels are initially elevated in endothelial cells in response to external stimuli, including ischemia, shear stress, bradykinin and acetylcholine, resulting in activation of eNOS. L-arginine is then converted to L-citrulline, also resulting in the production of NO, which can diffuse to adjacent cells in the vasculature. Subsequent activation of guanylate cyclase by NO elevates cyclic GMP (cGMP) production, resulting in smooth muscle relaxation, manifested for example, as vasodilatation. (B) During later stages of reperfusion, depletion of the eNOS substrate, L-arginine, allows eNOS to mediate the transfer of an electron from NADPH to molecular oxygen, thus producing the toxic oxygen species O_2^-.