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IS CYCLOXYGENASE-2 (COX-2) A MAJOR COMPONENT OF THE MECHANISM RESPONSIBLE FOR MICROVASCULAR REMODELING IN THE BRAIN?

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1. INTRODUCTION

The mammalian brain is exquisitely dependent on timely availability of both oxygen and glucose. Only minimal stores of either are present in the adult brain. Yet, mammals can exist and even flourish continuously at altitudes of up to 14,000 feet or more, and for brief periods at even higher elevations. The adaptive mechanisms that allow the brain to function over this wide range of environmental oxygen content reveal a complex metabolic and vascular control system, and an inherent structural and functional plasticity that appears to be driven by oxygen adequacy, i.e., the balance between delivery of oxygen and utilization of oxygen. These mechanisms are continuously active during normal physiological adaptation, and also play a significant role in the protective/restorative, as well as, the pathological responses to oxidative challenges.

Chronic adaptation to mild hypoxia includes a robust angiogenetic response[1]. The capillary density increases by almost double after 3 weeks of exposure to 0.5 ATM; the equivalent of an altitude of 5500m. The adaptive response is driven primarily by vascular endothelial growth factor (VEGF)[2,3] and angi`opoietin-2 (ang-2)[4]. VEGF is now well known to be upregulated by Hypoxia Inducible Factor-1 through activation of the hypoxic response element of the gene[5-7]. Upregulation of ang-2 has been less well understood until recently when it was shown that, in vitro, ang-2 is upregulated by prostaglandin E2 which comes from increased endothelial cyclooxygenase-2 (COX-2) activity[8]. Vascular remodeling depends on the balance between HIF-1 dependent and HIF-1 independent processes[9]. In this study we report preliminary evidence for in vivo upregulation of endothelial COX-2 in response to hypoxia.

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2. METHODS

2.1 Altitude Adaptation Model

All animals received humane care according to the US Department of Agriculture (USDA), Office for Laboratory Animal Welfare (OLAW), Public Health Services (PHS), and American Association for Accreditation of Laboratory Animal Care (AAALAC) using the guidelines set forth in the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals. Animals were kept on a standard light cycle and fed food and water ad libitum throughout the course of the experiments.

CD1 mice were kept for up to 3 weeks at 0.5 ATM in hypobaric chambers. Littermate controls were kept in the same room in a similar chamber under similar conditions except that they were kept at normobaric pressure for the 3 week duration of the experiment.

2.2 Immunohistochemistry

Mice were deeply anesthetized and transcardially perfused with ice-cold phosphate buffered saline (PBS, pH 7.2) followed by 4% paraformaldehyde in PBS. Brains were removed, post-fixed in the same fixative for 24 h at 4^{o}C and embedded in paraffin. Coronal serial sections (6μm) were deparaffinized, hydrated and subjected to antigen retrieval at 90^{o}C for 15 min using a Target Retrieval Solution (Dako). Antigens were detected through the use of a goat anti-rabbit secondary (1:150, Vector Laboratories), an avidin-biotin complex (Vectastain Elite ABC Kit, Vector Laboratories), and a diaminobenzidine peroxidase substrate (Vector Laboratories). Antibodies used included polyclonal rabbit anti-Glut1 (Dako) and rabbit anti-COX2 (Cayman Chemicals). Slides were dehydrated and coverslipped using Permount (Fisher).

2.3 Microvessel Density

Capillary diameters and densities were measured on tissues stained with antibodies against the glucose transporter, GLUT-1[10,11]. A photo montage spanning the full depth of the parietal cortex was created using a SPOT digital camera connected to a Nikon E600 Eclipse microscope with a 20X objective. A computer-aided interactive image analysis system (ImagePro Plus) was used to count marked antibody-positive capillaries between 4-25 μm in diameter and determine the number per unit area of brain tissue.

2.4 Western Analyses

Mice were deeply anesthetized using an intraperitoneal injection of sodium pentobarbital (65mg/kg). Control and hypoxic mouse cortical samples were dissected and homogenized in ice-cold lysis buffer (1x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100mM Na_3VO_4) or (50 mM Tris-HCl/1mM EDTA, pH=7.4) containing protease inhibitors (1 μg/ml leupeptin, 10 μg/ml aprotinin, 100 μg/ml PMSF, 1 μg/ml pepstatin). Homogenates were centrifuged at 20,000 g for 30 minutes at 4° C and the supernatants used for western blot analysis. Protein content in the supernatant was determined by a Bradford assay (Bio-Rad) with bovine serum albumin as a standard.