

PROTEOMIC ANALYSIS OF CAROTID BODY: A PRELIMINARY STUDY

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The oxygen-gradient diffusion of the capillary tissue level is essential for the cellular survival. Maintenance of oxygen homeostasis in the arterial blood is mediated by reflexes sensitive to oxygen decrease and by release of several factors. Carotid bodies (CBs) are the sensory organs to detect systemic hypoxia. CBs respond instantaneously in a few seconds required for initial transduction steps involving O₂ sensor and changing protein content or activity. Chronic hypoxia stimulates cellular growth and metabolism, CBs show hypertrophy of type I cells, increase in catecholamine synthesis and decreases in density of K⁺ ion channels. The regulation of genes encoding protein depends on accurate sensing of PO₂ and activation of HIF that is a key protein regulating cellular responses to hypoxia. HIF regulates several genes, including endothelin (ET-1) and vascular endothelial growth factor (VEGF). To better understand chemoreception we need to know which O₂ species are being sensed by cells and how cells sense oxygen. It would be helpful to have an overview of a specific set of proteins present in the CB and the functions of such proteins in signal transduction and adaptation during hypoxia. Here, we present a first proteomic analysis of the rat CB preparation by comparison between normoxia and hypoxia. Proteomic investigation would be helpful to identify the stress-induced protein during hypoxia and aging. Two groups of adult Wistar rats, weighing 200-250 g were used. One was kept in room air (21% O₂) as a control group, the other was kept in a Plexiglas chamber for 12 days in chronic hypoxia (10-11% inspired oxygen). Chamber temperature and CO₂ were kept in physiological ranges. Surgical procedures were carried out under Nembutal anesthesia and CBs were rapidly dissected and removed from anesthetized rats. CB specimens, grouped in two pools for both condition examined, were rinsed in PBS twice and incubated RT in proteinase inhibitors solution. The total protein extract for each lysated tissue was separated using a broad pH range nonlinear IPG strip (3-10) and the second dimension was performed on a 9-16% polyacrilamide gel. Using an Image Master 2D Platinum software, on the average, 67 and 45 protein spots were detected for normoxic and hypoxic samples, respectively. Exposure to hypoxia for 12 days produced a significant changes in the expression of proteins, providing an initial insight into the mechanism underlying differences in susceptibility to hypoxia at different age. Further investigation is needed to provide valuable biochemical information useful to elucidate the oxygen sensitive molecular mechanism.