

HYPOXIA-INDUCED METABOLIC STRESS IN BLADDER UROTHELIAL AND SMOOTH MUSCLE CELLS

Hypothesis / aims of study

Lower urinary tract symptoms are associated with the metabolic syndrome. The different components of metabolic syndrome contribute to end-organ dysfunction such as the bladder, possibly through endothelial dysfunction or decreased blood flow. Hypoxia is known to develop in tissues of obese animal models and disrupt dramatically cell metabolism and functions. The aim of our novel study is to determine if hypoxia causes changes at a cellular level that may be responsible for voiding dysfunction.

Study design, materials and methods

Urothelial and smooth muscle cells (SMC) were isolated from rat bladders using trypsin or collagenase IV respectively, and grown in petri dishes until passage between 2 and 5. After confluency, cells were exposed to oxygen 21% (normoxia) or 1% (hypoxia) for 24 hours then assessed for microscopy, immunohistochemistry and immunoblotting analysis. MTT test was carried out to evaluate survival of cells to hypoxic treatment. Lactic acid concentrations were measured using an enzymatic method.

Results

SMC were characterized by immunohistochemistry for myosin and smooth muscle actin- α before and after hypoxia. MTT test yielded similar results between normoxic and hypoxic SMC while urothelial cells show a decreased of 18.9% in hypoxia. Hypoxia led to an increase in lactic acid release in the media of both cell types (SMC +96%; urothelial cells +22%) (Figure 1).

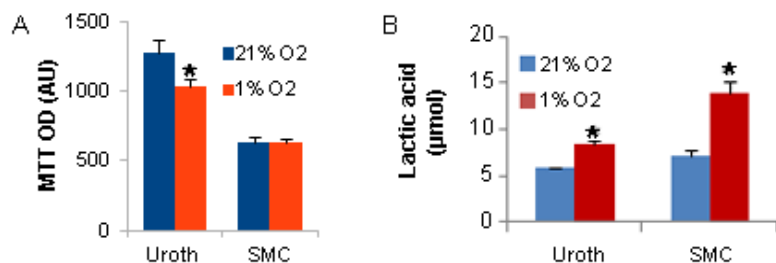


Figure 1. MTT optical densitometry (A) and lactic acid concentration (B) of urothelial and smooth muscle cells under hypoxia. MTT test reveals a sensitivity of urothelial cells (Uroth) to hypoxia while increases of lactic acid released in the culture media confirms the hypoxic states of both cell type. (* $P < 0.05$ student t-test)

GLUT-1 expression was increased in SMC, as revealed by immunohistochemistry (Figure 2) and immunoblotting, and in urothelial cells (Figure 5). Increase in HIF-1 α in SMC was also observed in SMC under immunohistochemistry (Figure 2).

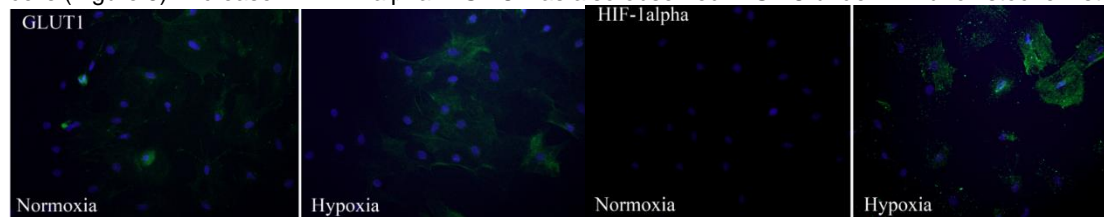


Figure 2. Immunohistochemistry of smooth muscle cells reveals an increase in GLUT-1 and HIF-1 α in hypoxic conditions.

Regulator of cytoskeleton RhoA and ROCK-1 were not affected by hypoxia. On the other hand, the activated protein c-Jun amino-terminal kinases (JNKs) JNK-P, involved in insulin signaling and cell survival, was decreased with stable expression of non-phosphorylated JNK in both urothelial and SMC (Figure 4).

The succinate receptor SUCNR1 (GPR91) was significantly reduced in SMC under hypoxia, with minor reduction in urothelial cells, as demonstrated by immunohistochemistry (Figure 3) and immunoblotting (Figure 4-5).

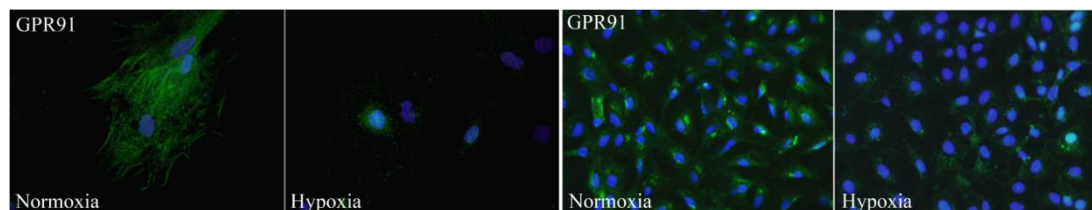


Figure 3. Immunohistochemistry of GPR91 in culture of SMC (left) and urothelial cells (right).

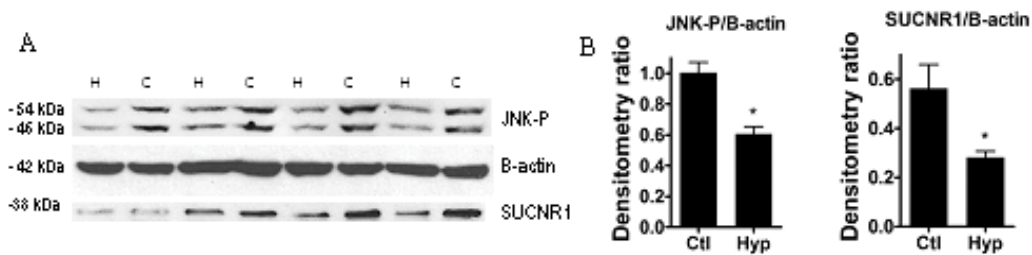


Figure 4. JNK-P and SUCNR1 expression in smooth muscle cells subjected to 24 hours of hypoxia (1% O₂). A: Levels of JNK-P and SUCNR1 measured by immunoblotting. B: Quantitative expression of densitometry ratio of JNK-P/B-actin and SUCNR1/B-actin. **P*<0.05 Statistically different from the control (normoxic) conditions.

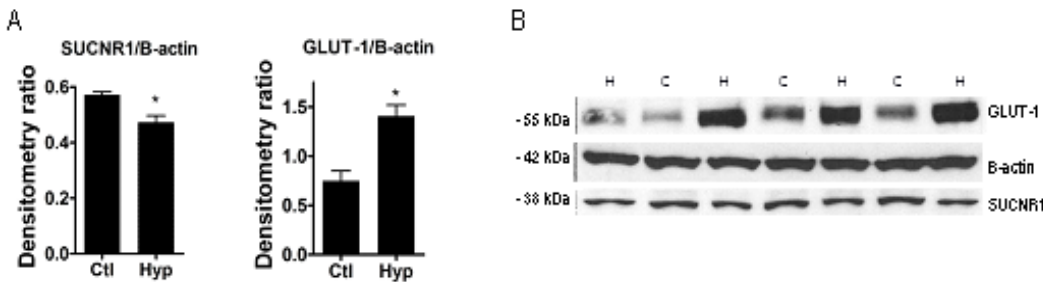


Figure 5. GLUT-1 and SUCNR1 expression in urothelial cells subjected to 24 hours of hypoxia (1% O₂). A: Levels of GLUT-1 and SUCNR1 measured by immunoblotting. B: Quantitative expression of densitometry ratio of GLUT-1/B-actin and SUCNR1/B-actin. **P*<0.05 Statistically different from the control (normoxic) conditions.

Interpretation of results

The increased expression of GLUT-1, HIF-1 alpha and the release of lactic acid confirms that the cells were under hypoxic stress. While short exposure to hypoxia does not affect SMC cytoskeleton integrity, decreased level of activated JNK suggests that hypoxia can contribute to the development of insulin resistance and increased vulnerability in urothelial and SMC. The dramatic reduction of SUCNR1 in SMC may be caused by a down-regulation from increased levels of succinate produced in hypoxia state.

Concluding message

Hypoxia induces metabolic and biochemical processes in urothelial and SMC of the bladder. While the cytoskeleton and contractile machinery of the muscle seem to be unaffected, hypoxia may affect bladder contraction by modifying the cross-talk between urothelial and smooth muscle cells, and by affecting intracellular signalling of smooth muscle cells.

Disclosures

Funding: Financial funding: Institutional, Quebec Diabetes, Canadian Urological Association Scholarship Foundation, Fonds de recherche Sante Quebec **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** McGill University Animal Care Committee