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Hyperoxia Upregulated Na,K-Adenosine Triphosphatase β_1 Gene Transcription*

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Alveolar sodium and fluid transport occur via type II cell apical sodium channels and basolateral Na,K-adenosine triphosphatases (ATPases), both of which are fundamental in resorbing edema fluid and restoring gas exchange following lung injury.¹ Na,K-ATPase gene expression is upregulated in the whole lung and type II cells in both *in vitro* and *in vivo* models of hyperoxic lung injury.^{2 3 4 5} This increase in Na,K-ATPase may serve as a homeostatic protective mechanism against alveolar flooding. Using a type II cell *in vitro* model of hyperoxic injury ($\geq 95\%$ O₂ for 48 h), we demonstrated a threefold and fivefold increase in steady-state levels of Na,K-ATPase α_1 and β_1 subunit messenger RNA (mRNA), respectively.^{2 3 6} In addition, hyperoxia did not alter messenger RNA stability of either subunit.⁷ To study the mechanism of Na,K-ATPase gene upregulation by hyperoxia, we developed an *in vitro* model using MDCK cells exposed to hyperoxia (95% O₂/5% CO₂ for 24 to 48 h).⁷

To measure transcription rates of the Na,K-ATPase subunits, nuclear run-on assays (NRAs) were performed with nuclei isolated from MDCK cells incubated in either normoxia or hyperoxia for 24 h. Slot blots containing the following plasmids were used for the NRAs: pGEM plasmid (control plasmid), actin (control), α_1 subunit complementary DNA, and β_1 subunit complementary DNA. NRAs revealed a 1.3-fold and 3.0-fold increase in α_1 and β_1 transcription with hyperoxia compared with normoxia. To identify hyperoxia regulatory regions within the promoter of the β_1 subunit, transient transfection

experiments using the 5'-flanking region of the Na,K-ATPase β_1 subunit linked to the reporter gene, luciferase, were performed in MDCK cells under hyperoxic and normoxic conditions (Table 1). The wild-type construct (β_1 -817) contained 817 basepairs (bp) of the 5' promoter region upstream from the transcription start site plus 151 bp of the first exon linked to a promoterless firefly luciferase expression vector (pXP1-luc). This construct was transfected via lipofection and revealed a 1.9-fold increase in promoter activity in hyperoxia compared with normoxia, confirming that hyperoxia induced Na,K-ATPase β_1 subunit transcription. To localize the region(s) necessary for the hyperoxia induction, MDCK cells were transfected with four different 5' deletion constructs of the β_1 promoter (Table 1).

Transfection of the deletion constructs in MDCK under normoxic conditions demonstrated a decrease in basal promoter activity with decreasing size of the deletion construct. The induction by hyperoxia was present in the β_1 -102 through β_1 -62 constructs; however, hyperoxia did not induce promoter activity in the β_1 -41 deletion construct. This localized a 21 bp regulatory region on the β_1 promoter between bp-41 and -62 that was necessary for the twofold induction by hyperoxia. Since the full induction by hyperoxia was not seen with transfection of the wild-type or deletion constructs, other regions outside of our constructs may be necessary for further hyperoxia induction.

View this table: Table 1. Effect of Hyperoxia on Na,K-ATPase β_1 Subunit Promoter Activity*
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To identify proteins that bind to this putative regulatory region, electromobility shift assays (EMSAs) were performed using whole cell extracts from MDCK cells under normoxic and hyperoxic conditions on an oligonucleotide spanning the 21 bp regulatory region identified from the transfection experiments. EMSAs revealed two bands that had increased binding in extracts obtained from hyperoxic cells compared with normoxic cells. In addition, cells treated with the thiol oxidizer, diamide, manifested mobility shift patterns identical to extracts from cells exposed to hyperoxia. This suggested that hyperoxia induced an increased protein binding within the regulatory region identified in the transfection experiments and that thiol oxidation played a role in the protein binding and therefore, the hyperoxia induction.

The Na,K-ATPase is an important protein for maintaining vectoral ion and fluid transport, along with normal cellular homeostasis.¹ This is especially important in the lung, where ion and fluid transport is necessary to maintain normal gas exchange, especially in the setting of lung injury. In our model system, we demonstrated that hyperoxia increased the gene expression of the Na,K-ATPase α_1 and β_1 subunits. Further, we determined that hyperoxia induced the transcription of the β_1 subunit and identified a 21 bp region within its promoter that is necessary for this induction. Further analysis with EMSA suggested that thiol oxidation may be playing a role in the upregulation by hyperoxia. This upregulation of the Na,K-ATPase by hyperoxia may help to maintain gas exchange in the injured lung that is undergoing alveolar flooding.

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Table 1. Effect of Hyperoxia on Na,K-ATPase β_1 Subunit Promoter Activity*

Construct	Normoxia	Hyperoxia	Hyperoxia/ Normoxia
β_1 -817	100	190	1.9
β_1 -102	62	120	2.0
β_1 -82	62	161	2.6
β_1 -62	21	39	2.1
β_1 -41	2.7	1.4	0.8

* Luciferase activity was normalized to either cytomegalovirus- β -galactosidase activity or protein concentration, then reported as a percent activity over control. Control is designated as the β_1 -817 promoter construct in normoxic conditions.