Isoflurane Affects CFTR Synthesis And Function Through ER Stress
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INTRODUCTION
- Anesthetic agents can induce protein misfolding, precipitate endoplasmic reticulum (ER) stress and activate the cellular Unfolded Protein Response (UPR).
- The clinical implications of these deleterious effects include the exacerbation of chronic neurodegenerative diseases and the observed onset of neurologic protein misfolding diseases like Congenital Central Hypoventilation Syndrome after Anesthesia.
- We have investigated what ER pathways are preferentially affected by isoflurane. In particular, how isoflurane exposure affects metastable and tenuously folded proteins, including the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR).

MATERIALS AND METHODS
Neuronal cells line SH-SY5Y were used to investigate isoflurane effects on ER-related pathways. The cells were exposed to isoflurane (1 MAC) for four hours before harvesting and isolating mRNA. Transcript levels were tested using a ER-specific RT-PCR array.

To test the effect of isoflurane on CFTR folding and function, HEK-293 cells were used. The cells were exposed to isoflurane (1 MAC) for the same four hours. CFTR expression was analyzed through western blotting of culture lysates. Functional analysis was performed through a fluorescent membrane potential chloride flux assay.

RESULTS
Isoflurane exposure caused:
- Increased expression of proteins involved in misfolded protein degradation (EDEM1, EDEM2), glycosyltransferases (UGGT1, UGGT2) and glycoprotein specific ubiquitin ligase Fbx06, as well as markers of ER stress and UPR.
- Increased misfolding of CFTR molecule, with more immature, ER-blocked CFTR present in isoflurane treated cells. A ~20% reduction in CFTR activity was also noticed.

CONCLUSION
- Isoflurane preferentially affects glycoprotein folding and degradation in the ER.
- Glycoproteins are particularly important in neuronal function, and misfolded glycoproteins are prominent in chronic neurodegenerative disease, indicating a potential mechanism by which anesthetics could alter the course or severity of these diseases.
- Isoflurane altered both CFTR synthesis and activity, indicating that difficult to fold, or tenuously folded, proteins could be particularly affected at other anatomic sites.
- Further research is needed to determine if CFTR function is altered in clinical samples, affecting lung function.