The Mechanism of Action of an Innovative Respiratory Stimulant, ENA-001

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BACKGROUND

Respiration is initiated in the brainstem and modified with input from the cortex and peripheral nerves. Chemoreceptors, sensitive to oxygen tension, carbon dioxide tension, pH, and other chemical stimuli, are located in both the brainstem and peripheral sites on the vasculature. The primary peripheral sensors for hypoxia are located on the type I glomus cells in the carotid bodies at the bifurcation of the internal and external carotid arteries. The carotid bodies send impulses along the carotid sinus nerve to the nucleus tractus solitarius (NTS) in the brainstem. The NTS is the principal site of termination for respiratory-related sensory afferents that come from the lungs, airways, and peripheral chemoreceptors and, connects to the pre-Bötzinger complex, which in rats at least, forms the primary pacemaker for normal respiration. Activation of several ion channels (e.g., BK, TASK-1, and TASK-3) in the glomus cells/carotid body have been associated with stimulation of the respiratory control arc in humans.

Interference with normal respiratory control is a common iatrogenic event in the peri-procedural setting and can be the result of the procedure (e.g., colonoscopy; surgery), drug treatment (e.g., anesthetic, benzodiazepine, opioid), or disease (e.g., central/sleep apnea) or combinations of these factors. In the post-procedural setting, it is not possible to predict the onset, duration, or severity of deleterious pulmonary complications (PPC) due to a number of contributing factors, including differing drug sensitivity and pharmacokinetics, occult pulmonary and CNS dysfunction, environmental activity level, and concomitant medications. Those patients with additional risk factors including advanced age, pre-existing respiratory pathology and sleep apnea are at particular risk for respiratory compromise, often leading to acute intervention, increased morbidity, and occasional mortality. Patients with additional risk factors for respiratory compromise who are housed on observational patient floors for routine post-operative care may have unrecognized and undiagnosed respiratory insufhciency, including opioid-induced respiratory depression. Often the acute response to respiratory compromise post-operatively is sudden reversal of opioids with specific antagonists.

Unfortunately, mu-opioid antagonists such as naloxone are non-selective for specific opioid effects and reverse not only respiratory depression but analgesia as well. The response to a sudden increase of pain in the previously sedated patients often requires increased doses of opioid to overcome the severe acute pain and the competitive inhibition of the mu-receptors, setting the stage for a rebound respiratory depression, particularly as naloxone effects wear off.

METHODS

Evaluate preclinical study data to explain the mechanism of action of ENA-001.

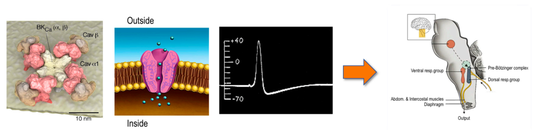
Carotid Body Detects Changes in Composition of Arterial Blood

- Located at the bifurcation of the carotid artery
- Contains glomus Type 1 cells
- Detects SpO_2, CO_2, blood pH, and temperature

OBJECTIVE

ENA-001 is being developed as a first in class, fast acting, and short-duration intravenous therapeutic agent for short to intermediate term use to stimulate ventilation for treatment of respiratory depression in post-operative patients including such conditions as central/ drug-induced respiratory depression, post-anesthetic atelectasis as well as respiratory depression associated with community drug overdose and apnea of prematurity

Perpherally-acting Stimulatory Signal to Brainstem via BK_{Ca} inhibition



CONCLUSION

Because its MOA is independent of opioid and other agents used for pain and anesthesia in the peri-operative environment, ENA-001 should provide reversal of respiratory depressant effects of these compounds without interfering with analgesic or anesthetic primary pharmacology.

RESULTS

The primary molecular mechanism underlying the ventilatory stimulant effects of ENA-001 appears to be functional inhibition of large-conductance Ca^{2+} and voltage-activated K* channels (Maxi-K, BK, BK(Ca^{2+}), KCNMA1, SIo1) in the carotid body to stimulate respiration and increase minute ventilation by primarily increasing tidal volume and secondarily through minor increases to respiratory rate. BK (Big Potassium) channels are ion channels characterized by their large conductance of potassium ions (K^+) through cell membranes. These channels are activated (opened) by changes in membrane electrical potential and/or by increases in concentration of intracellular calcium ion (Ca^{2+}).

Electrophysiology studies show that in inside-out patches pulled from rat pituitary GH3 cells ENA-001 causes a reversible, concentration-dependent inhibition of single BK channel activity. The concentrations required to block BK channels in this preparation are reasonably consistent with the free plasma levels of ENA-001 that produce increases in ventilation in vivo. Moreover, electrophysiology studies of whole cell currents in glomus cells isolated from the rat carotid body indicate that ENA-001 selectively inhibits the BK-like K^+ current in these cells.

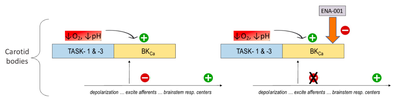
The beneficial effects of ENA-001 on respiration are assumed to derive by mimicking the hypoxic inhibition of BK channels: promoting depolarization of carotid body type I cells and precipitates voltage-gated Ca^{2+} influx where the resultant increase in [Ca^{2+}] elicits neurotransmitter release to activate sensory afferent discharge to the brainstem (via the carotid sinus nerve) and ultimately to corrective changes in breathing.

The proposed mechanism of action (MOA) for ENA-001 is further supported by work in rats with vs without carotid sinus nerve transection. Nerve transection resulted in an 80-90% blunting of the response to ENA-001 (2 mg/kg IV).

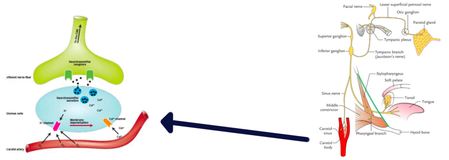
ENA-001: 1st-in-class BK_{Ca} Channel Antagonist

Mechanism of Action

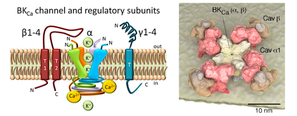
- Respiratory depression – due to any cause – leads to chemical changes in the bloodstream (O_2, CO_2, pH)
- These changes are detected in the carotid bodies by chemoreceptor cells and ion channels (e.g., TASK)[1]
- A feedback loop mediated via BK_{Ca} channels limits the magnitude of the response
- ENA-001 releases the negative feedback, enhancing the signal to the brainstem to restore respiration
  - The effect thus enhances the natural physiological restoration of respiration
  - And it is 'agnostic' to the cause of the respiratory depression



How the Carotid Body Sense and Respond to Arterial Oxygen



Big Calcium-Activated Potassium Channels (BK_{Ca})



REFERENCES

1. Golder FJ, Dax S, Baby SM, et a. Anesthesiology. 2015;123:1093—1104.
2. Dallas ML, Peers C, Golder FJ, et al. Adv. Exp. Med. Biol. 2015;860:361—370.
3. Andani R, Khan YS. Anatomy, Head and Neck: Carotid Sinus. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554378/figure/article-18972.image.f1/ Accessed August 1, 2024.
4. Ortega-Sáenz P, Levitsky KL, Marcos-Almaraz M, et al. J Gen Physiol. 2010;135:379-392.