The Role of Endothelin Converting Enzyme-1 and Neprilysin in Perivascular Sensory Nerve Dysfunction with Inflammatory Bowel Disease

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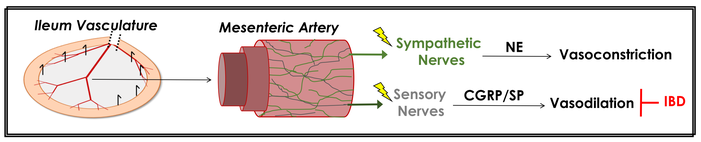
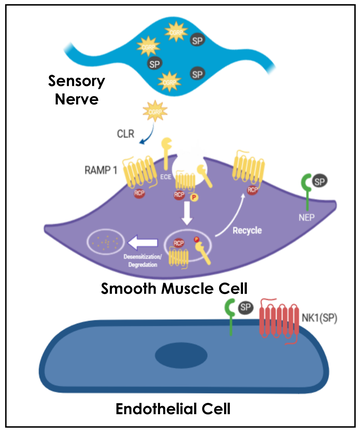
Abstract

Inflammatory Bowel Diseases (IBD) are chronic diseases that are diagnosed in around 70,000 Americans each year, and 1.6 million Americans in total. IBD is closely associated with cardiovascular complications, including arterial stiffening and ischemic heart disease. Blood flow to the intestines is impaired with IBD, and dilation of the mesenteric arteries is facilitated by perivascular sensory nerves, through the release of calcitonin gene related peptide (CGRP) and substance P (SP). Previous experiments suggest that CGRP and SP receptors function abnormally and fail to facilitate artery dilation during IBD. To study those pathways further, we looked at two components of the CGRP and SP signaling pathways. The metalloproteases endothelin-converting enzyme-1 (ECE) and neutral endopeptidase (NEP) are very important to the GCRP and SP pathways because they control the degradation and the recycling of CGRP and SP receptors. My preliminary experiments yielded two major results: (1) decreased ECE and NEP content in mesenteric arteries with IBD and (2) colocalization of surface and internalized CGRP and SP receptors with both ECE and NEP in isolated smooth muscle cells from mesenteric arteries. Therefore, we hypothesize that that IBD decreases receptor and metalloprotease colocalization and internalization after sensory nerve stimulation, leading to decreased receptor recycling and impaired sensory vasodilation. We also predict that IBD decreases the effect of NEP and ECE inhibition on sensory vasodilation in intact, cannulated arteries. To test these hypotheses, C57BL/6, IL10$^{-/-}$ mice will be inoculated with Helicobacter hepaticus by gastric gavage after weaning and develop IBD over 90 days. Non-gavaged C57BL/6 mice will serve as controls. Confocal imaging (Leica TCS SP8) of cannulated, immunolabeled mesenteric arteries will determine how IBD affects CGRP/SP receptor recycling and NEP/ECE expression and localization after exposure to one or both neuropeptides. In separate experiments, pressure myography will be used to examine how IBD affects the role of ECE and NEP in vasodilation of live vessels. Sensory vasodilation will be measured before and after pharmacological blockade of one or both metalloproteases. Myography data will be analyzed via GraphPad Prism. Previous studies suggest that CGRP and SP signaling and receptor trafficking is altered specifically in blood vessels (mesenteric arteries and aorta) and their directly adjacent tissues (perivascular adipose and serum). If results indicate that IBD alters the role of NEP and ECE expression and activity in sensory neurotransmitter signaling and vasodilation, targeting ECE and/or NEP activity in IBD patients may have the potential to eventually improve both blood flow and intestinal function.

Background



- Inflammatory Bowel Diseases (IBD) are chronic diseases that are diagnosed in around 70,000 Americans each year, and 1.6 million Americans in total.
- IBD is comorbid with cardiovascular disease where vascular dysfunction is common
- Perivascular sensory nerves that increase blood flow are impaired with IBD
- Sensory nerves release calcitonin gene-related peptide (**CGRP**) and substance P (**SP**) which bind downstream to CGRP (**RAMP1, CLR, RCP**) and SP (**NK1**) receptors
- Endothelin-converting enzyme-1 (**ECE-1**) and neutral endopeptidase (**NEP**) regulate CGRP and SP signaling pathways through peptide degradation and receptor recycling



Conclusions From Prior Findings

Decreased NEP and ECE-1 expression contributes to impaired perivascular sensory dilation and therefore decreased intestinal perfusion with IBD.

Previous Results

Tissue NEP Content

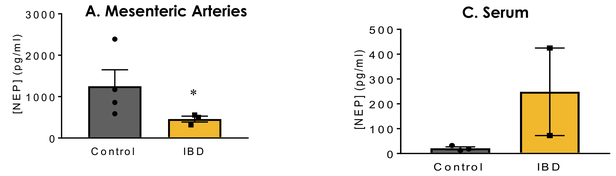


Figure 1: IBD alters NEP concentration in mesenteric arteries and serum.
Data are individual values and mean ± SE for [NEP] (pg/ml) in: **A**. Mesenteric Arteries (n=4, p≤0.05); **B**. Serum (n=2-3, 6 values below detection)

Tissue ECE-1 Content

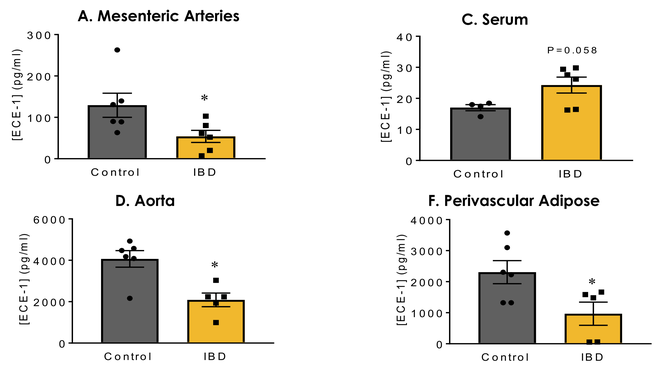


Figure 2: IBD alters ECE-1 concentration in mesenteric arteries, aorta, serum and perivascular adipose.
Data are individual values and mean ± SE for [NEP] (pg/ml) in: A. Mesenteric Arteries (n=6, p≤0.05); B. Serum (n=4-6, p=0.058); C. Aorta (n=5-6, p≤0.05); D. Perivascular Adipose (n=5-6, p≤0.05).

Previous Imaging Results

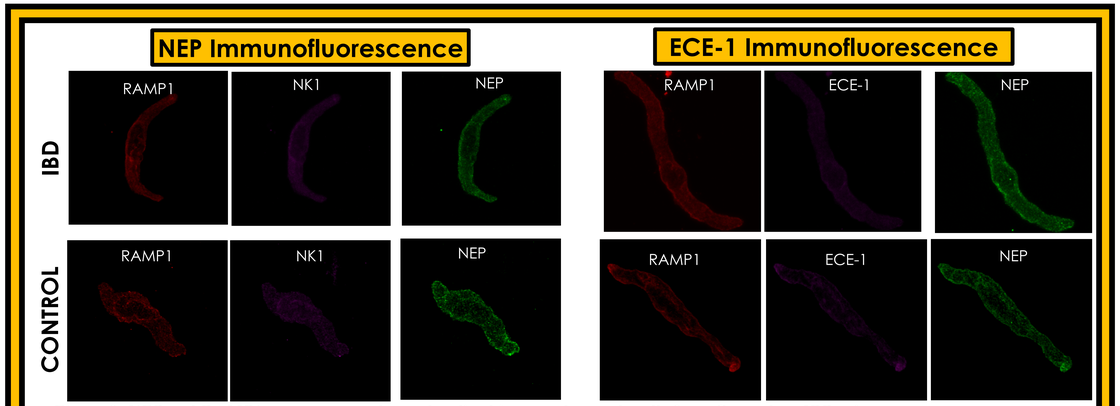


Figure 3: NEP and RAMP1 are more membrane localized in smooth muscle cells from Control vs. IBD mice.
Representative images of mesenteric artery smooth muscle cells from Control (Left Panel) and IBD (Right Panel) labeled for:

- NEP (right, green): labels NEP
- RAMP1 (left, red): labels CGRP receptors
- NK1 (middle, magenta): labels SP receptors

- NEP (right, green): labels NEP
- RAMP1 (left, red): labels CGRP receptors
- ECE-1 (middle, magenta): labels SP receptors

Hypotheses

IBD decreases receptor and metalloprotease colocalization and internalization after sensory nerve stimulation, leading to decreased receptor recycling and impaired sensory vasodilation

IBD decreases the effect of NEP and ECE inhibition on sensory vasodilation in intact, cannulated arteries

Current Methods

- C57BL/6, IL10$^{-/-}$ mice are inoculated with Helicobacter hepaticus by gastric gavage after weaning and develop IBD over 90 days. Non-gavaged C57BL/6 mice serve as controls.
- **Confocal imaging** (Leica TCS SP8) of cannulated, immunolabeled mesenteric arteries will determine how IBD affects CGRP/SP receptor recycling and NEP/ECE expression and localization after exposure to one or both neuropeptides.
- **Pressure myography** is used to examine how IBD affects the role of ECE and NEP in vasodilation of live vessels. Sensory vasodilation is measured before and after pharmacological blockade of one or both metalloproteases.
- Myography data is analyzed via GraphPad Prism.

Expected Results

- We expect that IBD alters the role of NEP and ECE expression and activity in sensory neurotransmitter signaling and vasodilation in live vessels, further supporting the results we saw in previous experiments
- Our methods will allow us to see how IBD alters these expressions in live vessels using pressure myography and confocal imaging of cannulated vessels
- If results support our hypothesis, targeting ECE and/or NEP activity in IBD patients may have the potential to eventually improve both blood flow and intestinal function.

Support

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