



**Karolinska  
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# The role of nitric oxide in the gastrointestinal tract

**AKADEMISK AVHANDLING**

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## ABSTRACT

Nitric oxide (NO) is an important second messenger involved in the regulation of a multitude of mechanisms in the body, such as neurotransmission, smooth muscle contractility, host defense and immune regulation. Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic disorders affecting the gastrointestinal (GI) tract with unknown etiology. These diseases are characterized by increased NO levels in the gut lumen, aberrant leukocyte recruitment to the inflamed tissue and changed motility pattern of the intestines.

This thesis aimed to investigate NO's involvement in inflammatory reactions as well as its regulatory role on motility in the GI tract by studying NO-related gene expression in IBD,  $\alpha_2$  integrin antibody treatment in comparison to conventional IBD drugs in experimental colitis, neuropeptide S (NPS) effects on motility, contractility and inflammation, as well as NO's regulation of the migrating motor complex (MMC) in relation to muscarinic and 5-HT<sub>3</sub> receptor blockade.

Cluster analysis of NO-related gene expression in CD and UC revealed common pathophysiological processes, with hypoxia-inducible factor 1 (HIF-1) as a central regulator of inflammation, angiogenesis and tissue fibrosis. Moreover, interaction analysis pinpointed the association of upregulated expression of IL-8 and ICAM-1 in both diseases, highlighting an exaggerated leukocyte infiltration in the pathophysiology of CD and UC.

In comparison to conventional IBD drugs, treatment with a function-blocking anti- $\alpha_2$  antibody by rectal administration showed alleviation of signs of colitis, such as reduced body weight loss, rectal bleeding, inflammation score and inflammatory biomarker expression including inducible NO synthase (iNOS). Although treatment with methotrexate also showed several signs of ameliorated colitis, these effects were not accompanied by a broad reduction in inflammatory marker expression. This study provides evidence for therapeutic use of integrin  $\alpha_2\beta_1$  as a novel drug target for treatment of IBD.

Infusion with NPS prolonged the MMC cycle length and the phase III duration in upper small intestine. Contractility studies on excised human muscle strips revealed a dampening of the amplitude, with NPS acting directly on small intestine circular muscle, while this effect seems mediated by prejunctional receptors in colon. These effects of NPS on motility and contractility are in agreement with the changes seen during inflammatory reactions in the intestine. Moreover, NPS induced the expression of inflammatory markers iNOS, IL-1 $\beta$  and CXCL1, further supporting a role of NPS in NO-dependent induction of inflammation in the GI tract.

Studies with the NOS inhibitor L-NMMA suggested marked effects of NO on motility. L-NMMA, shown to inhibit NO, initiated phase III MMC activity, while additional muscarinic and 5-HT<sub>3</sub> receptor blockades revealed that the transition from phase I to phase II activity seem regulated as a balance between inhibitory nitroergic and excitatory cholinergic and serotonergic pathways.

These results demonstrate increased iNOS expression during inflammatory reactions in the GI tract, with the resulting increase of NO as a pathophysiological inhibitor of motility seen in inflammatory disorders of the GI tract.