Summary

Enteric neuropathies, disorders in which the enteric nervous system is compromised, may have severe effects on gastrointestinal function. Interestingly, neurons containing nitric oxide synthase appear to be more susceptible in a number of conditions including esophageal achalasia, hypertrophic pyloric stenosis, gastroparesis, Hirschsprung’s, Chagas, and Prion disease, as well as in intestinal ischemia/reperfusion injury.

Keywords: Nitric oxide, ischemia, enteric nervous system

Introduction

Damage following ischemia and reperfusion (I/R) injury is common in the intestine, and can be caused during abdominal surgery, in several disease states and following intestinal transplantation. I/R results in initial tissue damage, and prolonged changes in motility, most often slowed transit. We have observed swelling of NOS immunoreactive neurons (Fig. 1) and accumulation of nitrosylated protein aggregates following I/R. Intestinal I/R may be a useful model to investigate the involvement of NOS neurons in enteric neuropathies.

Material and Methods

A branch of the superior mesenteric artery of anaesthetised mice was occluded for two hours and the animals were allowed to recover from three hours to one day before tissue was taken for structural analysis and in vitro muscle recording. In other experiments, occlusion was for 1 hr and reperfusion was up to 28 days.

Results and Discussion

After 2 hr occlusion, histological analysis revealed damage to the mucosa, muscle, and neurons. The mucosal surface was sloughed off 3 hours following I/R but the epithelium appeared normal by 1 day in both nNOS KO and WT mice (Fig. 2 b, c). More severe degenerative changes were observed in both the longitudinal and circular muscle layers of nNOS KO mice compared to WT mice (n=3) (Fig. 2). After 1 hr occlusion, muscle and mucosal damage repaired, while damage to NOS neurons persisted up to at least 28 days. Data to date indicate a reduction in the spontaneous
muscle activity and amplitude of muscle contraction in response to electrical field stimulation (2, 5, and 20Hz/60V/100 pulses/0.5ms), 3 and 24 hours following 2 hr I/R.

Fig. 1 Effects of I/R on NOS neurons in mouse myenteric ganglia
a: NOS neurons from sham-operated mice have irregular shapes without prominent dendrites. This is similar to control. b: After 1 hr ischemia followed by reperfusion (here for 24 hr) dendrites are prominent and the neurons are swollen. c, d and c', d' (enlarged images): Vacuoles (examples at arrows) and distorted dendrites (asterisks) in NOS neurons, 2 days following 1 hour ischemia were prominent in both the non-occluded (c, c’) and occluded regions (d, d’) of I/R mice. Scale bars: a, b, c, & d: 20 µm (bar on d), c’ and d’: 10 µm (bar on d’)

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Fig. 2 Effects of 2 hr ischemia followed by reperfusion for 3 hr to 24 hr, on the histological appearance of the ileum.  
**a** In the control, the villi and the longitudinal muscle have normal micromorphology.  
**b, c** After 3 hours of reperfusion, both KO and WT animals had patches of the epithelium missing and tissue remnants can be found in the lumen. **b'** A higher power view of the external muscle showing severe circular muscle damage in KO animals, 3 hr after reperfusion. **c'** A higher power view of the external muscle showing patches of longitudinal muscle damage in WT animals, 3 hours after reperfusion. **d** 24 hr after reperfusion, substantial longitudinal muscle damage can be seen in KO animals compared to WT animals (e).  
Scale bars: a: 100 µm; b and c: 50 µm (bar on c), b', c', d, e: 25 µm (bar on e)
Conclusions
This work indicates that I/R causes severe longitudinal and circular muscle damage that repairs. Longer-term effects on neurons may contribute to persistent dysmotility. NOS neurons are more susceptible than other neuron types. A theory that may explain the susceptibility of NOS neurons has been developed from these I/R studies.

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References

