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Adaptation of iron absorption in the 5/6 nephrectomy animal model reflects the pathological features of chronic renal failure in humans

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Anaemia is present in the majority of patients with chronic renal failure (CRF) and is caused predominately by the lack of adequate erythropoietin (EPO) secretion. Patients treated with erythropoietin often require iron supplementation; however, oral iron therapy is not always effective, indicative of inadequate iron absorption in CRF. This present study investigated the effect of renal failure and EPO treatment on intestinal iron absorption *in vivo*, using the 5/6 nephrectomy model.

Male Sprague-Dawley rats (240g) underwent a 2-stage, 5/6 nephrectomy or sham operation under halothane anesthesia (2-3% halothane in 100% oxygen). Analgesic (Rimadyl, 5mg/kg s.c.) was administered immediately after surgery and after a further 6 hours and the well being of the animals monitored daily. Animals were administered 100 i.u./kg recombinant human EPO or saline i.p. twice per week. After 5 weeks, animals were anaesthetised with pentobarbitone sodium (50mg/kg i.p.) and the femoral artery cannulated. Segments of duodenum (5cm beginning 1cm from the stomach) were selected and flushed with warm 0.9% saline, followed by air. Uptake buffer (250µl) containing 200µM iron labelled with ^{59}Fe was instilled into the lumen and the segment was tied off. Blood (0.3ml) was collected at 5, 10, 15 and 30 min intervals and iron absorption calculated from the ^{59}Fe activity of the uptake buffer and blood. Induction of renal failure was established by measurement of packed cell volume (PCV) and plasma urea and creatinine concentration.

Animals that had undergone a 5/6 nephrectomy had a reduced PCV and increased plasma urea and creatinine concentration compared to sham operated animals, confirming induction of CRF. Intestinal iron absorption was significantly decreased in nephrectomised animals. EPO treatment normalized PCV, although, iron absorption was only partially restored (Fig. 1). These results suggest that changes in intestinal iron absorption in the 5/6 nephrectomy model reflect those occurring in patients with CRF, demonstrating this to be an appropriate model for investigating the underlying mechanisms of iron homeostasis in CRF.

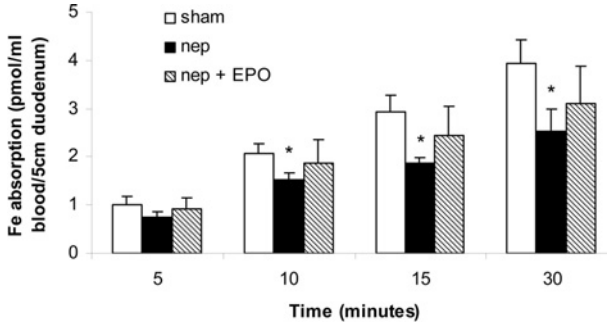

Figure 1. *In vivo* iron absorption by the duodenum of sham, nephrectomised and EPO treated nephrectomised rats. Results are expressed as mean ± SEM of 6 animals per group. * P<0.05 compared to sham using ANOVA.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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Effects of neurotensin on small intestinal afferent fibre activity in the rat

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The tridecapeptide neurotensin is located in high concentrations within specialised neuroendocrine cells (N cells) predominantly within the ileum. Neurotensin is released from the intestine in response to lipid infusion; however, the effects of neurotensin on extrinsic sensory nerves innervating the gut have not been characterised.

Experiments were performed in male Sprague Dawley rats (300-450g) anaesthetised with sodium pentobarbitone (bolus 60 mg/kg i.p.; followed by 10-50 mg/kg/h i.v.). Femoral artery and vein were cannulated to facilitate the measurement of blood pressure and administration of anaesthetic, respectively. The jugular vein was cannulated for drug administration and the trachea cannulated to maintain a patent airway. A laparotomy was performed and a 5 cm segment of small intestine cannulated with saline-filled tubing. Resting intestinal pressure was raised to 2mmHg with saline. A mesenteric nerve bundle was dissected and attached to one pole of a platinum wire electrode with a strip of mesentery attached to the second pole. The nerve activity was amplified, filtered and recorded as a rate histogram. Changes from baseline in nerve activity and mean blood pressure are expressed as mean ± sem. Statistical comparisons were performed using Student's paired t test.

Neurotensin (3 nmol/kg i.v. n=5) elicited a robust increase in afferent activity (101.1 ± 10.6 spikes/s) and a decrease in mean blood pressure (46.3 ± 2.9 mmHg). A significant ($p<0.05$) desensitisation of the afferent fibre (70.6 ± 12.0 spikes/s) and depressor response (18.6 ± 3.8 mmHg) to neurotensin was observed following a second repeat injection. However, subsequent injections of neurotensin showed no further desensitisation enabling a dose-response relationship to be determined.

Injection of increasing doses of neurotensin (0.1, 0.3, 1.0, 3.0 and 10 nmol/kg i.v., n=5) elicited a dose-related increase in afferent nerve activity with the exception of 10 nmol/kg at which no further increase was observed (e.g. 18.5 ± 3.0 spikes/s at 0.1 nmol/kg; 57.7 ± 4.6 spikes/s at 1.0 nmol/kg and 45.6 ± 4.2 spikes/s at 10 nmol/kg).

The results of this study demonstrate that exogenous application of neurotensin excites visceral afferent fibres innervating the small intestine. These data support the hypothesis that neurotensin has a role in signalling sensory information from the gastrointestinal tract.