Chapter I – Literature Survey. Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme, converting heme to CO, biliverdin and free iron. Biliverdin is subsequently reduced to bilirubin by biliverdin reductase (BVR). Three HO isoforms have been identified: the stress-inducible HO-1 and the constitutive HO-2/HO-3 isoforms which are expressed under basal conditions. The HO-2 isoform is widely expressed in the gastrointestinal (GI) tract and more specifically in myenteric neurons, interstitial cells of Cajal (ICCs) and mucosal epithelial cells; co-localisation of HO-2 with neuronal NO synthase (nNOS) has been reported in different GI tissue preparations. Based on the observation that enteric non-adrenergic non-cholinergic (NANC) neurotransmission was impaired in HO-$2^{-/-}$ mice, the suggestion was made that CO might function as an inhibitory (i)-NANC neurotransmitter in the enteric nervous system (ENS). Furthermore, other studies reported a possible interaction between the HO/CO and NOS/NO signalling pathways. The adaptive response of HO-1 to various stimuli suggests that HO-1 may play an important role in conferring protection against different types of stress. Accordingly, HO-$1^{-/-}$ mice show an increased systemic inflammatory response and decreased survival after lipopolysaccharide (LPS) challenge and renal ischemia, whereas HO-1 induction has been shown to protect tissues against inflammatory and oxidative stress injury. Emerging evidence reveals that CO can exert diverse biological and cytoprotective effects. In the GI tract, CO inhalation has been shown to reduce ischemia/reperfusion (I/R) injury of intestinal grafts and chronic colitis in IL-$10^{-/-}$ mice. In addition, it has been reported that CO inhalation protects against the development of postoperative ileus and necrotising enterocolitis. The recent discovery that certain transition metal carbonyls function as CO-releasing molecules (CO-RMs) in biological systems highlighted the potential use of this class of compounds as stratagem to deliver CO for therapeutic purposes.

Chapter II. The **aim** of our experimental work was to investigate the role of the HO/CO signalling pathway in the GI tract under normal and pathological conditions; the latter was investigated in a murine model of postoperative ileus (POI).

Chapter III. In a first study, we investigated the role of the HO/BVR system in i-NANC neurotransmission in murine gastric fundus and jejunum. Previous studies have shown that both HO-2 and BVR are expressed in ICCs and co-localized with nNOS in a large proportion of myenteric neurons along the GI tract. Neither HO inhibition by chromium mesoporphyrin (CrMP) nor co-incubation with CO or biliverdin/bilirubin affected nitrergic neurotransmission – i.e. relaxations induced by NANC nerve stimulation or exogenous NO – under normal physiological conditions. However, biliverdin/bilirubin reversed the inhibitory effect of the superoxide generator LY83583 on exogenous NO-induced relaxations in both tissues. When gastric fundus muscle strips were depleted of the endogenous antioxidant Cu/Zn superoxide dismutase (SOD) by the Cu-chelator DETCA, electrically induced NANC relaxations were also affected by LY82583; however, biliverdin/bilirubin could not substitute for the loss of Cu/Zn SOD when this specific antioxidant enzyme was depleted. In jejunal muscle strips, the combination DETCA plus LY83583 nearly abolished contractile phasic activity and, hence, did not allow studying nitrergic relaxation in these experimental conditions. In conclusion, our data do not establish a role for HO/CO in enteric NANC neurotransmission under normal physiological conditions. However, the antioxidants biliverdin/bilirubin might play an important role in the protection of the nitrergic neurotransmitter against oxidative stress.

Chapter IV. Although we did not confirm a role for CO as an endogenous NANC neurotransmitter in the GI tract, it is well known that CO relaxes a number of GI preparations when administered exogenously. Similar to NO, CO has been reported to act via activation of soluble guanylyl cyclase (sGC), leading to an increase in cGMP levels. Our study confirms the involvement of sGC in CO-evoked responses, as 1/ the sGC inhibitor ODQ (10 µM) abolished CO-induced relaxations; 2/ the sGC sensitizer YC-1 was able to potentiate CO-evoked inhibitory responses; and 3/ the relaxant response evoked by CO (300 µM) was associated with a significant increase in cGMP. Remarkably, CORM-2-induced relaxations were only partially reduced by ODQ – even at 100 µM – suggesting that CORM-2 mediates GI smooth muscle relaxation in both a sGC-dependent and sGC-independent manner. The mechanism(s) of relaxation 'downstream of sGC/cGMP' involved K_{Ca} channel activation as well as other unspecified mechanisms. Remarkably, the NOS inhibitor L-NAME also significantly reduced CO- and CORM-2-evoked relaxations in jejunum, but had no effect on the relaxations in gastric fundus. Collectively, these results indicate that CO and CORM-2 cause enteric relaxation via sGC and the subsequent activation of K_{Ca} channels, but an additional sGC-independent mechanism is required for CORM-2; in addition, a NO-mediated amplification of CO signalling in jejunum was suggested.

Chapter V. As sGC was identified to be the main target of CO-mediated relaxations in the GI tract, we wanted to investigate the role of the $sGC\alpha_1\beta_1$ and $sGC\alpha_2\beta_1$ isoforms in the relaxant effect of CO and CORM-2 in murine gastric fundus using wild-type and $sGC\alpha_1^{-/-}$ mice. In wild-type mice, CO (bolus)-induced relaxations were abolished by the sGC inhibitor ODQ, while CORM-2- and CO (infusion)-induced relaxations were only partially inhibited by ODQ. In $sGC\alpha_1^{-/-}$ mice, relaxant responses to CO and CORM-2 were significantly reduced when compared to wild-type mice, but ODQ still had an inhibitory effect. The sGC sensitizer YC-1 was able to potentiate CO- and CORM-2-induced relaxations in wild-type mice, but lost this potentiating effect in $sGC\alpha_1^{-/-}$ mice. Both in wild-type and $sGC\alpha_1^{-/-}$ mice, CO-evoked relaxations were associated with a significant cGMP increase; however, basal and CO-elicited cGMP levels were markedly lower in $sGC\alpha_1^{-/-}$ mice. These data indicate that besides the predominant $sGC\alpha_1\beta_1$ also the less abundantly expressed $sGC\alpha_2\beta_1$ isoform plays an important role in the relaxant effect of CO in murine gastric fundus; however, the sGC stimulator YC-1 loses its potentiating effect towards CO in $sGC\alpha_1^{-/-}$ mice. Prolonged administration of CO – either by the addition of CORM-2 *or* by continuous infusion of CO – mediates gastric fundus relaxation in both a sGC-dependent and sGC-independent manner.

Chapter VI. A novel method for the evaluation of intestinal transit and contractility in mice using fluorescence imaging and spatiotemporal motility mapping was introduced in order to facilitate the study of GI motility in our following experiments.

Chapter VII. Recent evidence indicates that a complex cascade of inflammatory responses within the intestinal muscularis can be attributed as the root cause of POI following intestinal manipulation (IM). The anti-inflammatory mediator HO-1 is induced as part of the postoperative muscularis inflammatory milieu and treatment of mice, rats, and pigs with inhaled CO can substantially prevent POI. In our study, IP administration of water-soluble CO-releasing molecules significantly reduced the development of POI in mice; the inactive compounds (iCO-RMs) – which do not release CO – did not provide any protection. When studying the mechanism(s) of action of the most effective compound – i.e. CORM-3 – we demonstrated that CORM-mediated protection against POI was associated with a down-regulation of pro-inflammatory mediators, iNOS activity as well as a decrease in leukocyte recruitment into the muscularis externa of the manipulated bowel. Importantly, these protective effects appear to be, at least in part, mediated through induction of HO-1 – in a p38-dependent manner – and a reduction of IM-induced ERK1/2 activation. Administration of CORM-3 was also shown to reduce the early 'oxidative burst' in the mucosa following IM, thereby preserving the mucosal barrier integrity.

Chapter VIII. Recent studies reported that CO might exert its anti-inflammatory effects through the induction of PPARγ, a member of the nuclear hormone receptor superfamily. Besides its well-known role in glucose metabolism, PPARγ has indeed been shown to play a pivotal role in the regulation of inflammatory/immune responses. Surgical manipulation induced a rapid phosphorylation and subsequent degradation of PPARγ within both the mucosa and muscularis of the manipulated colon. Accompanying these modifications, there was a decrease in PPARγ DNA-binding activity which was significantly restored by rosiglitazone treatment. The functional severity of POI was significantly ameliorated in mice pre-treated with rosiglitazone; this was associated with a down-regulation of inflammatory parameters, iNOS/COX-2 enzyme activity as well as a decrease in leukocyte recruitment into the intestinal muscularis of both colon and jejunum. These anti-inflammatory effects were preceded by a PPARγ-dependent inhibition of Egr-1, a key regulator of inflammatory gene expression. In addition, rosiglitazone markedly reduced the early 'oxidative burst' in the mucosa following colonic manipulation; an effect that appeared to be independent of PPARγ. In conclusion, these data demonstrate that PPARγ occupies a key role in the pathogenesis of POI and that rosiglitazone prevents POI by suppression of the muscularis inflammatory cascade through a PPARγ-dependent down-regulation of Egr-1.

In conclusion, our results do not establish a role for the HO/CO signalling pathway in i-NANC neurotransmission in murine gastric fundus and jejunum under normal physiological conditions. However, both our *in vitro* and *in vivo* data suggest that the HO/CO pathway may be of benefit in the prevention and/or treatment of GI motility disorders caused by free radicals such as POI, septic ileus, intestinal I/R injury and diabetic gastroparesis. Future studies should further unravel the underlying molecular mechanism involved in HO/CO-mediated cytoprotection, with the main focus on heme-containing proteins (sGC, cytochrome *c* oxidase, Nox) and several transcription factors (PPARγ, Egr-1, HIF-1α).