Pathophysiology of stress-induced visceral hypersensitivity
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Chapter 6

Summary and conclusions
SUMMARY AND CONCLUSIONS

Next to being one of the most common, irritable bowel syndrome (IBS) is also one of the least understood gastrointestinal disorders. It is a functional bowel disorders identified only by symptoms. Patients experience abdominal pain or discomfort, associated with defecation or a change in bowel habit. Importantly, visceral hypersensitivity (i.e. increased perception of gastrointestinal stimuli) is observed in the majority of patients and considered a pathophysiological mechanism. Although reversal of visceral hypersensitivity may proof beneficial for these patients, effective treatment options are lacking because mechanisms relevant to hypersensitivity are ill defined. Stress, however, was shown to induce enhanced sensitivity to distension in IBS patients and this knowledge can be used as a starting point for novel directions in research. We used the maternal separation model in rat, combined with acute stress at adult age, to obtain a better understanding of mechanisms relevant to visceral hypersensitivity.

In chapter 2 we confirmed earlier reports on the relevance of mast cell degranulation for post stress visceral hypersensitivity; pre-stress administration of the mast cell stabilizer doxantrazole prevented its occurrence. A similar result was obtained by peri-stress nerve growth factor (NGF) neutralization with antiserum. These results substantiated earlier observations obtained by Barreau et al., who already suggested that mast cell derived NGF is relevant to the observed phenotype. Importantly, NGF is a modulator of the transient receptor ion channel 1 (TRPV1) and, on the basis of enhanced immunohistochemical staining in tissue samples, Akbar et al. suggested that TRPV1 may be relevant in IBS pain perception. Therefore, we next assessed TRPV1 expression levels and its in vivo relevance in our rat model. Evaluating retrograde labelled and microdissected dorsal root ganglia sensory neurons we could not show enhanced TRPV1 transcription in MS rats. Neither did we observe increased numbers of TRPV1 positive neurons by immunohistochemical staining. Nevertheless, the use of capsazepine as well as the selective antagonist SB-705498 indicated that mast cell induced hypersensitivity to distension does depend on TRPV1. Together these data suggested that mast cells, possibly via the release of NGF, modulated TRPV1 responses without affecting TRPV1 expression levels. We herewith identified mast cells and TRPV1 as possible targets for future therapeutical intervention strategies in IBS.

Based on investigations in preclinical stress models others suggested that peripheral corticotrophin releasing hormone (CRH) is an important trigger for stress-induced mast cell degranulation in colon. Earlier animal experiments already indicated the relevance of brain expressed CRH for the post stress
phenotype. Consequently, two large clinical trials were performed with CRH-receptor antagonists but, surprisingly, they both failed to show the expected results.\textsuperscript{11,12} In response to these failures, Professor Michael Camilleri (Mayo Clinic, Rochester, USA) suggested that ‘the degree of stress experienced by patients attending a clinic is not as severe as that of a rat avoiding water!’\textsuperscript{13} In other words, animal models of stress-induced IBS-like complaints may not be relevant for the human situation. In \textbf{chapter 3} we set out to establish why these earlier experiments failed to predict the negative outcome of CRH-receptor antagonist studies in IBS patients. Interestingly, although a range of different stress protocols was used in these previous studies, they showed one important commonality; successfully tested CRH-receptor antagonists were always given in a pre-stress setting. Using $\alpha$-helical CRF (9-41) we were able to confirm these findings. However, when we used the same antagonist in a post-stress treatment protocol we failed to reverse established visceral hypersensitivity. In contrast, the mast cell stabilizer doxantrazole was capable of reversing the hypersensitive phenotype. Two important conclusions can be drawn from this study a) results obtained in older CRH receptor antagonist experiments were correct but probably not relevant because patients require reversal instead of prevention. b) If CRH is not required for post stress mast cell activation, other factors are. We hypothesized, but did not investigate, that these could be luminal antigens (bacterial or food derived). Future identification of receptors relevant to recognition of such antigens may provide novel therapeutic targets for IBS.

In line with preclinical animal investigations, several lines of evidence obtained with human tissue also suggested that mast cells are relevant in IBS.\textsuperscript{14-16} Therefore, our group at the AMC conducted a double blind placebo controlled trial with the mast cell stabilizer ketotifen.\textsuperscript{17} Although clinical results obtained in this study were positive, \textit{ex vivo} investigation also suggested that ketotifen did not act as a mast cell stabilizer. Since ketotifen is also a histamine-1-receptor (H1R) antagonist we hypothesized that this was the more likely mechanism of action. However, ketotifen does not have high specificity for the H1R and, because it crosses the blood brain barrier, may cause central side effects. Therefore, in \textbf{chapter 4}, we decided to evaluate second generation H1R antagonists in our animal model. These antagonists show enhanced H1R specificity and are peripherally restricted.\textsuperscript{18} In chapter 3 we already showed that 1 hour of water avoidance stress was able to induce long term (up to one month) mast cell dependent visceral hypersensitivity in maternal separated rats. Since reversal of hypersensitivity is the ultimate goal in patients, we evaluated fexofenadine and ebastine in a post stress intervention protocol. Both compounds successfully reversed the IBS-like phenotype. Further, although we only obtained a limited set of data (on expression of occludin), our results also suggested that barrier function is restored by antagonist treatment. Based on these results we suggested that H1R antagonists, initially developed for the treatment of allergic rhinitis, should also be evaluated in IBS. This trial was recently
conducted at the Catholic University of Leuven by the group of Prof GE Boeckxstaens. The use of ebastine resulted in significant improvement in global symptom relief, abdominal pain and quality of life compared to placebo.19

Whether the most important contributing factors in IBS are genetic or environmental in nature is an important question that influences general directions in IBS research. So far, the search for single nucleotide polymorphisms associated with increased risk for IBS was not very successful20,21 and twin studies also suggested that environmental triggers are probably more important than genetic factors.22,23 Nevertheless, IBS does cluster in families.24,25 This may suggest that each generation is exposed to a trigger factor independent of the previous generation, or, the environment induced phenotype can be transferred vertically from one generation to the next. We investigated the latter hypothesis in our rat model of maternal separation (discussed in chapter 5). In contrast to humans, a rodent model has the advantage that subsequent generations can be investigated under controlled conditions and in a relatively short timeframe. We could show that susceptibility to stress induced visceral hypersensitivity in maternally separated (F1) rats can be transferred to the next (F2) generation without further separation protocols. Our cross-fostering experiments indicated that the observed transfer depends on maternal care: pups adapted to the phenotype of the foster mother. This suggests that in utero mechanism do not play an important role. In our experiments we did not investigate other, possibly causal, mechanisms like e.g. transfer of milk born factors or an ‘IBS micro biome’ from mother to child. We did however explore the role of mast cells in post-stress visceral hypersensitivity of the F2 offspring. Similar to earlier findings in F1 rats, the mast cell stabilizer doxantrazole was capable of reversing the IBS-like phenotype in F2 animals. Together these findings indicate that maternal separation can be used to investigate cross-generational effects of environmental IBS-triggers.

In conclusion, pre-clinical investigations presented in the current thesis indicated that stress-induced visceral hypersensitivity depends on mast cell activation, initially triggered by peripheral CRH. We also showed that prolonged, post stress activation of these cells no longer depends on this stress hormone and suggested that this may explain the failure of clinical trials that were carried out with CRH-receptor antagonists. Consequently, we strongly feel that future identification of post stress triggers for mast cell activation will lead to novel therapeutic strategies. An alternative approach to the identification of novel treatments is to define mast cell mediator/receptor interactions relevant to the observed hypersensitive phenotype. Here, we presented evidence that the H1R is an imported target and this was recently confirmed in a clinical trial. Histamine as well as NGF may exert their effect via modulation of TRPV1. Although our results with a specific TRPV1 antagonist suggest that this ion channel could also be a target, its role in the regulation of body temperature may block future use of
this type of drug in IBS. Finally, an important strategy that is often neglected in IBS research is to aim for prevention instead of treatment. Our model of transfer across generations can be used to delineate and intervene with environmental triggers and mechanisms that may be relevant to IBS clustering in families.
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