Substance P and the neurokinin 1 receptor

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Summary and Conclusions

This thesis discussed the function of the neuropeptide Substance P from the molecular to the behavioral level. Substance P is abundantly distributed in the brain and belongs to the neurokinine family. When it was discovered that antagonists for the neurokinine NK_1 receptor, to which Substance P preferentially binds, have antidepressant properties in animal models, both the preclinical and clinical research was intensified. The first open label clinical studies with the NK_1 antagonist MK 869 were indeed promising, but the initial results could not be reproduced in a larger double-blind placebo-controlled study. Two factors may have contributed to the disappointing results in the latter study. Firstly, the focus in antidepressant research is primarily on monoaminergic systems. This focus has been ongoing for so many years that this may have led to a certain emphasis on monoamine related symptoms of depression, excluding other factors that could play a role in the biological mechanisms which are responsible for major depression. This would have possibly left some marks on the questionnaires used in clinical studies, which are used for measuring the effectiveness of antidepressant treatment. The tree-shrew studies in the first part of this thesis may also be instructive in this respect. The classic monoamine antidepressant clomipramine, a drug that primarily affects the monoaminergic system, was used as a control compound. Initially it shows that an NK_1 antagonist displays similar efficacy when compared with important aspects of our model. However, some differences were observed, especially on immune system function parameters. This might indicate that NK_1 antagonists are less efficacious with certain symptoms of depression, but it also illustrates that potential new types of antidepressants, which target other neuronal systems, can't shown the same efficacy in treating depression, since they target other, not taken into account, symptoms. The disappointing results with glucocorticoid receptor antagonists in major depression may also point in that direction. Secondly, the efficacy of the traditional monoamine-based antidepressants is far from satisfactory. The effectiveness of antidepressants barely exceeds placebo response according to a large meta-analysis of clinical studies in major depression. This is not only caused by the complexity and considerable heterogeneity of the disease, but it also indicates that it is far from trivial to demonstrate antidepressant efficacy in clinical studies. The moderate efficacy of the classic antidepressants and the lag time of the therapeutic response have inspired researchers to investigate augmentation strategies to improve their antidepressant effect. When it was discovered that an NK_1 antagonist augmented the effect of an SSRI on extracellular 5-HT levels in prefrontal cortex of mice, a revived interest in NK_1 antagonists for treating depression occurred. Augmentation strategy for treating depression is already pursued by targeting classical antidepressant in combination with compounds that exert their effect on monoaminergic receptors. However, preclinical research continued and it showed that large differences exist with respect to the NK_1 receptor between species. The same turned out to be the case for SP neuronal architecture. This species variation is likely to have a major impact on the pharmacology of NK_1 antagonists. In addition, the differences in neuronal architecture suggest that Substance P is

involved in different central processes in the various species. This is indeed supported by our study in guinea pigs wherein augmentation of 5-HT levels was observed in ventral hippocampus but not prefrontal cortex, indicating that one should be cautious with generalizing SP data from mice to other species. The study in guinea pigs also demonstrates the need for more fundamental research to fully comprehend the role of Substance P in the brain. This requires a reliable *in vivo* assessment of Substance P, with both the sampling and the analysis being optimized.

In chapters 2-4 of this thesis the effect of chronic treatment with the NK_1 antagonist L-760,735 on psychosocial stress in tree shrews is studied, using the antidepressant clomipramine as a reference. Phylogenetically, tree shrews are in between insectivores and primates and thus more closely related to humans than rodents are. The tree shrew model is based on social defeat and has been validated extensively with antidepressants from various pharmacological classes. Moreover, it has excellent construct and face validity and a variety of behavioral and biological parameters can be studied.

In chapter 2 the endocrine and behavioral responses are studied. Psychosocial stress in tree shrews induces HPA-axis hyperactivity, which is also seen during chronic stress and depression in humans. Furthermore a traumatic or stressful life event is seen as a trigger for depression. Behaviorally, locomotor activity, marking and grooming behavior are reduced by chronic psychosocial stress in tree shrews. Moreover, submissive animals display significantly more freezing behavior when confronted with a dominant male. Both clomipramine and L-760,735 were capable of reversing the impaired HPA-axis activity and behavior. The normalization of the behavioral parameters by clomipramine can be explained by its antidepressant properties, since anxiolytics were not capable of normalizing of the chronic psychosocial stress induced changes, indicating that L-760,735 has antidepressant properties.

In chapter 3 the effects of L-760,735 on neuroplasticity and brain metabolism are studied. Psychosocial stress in tree shrews has a major effect on brain plasticity: the hippocampus decreases in size and neurogenesis is diminished in dentate gyrus. Brain metabolism, as measured by magnetic resonance spectroscopy, is also reduced in stressed animals. Treatment with clomipramine or L-760,735 was able to partially restore brain plasticity and metabolism. L-760,735 even appeared to be more effective than clomipramine.

In chapter 4 the effect of L-760,735 on the disturbed immune function caused by psychosocial stress was studied and compared to effects of the antidepressants clomipramine, fluoxetine and tianeptine. Immune function was determined by *in vitro* activation of splenocyte proliferation by the T-cell mitogen concavalin A. In control animals chronic psychosocial stress increased [^{3}H]thymidine uptake in splenocytes approximately 5 fold. Only fluoxetine and tianeptine were able to significantly reverse the increased proliferation. Spleen weight was found to be reduced in stressed animals as well, but only clomipramine and tianeptine were able to normalize this effect.

In chapter 5 the effect of combined administration of a SSRI with a NK_1 antagonist on extracellular serotonin levels was investigated in the guinea pig prefrontal cortex and ventral hippocampus. Most antidepressants exerts their pharmacological effect on the serotonin system and previous microdialysis studies had demonstrated that NK_1 antagonists are capable of augmenting the effect of SSRIs on extracellular serotonin levels in the prefrontal cortex of rats and mice, it was suggested that NK_1 antagonists might improve therapeutic efficacy of SSRIs. We were able to replicate the rodent findings using fluoxetine and the NK_1 antagonist GR 205171 in the guinea pig ventral hippocampus but not in prefrontal cortex. These data support the idea that SP neuronal architecture varies among species. Also, they imply that extreme caution must be taken when translating animal data to different species, and to the clinic.

Chapter 6 investigates dynamic behavior of Substance P in microdialysis and the *in vitro* recovery. *In vivo* concentration determination of Substance P is indispensable for full comprehension of the neuropeptide's role in the central nervous system. Although several microdialysis studies on Substance P have been previously reported, microdialysis has never been properly validated for neuropeptides. To this end we investigated the performance of several microdialysis membranes. It appeared that the physicochemical properties of the membrane and their pore size are critical to achieve detectable levels of Substance P to measurable dynamic behavior of the neuropeptide. It was possible to achieve detectable levels of Substance P with both poly-ether-sulphon (PES) and cellulose membranes, but only when bovine serum albumin was included in the perfusate to minimize non-specific binding of Substance P to the sampling system. However, PES was the only membrane that also met our criteria regarding the dynamic response to potassium stimulation *in vivo*.

Chapter 7 compares the conventional RIA analysis of Substance P with a newly developed LC-MS/MS method. RIA is sensitive but cross reactivity with related peptides or degradation products complicates interpretation of data. Moreover,

changes in ion strength that occur during *in vivo* assessment might influence the affinity of the antibody for the peptide. We have developed a coupled LC-MS/MS method that is both more sensitive and more selective than RIA for Substance P. The methods were compared, using microdialysates from striatum and dorsal raphe nucleus of freely moving rats. On average, levels of Substance P were four times higher in RIA then when analyzed by LC-MS/MS. However, the relative increase following potassium stimulation in dorsal raphe nucleus was comparable between the methods. This suggests that with RIA degradation products of Substance P are also cross-reacting when determining Substance P levels. Infusion of tetrodotoxin, elevation of the calcium ion concentration or omission of calcium from the perfusate had no effect on Substance P dialysate levels, indicating that the detection of neurotransmission of Substance P does not meet the classical neurotransmitter release criteria. Our data indicate that critical evaluation of the analysis method is important when determining neuropeptide levels in *in vivo* experiments.

Conclusion

Several aspects of Substance P have been investigated in this thesis. NK_1 antagonism appeared to fulfill several criteria for antidepressant-like behavior in the tree shrew model. The NK_1 antagonist was able to augment the effect of SSRIs on extracellular serotonin levels in the ventral hippocampus of the guinea pig but not in the prefrontal cortex. Some inconsistencies in the current literature may be related to species differences in NK_1 receptor function and Substance P neural architecture. Clearly, more research is required to fully understand the role of Substance P in the central nervous system. One important step forward in neuropeptide research, as presented in this thesis, is the development of an *in vivo* assessment method for Substance P that can be used to quantify the peptide reliably in freely moving animals.