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The Brain-derived Neurotrophic Factor / TrkB-Receptor Pathway as a Possible New Target for the Pharmacotherapy of Anorexia Nervosa
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Medical background
Anorexia nervosa is a life-threatening disease which affects mostly young females. It starts as a psychiatric disorder but often ends as a serious metabolic syndrome. It has the highest mortality rate of all psychiatric disorders which can mainly be attributed to metabolic disturbances. The loss of body weight is due to a reduced fat and lean body mass as a consequence of a negative energy balance. This is mainly caused by the patients' deliberate reduction in food intake. In a large proportion of patients energy expenditure is increased by exaggerated physical activity and contributes to the negative energy balance.

Currently patients are treated with psychotherapy. Neuroleptics are occasionally used but there is no effective pharmacotherapy available. Effective drug treatment of anorexia nervosa would be highly desirable at least to break the vicious cycle in severely ill patients and to stabilize their condition to such an extent that they can further improve on continued psychotherapy.

Experimental background
In recent years remarkable progress has been made in the understanding of the regulation of energy balance. Several central mediators that influence energy intake or expenditure have been identified and characterized. One of the most important pathways starts with the synthesis of leptin, a small protein, in adipose tissue (for review see: Hofbauer et al., 2007). Leptin is produced in direct relation to the number and amount of fat cells. After being released into the blood it reaches the brain where it activates specific receptors. Subsequently this signal is translated via downstream mechanisms and finally results in a reduction in appetite.

The first of these downstream mechanisms is the synthesis of pro-opiomelanocortin, the precursor of alpha-melanocyte stimulating hormone. This peptide acts as an agonist of the melanocortin-4 receptor (MC4R) in the hypothalamus, reduces appetite and increases energy expenditure and thereby in the long term leads to a reduction in body weight. Conversely, blockade of the MC4R increases appetite and body weight.

In previous studies we confirmed earlier observations that brain-derived neurotrophic factor (BDNF) is the second of these downstream mechanisms and mediates the activation of the MC4R by stimulating so-called TrkB receptors (TrkBR). Our results

indicated that most if not all of the effects of the MC4R on appetite are mediated via BDNF and its TrkBR (Nicholson et al., 2007).

Working hypothesis

The molecular mechanisms underlying the dysregulation of energy balance in anorexia nervosa have not yet been identified but central appetite-regulating pathways are likely to play an important role in its pathophysiology. Patients with anorexia nervosa deliberately suppress their appetite. It can be assumed that this involves the stimulation of endogenous appetite suppressing systems, i.e. the leptin pathway. Blockers of this pathway should therefore oppose the appetite suppressing behavior and thereby increase energy consumption. Moreover, such blockers may also influence the energy expenditure of these patients by reducing their increased physical activity.

The MC4R and TrkBR would be suitable pharmacological targets for the blockade of the leptin signaling pathway. A lack of function of one of these receptors due to genetic disorders results in severe childhood obesity. Conversely it may be expected that pharmacological blockade of these receptors in anorexia nervosa may lead to an increase in body weight.

In a recent study on an experimental model of anorexia nervosa evidence for an over-expression of hypothalamic MC4Rs was found and the authors concluded that “MC4R antagonists … could improve the clinical management” of this disease (Gutiérrez et al., 2009). This notion is supported by our previous finding that a synthetic MC4R antagonist prevented the loss of lean body mass in a cancer cachexia model (Nicholson et al., 2006).

Blockade of the BDNF/TrkBR mechanism could also be therapeutically relevant (Bariohay et al., 2009). A contribution of BDNF to the pathophysiology of anorexia nervosa seems possible (Mercader et al., 2007; Ehrlich et al., 2009; Saito et al., 2009) but its precise role has not yet been elucidated.

Aims of the project

The main aim of the project was to evaluate two possible targets for pharmacotherapy, the MC4R and the TrkBR, by using antibodies (Abs) as pharmacological tools. In a previous study we demonstrated that immunization of rats against the N-terminal domain of the MC4R, a domain which is essential for the basal, constitutive activity of the MC4R, induced a mild obese phenotype associated with insulin resistance (Peter et al., 2007). In subsequent experiments we have produced monoclonal antibodies (mAbs) directed against the same sequence of the MC4R. One of these mAbs, 1E8a, showed pharmacological activity as an inverse agonist and non-competitive antagonist and reduced the activity of the MC4R in vitro. From this mAb we prepared a scFv fragment and demonstrated that it crossed the blood brain barrier, reached the hypothalamus and increased food intake and body weight after intravenous administration (Hofbauer et al., 2008, Peter et al., 2012) patent application filed).

Experimental plan

Since it is known that a high percentage of patients with anorexia nervosa not only restrict their energy intake but also increase their energy expenditure by an exaggerated physical activity an efficient pharmacotherapy should correct both disturbances. The existing

literature suggests that MC4R-blockade may lead to an increase in energy intake but has no effect on physical activity. By contrast TrkBR-blockade may also reduce physical activity. For this reason our project focused on anti-TrkBR antibodies. The following subprojects were planned:

- Generation and pharmacological characterization of polyclonal Abs, mAbs and scFv derivatives against the TrkBR.

- Comparative target validation studies of MC4R- and TrkBR-blockade in an acute cachexia model.

- Proof-of-concept studies in an experimental model of anorexia nervosa in collaboration with Prof. M.J. Kas at the University of Utrecht, NL.

These studies should allow us to find out whether either or both of these two principles, MC4R- and/or TrkBR-blockade, might be suitable concepts for the clinical treatment of anorexia nervosa**.**

Experimental results

Financing by the SANS made it possible to work on all three planned subprojects. The results are presented in detail in two reports, the interim report of 24.02.2011 and the final report of 24.07.2012 and are briefly summarized below:

- Immunization against a peptide fragment of the TrkBR in rats resulted in the generation of polyclonal antibodies from which Fab fragments could be produced. As expected, the antibodies and their Fab derivatives inhibited the activity of TrkBR *in vitro*. Immunization of mice with the same peptide resulted in the generation of an inhibitory mAb against the TrkBR from which a scFv derivative was produced.

- In *in vivo* experiments polyclonal anti-TrkBR antibodies stimulated food intake in rats under basal conditions. The mAb and its scFv derivative showed comparable effects.

- In a mouse model of anorexia nervosa, the so-called activity-based anorexia (ABA) model polyclonal anti-TrkBR antibodies were applied into the cerebral ventricles and compared with anti-MC4R antibodies. Neither the anti-MC4R- nor the anti-TrkBR antibodies had an influence on food intake. However, the mice treated with the anti-TrkBR antibodies showed a significant reduction of their exaggerated locomotor activity. This behavioral effect could be of therapeutic benefit in the treatment of patients with anorexia nervosa.

These experimental results are subject of the following two publications:

Peter, J.-C. et al., Anti-TrkB antibodies as pharmacological tools to study the function of the TrkB receptor and its role in the regulation of food intake, Pharmacology, in press

Peter, J.-C. et al, Protective effects of an anti-melanocortin-4 receptor scFv derivative in lipopolysaccharide-induced cachexia in rats. J Cachexia Sarcopenia Muscle, published online: 22 August 2012

A patent has been filed in collaboration with the Technology Transfer Unit of the University of Basel:

Antibodies against tropomyosin-related kinase B receptors, US patent application filed, 30.06.2012.

Conclusions

Our experiments provided several positive results:

- We succeeded in producing novel, pharmacologically active anti-TrkBR antibodies and their derivatives.
- These antibodies proved to be effective inhibitors of the TrkBR in various *in vitro* and *in vivo* models.
- In the ABA model in mice the anti-TrkBR antibodies showed a therapeutically useful behavioral effect.
- The scientific value of our observations is demonstrated by the acceptance of two manuscripts in international journals.
- A patent application secures the rights for further development and possible therapeutic application in patients with anorexia nervosa.

An unexpected negative result concerned the lack of effect of anti-MC4R and anti-TrkBR antibodies on food intake in the ABA model. This may be due to specific properties of this disease model, the mouse strain used in these experiments or a species difference between mice and rats, the species in which effects on food intake had been observed under basal conditions and in an acute cachexia model. These open questions should be addressed in further studies which are summarized in the subsequent section.

Outlook

Since anti-TrkBR antibodies showed a therapeutically relevant behavioral effect in the ABA model and are protected by a patent application the further evaluation of their potential seems warranted. For this reason the preclinical proof-of-concept should be continued to provide rational decision criteria for further development.. These studies should be performed in the laboratories of Prof. M. Kas in Utrecht and are summarized in his project proposal „Request for financing of studies in a rodent model of anorexia nervosa. M.J. Kas, 23.07.2012”. This proposal includes studies to reproduce the previously observed effects in the same and in a different mouse strain and studies in a rat model to find out whether food intake may be influenced by the treatment with anti-MC4R and anti-TrkBR antibodies in this species.

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