Author's response to reviews

Title: Central neuropeptide Y receptors are involved in 3rd ventricular ghrelin induced fasted motor activity of the colon in conscious fed rats.

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Author's response to reviews: see over
Dear Sirs,

Please find enclosed our revised manuscript “Central neuropeptide Y receptors are involved in 3rd ventricular ghrelin induced alteration of colonic transit time in conscious fed rats” by JJ. Tebbe, CG. Tebbe, S. Mronga, M. Ritter, and M. K-H. Schäfer which have revised according to the reviewers´criticisms.

Here is our detailed response to reviewers´concerns.

Response to reviewer #1 (Akio Inui):

Point 1: - The authors used the word “fasted motor activity” in the title, but they actually measured colonic transit time.

**Response:** We have revised the title according to the reviewers criticisms as follows:

“Central neuropeptide Y receptors are involved in 3rd ventricular ghrelin induced alteration of colonic transit time in conscious fed rats”.

Point 2: - BIBP-3226 may produce central depressive effects in contrast to BIBO-3304 developed later. Could the authors show the evidence that BIBP-3226 acted specifically in their system at the dose used?

**Response:** In the present study we used the NPY Y1 receptor antagonist BIBP-3226 in a supraphysiological concentration. We have demonstrated in recent studies that the dosages used are effective to block the Y1 receptor in the CNS [Tebbe JJ. et al.: Am J Physiol Gastrointest Liver Physiol. 2003 Dec;285(6):G1075-83]. This is supported by binding-studies released by Raposino et al. [Endocrinology. 1999 Sep;140(9):4046-55]. Moreover it has been shown that the selective NPY-1 receptor antagonist BIBO-3304 is less effective at the Y1 receptor in comparison to the selective NPY-1 receptor antagonist BIBP-3226 [Kask A. et al.: Can J Physiol Pharmacol. 2000 Feb;78(2):143-9]. Thus we used the Y1 receptor...
antagonist BIBP-3226 to be on the safe side to get the most effective blockage of the NPY Y1 receptor. In our hands no central depressive effects or conspicuous behavior was observed after BIBP-3226 treatment in awake rats [Tebbe JJ. et al.: Am J Physiol Gastrointest Liver Physiol. 2003 Dec;285(6):G1075-83].

We have included this points in the methods (page: 5 and 6) as follows:

“The used intracerebroventricular concentrations of the receptor antagonists were comparable with the ICV-dosages used by other groups in rodents [20].” (page 5)

and

“In our hands nanomolar concentrations of BIBP-3226 and BIIE-0246 were effective in antagonization of NPY receptor subtypes without any side effects. In particular no central depressive effects or conspicuous behavior was observed after BIBP-3226 treatment [18].” (page 5-6)

**Point 3:** - *Labeling of figures 1 and 2 is wrong.*

**Response:** We agree with the reviewer that the label of figure 1 and 2 are interchanged. The figure labels have been corrected as suggested by the referee. (see figure legends)

**Point 4:** - *The authors should refer not only Ref. 23 but also Ref. 1 (and potentially …) to indicate that NPY Y1 receptor mediates ghrelin induced feeding.*

**Response:** The citations have been corrected. In addition we have followed the reviewer advice suggestions to use more appropriate citations to support our statements and have changed the manuscript accordingly. (see: Background)

**Point 5:** - *It is already reported that ghrelin induces fasted motor activity in the stomach and the duodenum of conscious fed rats via the vagus nerve. NPY Y2 receptors appears to be involved in this action. The authors need to include these findings and to discuss in more detail the potential mechanisms by which ghrelin stimulates colonic motor functions.*

**Response:** We have revised the discussion according to the reviewers criticisms and incorporated the facts mentioned above as follows:

“(…) In this context Fujino et al. have recently demonstrated that the ghrelin induced fasted gastroduodenal motor activity in rats is blocked by ICV injection of GHS-R antagonist as well as NPY antiserum [9]. The results presented by Fujino et al. also suggest that the vagal pathway may mediate the action of centrally administered ghrelin on gastroduodenal motility [9]. Thus we can speculate that central NPY pathways, e.g. centrally NPY receptor activation, are the primary downstream mediator of circulating ghrelin. (…)” (see page 13)

and

For example NPY released from ARC neurons activates NPY-Y1 receptors in the hypothalamus, e.g. the PVN, and results in the stimulation of GI motor function [18]. Furthermore arcuate NPYergic neurons have been thought to regulate feeding behavior by NPY receptor subtypes Y1 and Y5 in the PVN and adjacent areas [17]. Pretreatment with a Y1, but not other receptor antagonist markedly inhibited ghrelin-induced feeding, pointing to NPY receptor Y1 as one of the downstream pathways (…)Thus we can speculate that circulating ghrelin modulates gastrointestinal motility via activation of hypothalamic, in
particular by using NPYergic pathways via activation of NPY-Y1 receptors, in the arcuate nucleus. (…)” (see page 14)

Response to reviewer #2 (Theo Peeters):

Point 1: - Background. Omit reference 10 at line 13

Response: We have omitted the reference 10 at line 13 in the revised manuscript.

Point 2: - Results. Figures 1 and 2 are interchanged. Vertical axis legends overlap with labels. Horizontal axis labels to figure 1 should be doses instead of A, B, C etc.. Abbreviation MI is not in general list and it took me a while to realize it was micro-injection and is explained in the legend. Actually it is superfluous. Drop it.

Response: We agree with the reviewer that the label of figure 1 and 2 are interchanged. The figure labels have been corrected as suggested by the referee.

Point 3: - Tables give the same data as the figures. Drop the tables.

Response: We have omitted the tables as suggested by the referee.

Point 4: - Discussion. “…as well as NPY antiserum [10]” Should be [11].

Response: The citation has been corrected as suggested by the referee.

Point 5: - Put always periods after brackets of reference numbers.

Response: The position of the periods have been corrected as suggested by the referee.

Point 6: - I think the data, as well as other studies, suggest that ghrelin acts on NPY neurons. It is therefore in my opinion nor correct to say that “… ghrelin unfolds….by acting on central NPY1 but NPY2 receptors”. Besides the word NOT is missing there. This kind of confusing is repeated a few times in the discussion. See also the paragraph conclusions for example.

Response: We have now focused our discussion in the revised manuscript on the different role of NPY Y1 and Y2 receptor subtypes in central effects of ghrelin. In this context the description is now unambiguous. (see discussion).
We hope that our revised manuscript can now be judged suitable for publication in BMC Gastroenterology and would like to thank you and the reviewers' again for their constructive critical comments.

Yours sincerely,

Dr. Johannes J. Tebbe