Author’s response to reviews

Title: Liraglutide modulates GABAergic signaling in rat hippocampal CA3 pyramidal neurons predominantly by presynaptic mechanism

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Version: 1 Date: 19 Sep 2017

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"The authors' response letter has been included as a supplementary file"

Uppsala, Sweden
2017-09-19.

Dear Editor,

We thank the reviewers for their comments and suggestions. Our response to the reviewers is here below. In the manuscript we have highlighted in yellow our changes and the text that is relevant to our answers. We have also added Fig. 5 that shows the amino acid sequence of GLP-1, exendin-4 and liraglutide.

We hope the manuscript can now be accepted by BMC Pharmacology and Toxicology.

Best regards,

Bryndis Birnir,
Response to Reviewers

Francesco Clementi (Reviewer 1)

The AA report a further detail on the possible GABA release from CA3 neurons of the hippocampus slices after application of liraglutide, a GLP-1 analog. These results confirm and enlarge those already published by the same AA with the use of GLP-1 and exenatide.

The involvement of GLP-1 in the control of hippocampal functions is an interesting issue that deserves to be investigated. The new finding here reported is that in a limited population of neurons liraglutide probably increases the frequency and amplitude of iPSCs and that this effect is not affected by TTX. Why do you need kinurenic act?

Kynurenic acid is an antagonist at excitatory glutamate receptors; AMPA, NMDA and kainate receptors. In order to study only the GABAergic synaptic transmission kynurenic acid is used to block the glutamate synaptic transmission. This is a standard procedure in the synaptic transmission field and is now clarified on page 4, line 83.

I found that the scientific information supplied by this paper is not sufficient to merit an independent publication. The paper does not give any indications of the possible nature of the neurons involved, on the mechanisms by which liraglutide induces GABA release, on the reasons why this drug is less potent than GLP-1 and exenatide and the meaning of this difference.

Although the GLP-1 analogs are used interchangeable there is a growing literature showing that effects of these compounds may differ somewhat. CA3 pyramidal neurons in the rat hippocampus are primary relay neurons but vary in size and some electrical properties. We found that only in a part of the CA3 neurons can liraglutide modulate the GABA signaling. These results were unexpected although even GLP-1 and exenatide varied somewhat in their effects but not as strikingly as liraglutide. That agonists differ in potency/efficacy at receptors is common although the mechanisms for this varies widely e.g. ease of induced conformational changes, membrane fluidity where the receptor resides, access to binding site, ease of activation of intracellular cascades as induced conformations may vary and may also vary in stability. The mechanism by which liraglutide induces GABA release may be somewhat similar to mechanism by which activation of GLP-1 receptor leads to insulin secretion described for pancreatic beta-
cells and we refer to (see e.g. ref. [29]). In brief, binding of GLP-1 receptor agonist to its receptor in beta-cell activates the G-alpha type of G protein which activates adenyl cyclase, induces cAMP level elevation and eventually stimulates hormone secretion, see page 10, line 180.

Whether we have identified a particular subpopulation of CA3 neurons or if the effect is related to concentration of the intracellular messenger proteins or something else awaits further studies. But it is very important to acknowledge now that the effects of the GLP-1 receptor agonists on the brain can vary as these drugs are already in clinical use, see page 10 line, line 188.

Fang Xie (Reviewer 2)

In this manuscript, authors examined effects of a GLP-1 analog liraglutide on GABA signaling in CA3 hippocampal pyramidal neurons. Only 100 nM liraglutide could change the sIPSC frequency and the most probable amplitudes significantly. Furthermore, these effects in neuron induced by 100nM liraglutide were inhibited upon tetrodotoxin treatment. Authors concluded that liraglutide regulation of GABA signaling of CA3 pyramidal neuron is predominantly presynaptic, but limited compared with GLP-1 and exendin-4.

1. To help potential readers to better understand these chemicals, you might consider to add a specific figure that aligning/showing the molecular structures of GLP-1, exendin-4 and liraglutide.

We have now added a cartoon highlighting the differences between the GLP-1 receptors agonists. See here below and page 9, Line 165.

Figure 5. Amino acid sequences of GLP-1, exendin-4 and liraglutide. Differences in amino acids are highlighted with orange ovals and violet letters. DPP-IV protease cleavage site is marked. C-16 fatty acid (palmitic acid) is linked to the peptide through glutamic acid spacer enabling binding to albumin and, thereby, preventing degradation by DPP-IV.

2. You concluded that liraglutide regulation of GABA signaling of CA3 pyramidal neuron is more limited than has been observed for GLP-1 and exendin-4 in hippocampal neurons. References about GLP-1 and exendin were also cited, but why didn't you compare GLP-1 and exendin with liraglutide in your experiments? Both chemicals would be very important controls to support your current conclusions.

Once the GLP-1 receptor is activated, the intracellular cascades are started and will last for some time. If we apply GLP-1 first, an effect or not of liraglutide is unconclusive as we can never be sure if: (1) the effect was related to the first compound applied i.e. GLP-1, or (2) if no effect is
observed, then if the first compound i.e. GLP-1, has desensitized the receptor or caused it to be internalized. Therefore applying two or more of these agonists to the same cell does not result in clean answers and, therefore, we have not done these experiments.

3. Would you like to discuss more about why only 100 nM liraglutide could take significant effects (sIPSC and the most probable amplitudes) in pyramidal neurons?

It seems that concentrations lower than the 100 nM used in our experiments are not high enough to trigger the response that modulates the GABA signaling system, and concentrations larger than 100 nM may desensitize the GLP-1 receptor, and thus, we again have no effect of liraglutide on GABA signaling. We have now clarified this in the article, page 10, line 176.

Sisi Qin (Reviewer 3):

This paper reported the modulation of liraglutide in GABA signaling. The study is well controlled and designed. The only unclear thing is there are two sources to obtain liraglutide in the method. It will be better to specify the source of liraglutide for each different experiment, in order to get a more reliable comparison and consistent conclusion.

First, we did experiments with the liraglutide from Bachem, and saw effects on GABA signaling only with 100 nM liraglutide and no other concentrations. We decided to confirm these results using liraglutide from another source and that is why we asked for the compound from the producer Novo Nordisk. The results were very similar with liraglutide from the two sources and are mixed together in our results/Figures.