Whole-brain distributional differences of lipidated Exendin-4 peptides

Grethe Skovbjerg [1], Henrik H. Hansen [1], Urmas Roostalu [1], Casper G. Salinas [1], Jacob L. Skytte [1], Jacob Jelsing [1], Lisbeth Elster [1], Camilla K. Frich [1], Jacob Hecksher-Sørensen[1] . Contact of the corresponding author: gsk@gubra.dk

1: Gubra ApS, 2970, Hørsholm, Denmark

Background

Peptide drug development is challenged by the presence of the blood-brain barrier (BBB) that effectively blocks most neurotherapeutic peptides from reaching their target receptors. Accordingly, centrally acting peptide based anti-obesity drugs show suboptimal efficacy due limited CNS access. While peptide lipidations are typically applied for increasing circulating half-life through binding to albumin, such modifications may also significantly influence CNS drug delivery.

Objectives

Using whole-brain quantitative 3D light-sheet fluorescence microscopy (LSFM) imaging, we compared brain-wide distribution patterns of lipidated isoforms of Exendin-4, an anorectic glucagon-like peptide 1 (GLP-1) receptor agonist.

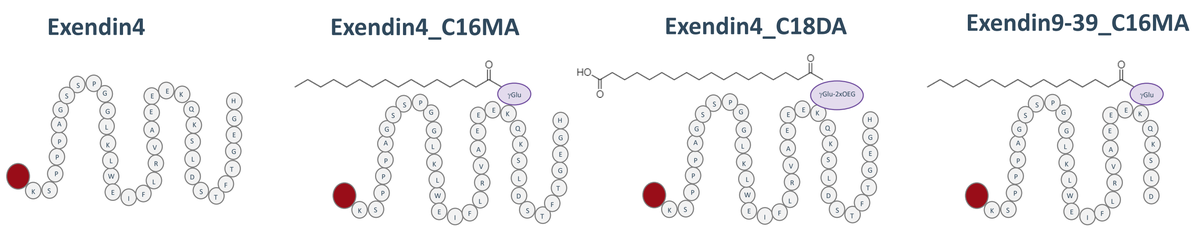


Figure 1: Structures of the fluorescently labelled peptides. ●: IR800 fluorophore ○: chemical linker

Methods

In vivo distribution of lipidated Exendin-4 peptides

30 C57BL/6JRj mice were intravenously injected with either vehicle or 100 nmol/kg fluorescently labelled Exendin peptides with different lipidations 2h before termination. The study involved 5 groups of mice with 5 animals in every group (Ex4, Ex4_C16MA, Ex4_C18DA, Ex9-39_C16MA).

Sample treatment

At termination, the mice were perfusion fixed and brains and pancreata were dissected. The tissues were cleared according to iDISCO protocol. Subsequently, the samples were imaged using LaVision Ultramicroscope II setup. Images were acquired in a z-stack at 10 µm intervals in autofluorescence and peptide-specific channel. Image analysis was used to quantify signal intensity per brain region and within beta-cell islets of the pancreata.

Statistical analysis

Following sample processing and imaging, an optimized LSFM mouse brain atlas was aligned to individual mouse brain datasets (Perens et al., Neuroinformatics, 2021)[1] and the total fluorescence signal was quantified for every brain region. For statistical analysis, a negative bionomial generalized linear model was fitted to the data and Dunnett's test as well as p-value adjustment was performed.

Results

Whole-brain peptide distribution

Group overview

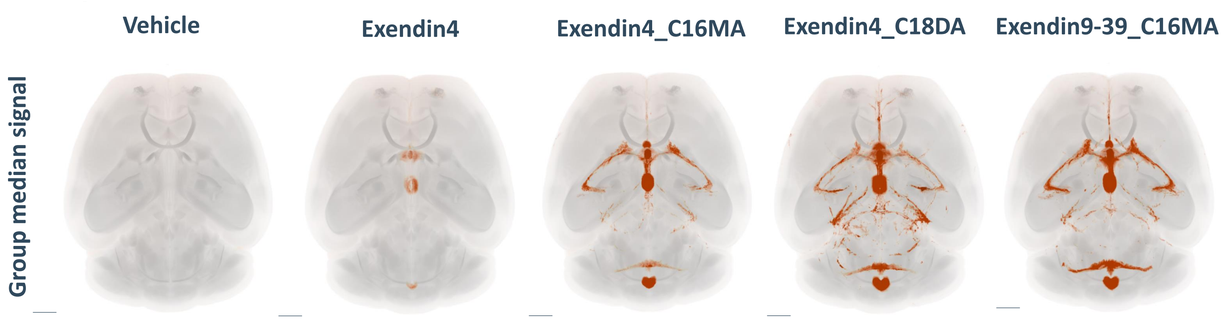


Figure 2: Overview of the median IR800 signal in each group (n=5 per group) mapped to the average mouse brain atlas. Scale bar 500 µm.

Brain regions showing accumulation of fluorescently labelled drug

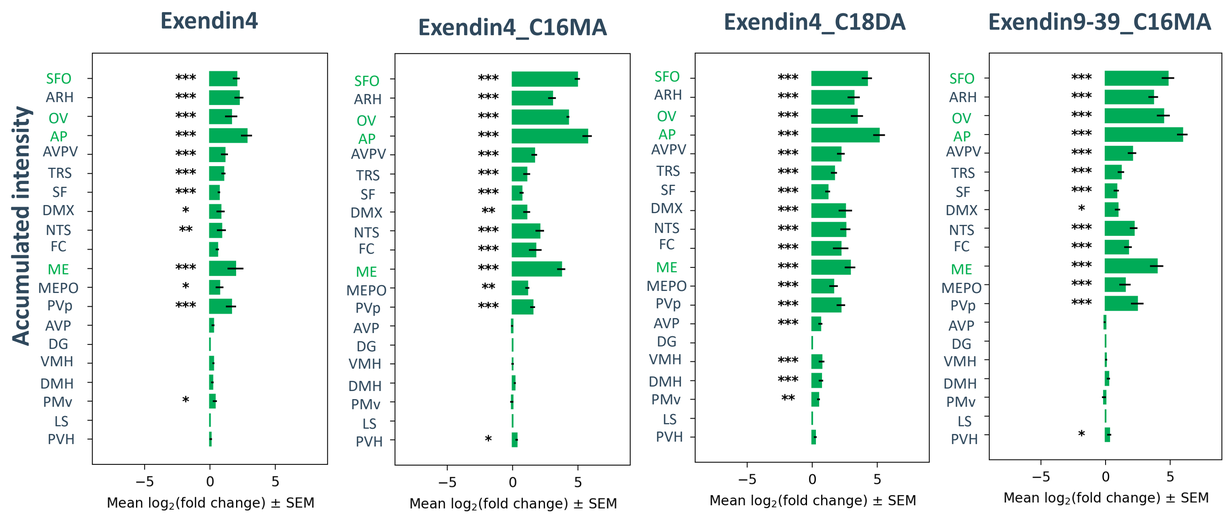


Figure 3: Top 20 brain regions with significantly altered accumulated fluorescence signal intensity for every group compared to vehicle. Brain regions noted in green refer to circumventricular organs. Brain region abbreviations obtained from Allens Brain Atlas.

Fluorescently labelled drug accumulation in the PVH

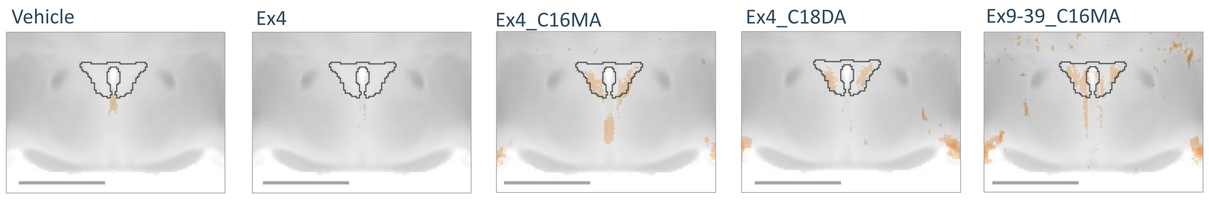
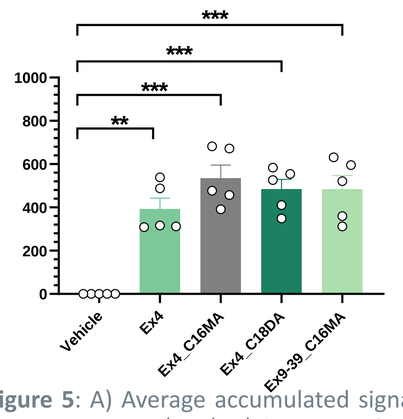


Figure 4: Average fluorescence signal for every group in the PVH. Significantly increased fluorescence signal observed for lipidated exednin4 and exendin9-39. Scale bar 500 µm.

Receptor potency and pancreatic labelling

A) Mean pancreatic β-cell intensity



B) GLP-1 receptor potency

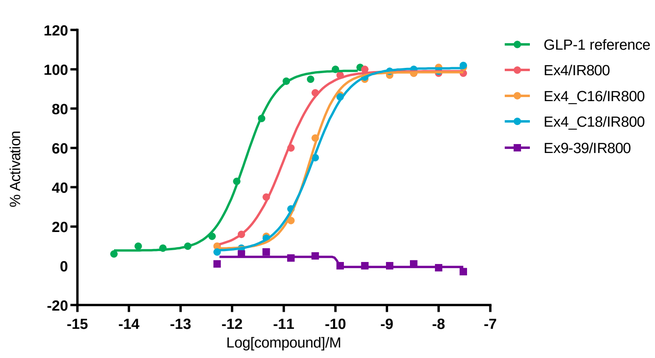


Figure 5: A) Average accumulated signal intensity across pancreatic beta-cell islets from each animal compared between groups (n=5). B) Concentration-response curves for each fluorescently-labelled peptide tested in a cAMP in vitro assay on GLP-1 receptor expressing cell line. Native human GLP-1 used as reference. Retained agonistic profile observed for C16MA and C18DA lipidated exendin4. No cAMP stimulation observed for the antagonist (Ex9-39_C16MA).

Differential drug distribution in circumventricular organs

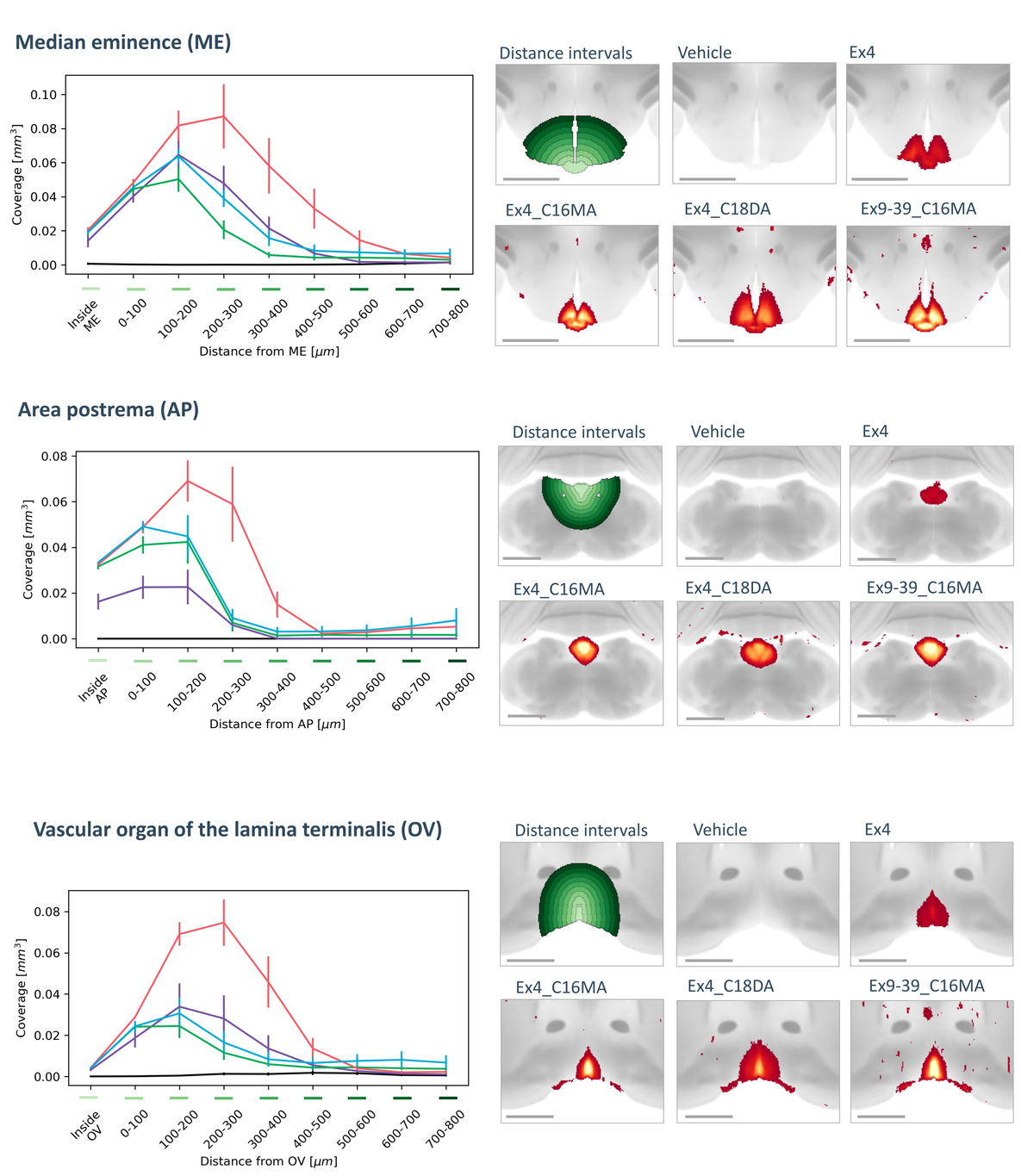


Figure 6: (Left) Coverage vs. distance plots for circumventricular organs. The distance from region center is divided in intervals with color codes corresponding to snapshot illustration. (Right) Coronal sections illustrating signal coverage of each group. Scale bar 500 µm.

Conclusion

- Unmodified Exendin4 distributes to several permeable circumventricular brain areas, including the AP, NTS and ARH.
- 16-carbon monocarboxylic acid side-chain lipidation of Exendin-4 (Exendin4_C16MA) significantly increased brain distribution of Exendin-4 with signal accumulation in the PVH.
- 18-carbon dicarboxylic acid sidechain lipidation of Exendin-4 (Exendin4_C18DA) further increases distribution of Exendin4 from the CVOS into deeper lying brain structures, including the DMH and VMH.

[1]Perens, J., Salinas, C.G., Skytte, J.L. et al. An Optimized Mouse Brain Atlas for Automated Mapping and Quantification of Neuronal Activity Using iDISCO+ and Light Sheet Fluorescence Microscopy. Neuroinform 19, 433–446 (2021). https://doi.org/10.1007/s12021-020-09490-8