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FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

Functional differences of iodinated obestatin peptides: in vitro metabolic stability profile.

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INTRODUCTION

Peptides constitute a new group of promising drugs, with a diverse range of pharmacological activities. One of their most promising targets is the Central Nervous System (CNS), where the Blood-Brain Barrier (BBB) constitutes an important flux-regulating compartment. In order to measure BBB transport influx constants, radiolabeld peptide tracers are used. However, it is reported that enzymatic degradation as well as deiodination occurs *in vitro* and *in vivo* [2, 3]. This phenomenon should be taken into account since the deiodinated metabolite(s) can cause the biologic activity. Therefore, the *in vitro* stability of different iodinated mouse obestatin derivatives is characterised in the main metabolic compartments: plasma, liver and kidney.

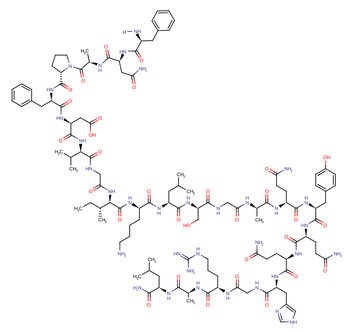
EXPERIMENTAL

In-vitro metabolic stability

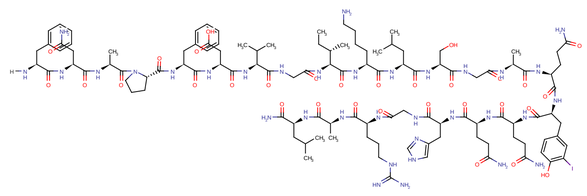
Peptides are incubated at 37°C in mice brain, plasma, liver and kidney tissue homogenates using a mixing block heater. Aliquots are taken at suitable time points, and analyzed (after acidification, heating at 95°C and centrifugation) using HPLC. Results are expressed as half-life times, calculated from the regression curves.

Typical HPLC-UV conditions

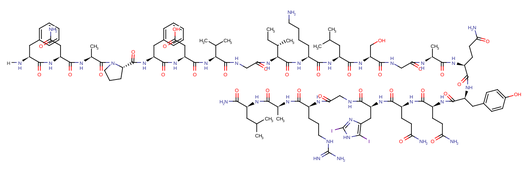
Everest C_{18} 238EV54 (250 × 4.6 mm, 300 Å, 5 µm) column (Grace Vydac) at 30°C, with (A) 0.1% formic acid in water, and (B) 0.1% formic acid in acetonitrile. A gradient program was used and the flow rate was set at 1.0 ml/min. Detection was permorded with PDA (190-400 nm).



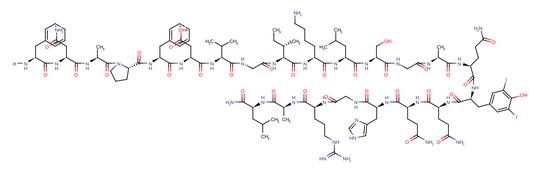
Mouse obestatin



I_1Y mouse obestatin



I_2H mouse obestatin



I_2Y mouse obestatin

RESULTS AND DISCUSSION

Typical results are given here. For the BBB transport, only the results for EM-2 are given here, as the other peptides were successfully analyzed using the same methodology (data not given in this presentation).

Table 1: In-vitro metabolic stability half-lives

T ½ (min)	EM-1	EM-2	CTAP
Liver	13	18	225
Brain	205	88	119
Kidney	8	14	101
Plasma	7	4	60

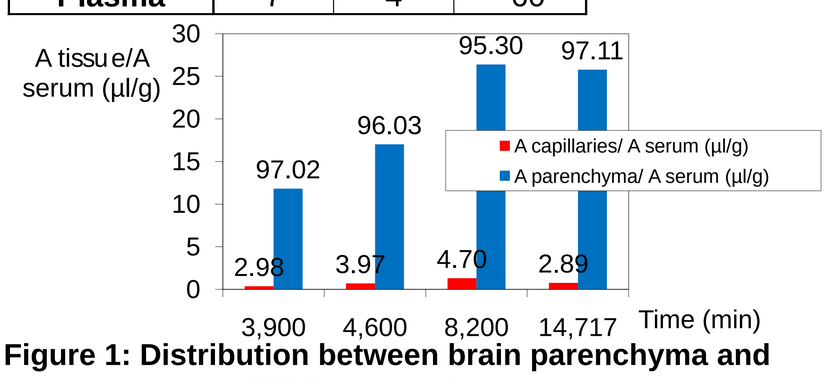


Figure 1: Distribution between brain parenchyma and capillaries in the EM-2 brain influx experiment

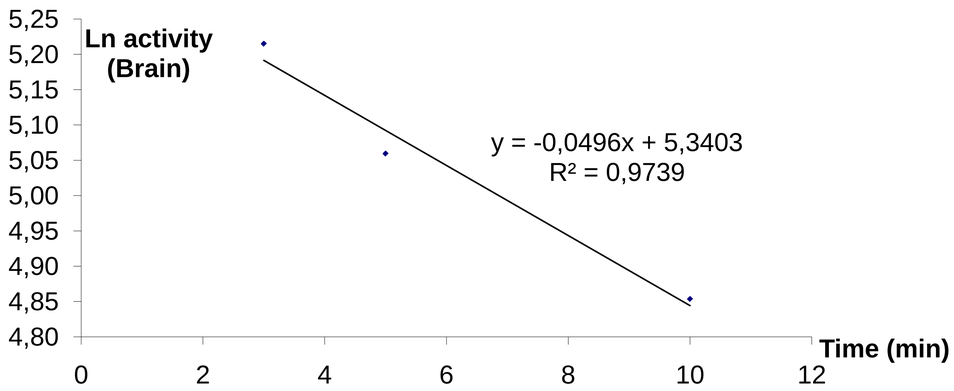


Figure 2: The efflux of EM-2 from brain.

CONCLUSIONS

Significant differences in the degradation kinetics of the iodinated peptides, arising from both enzymatic proteolysis and deiodination, were found when compared to the native, unmodified peptide. LC-MS/MS analysis demonstrated that the cleavage sites were dependent upon the biological matrix and the location of the amino acid residue incorporating the iodine atom(s). The degrading enzymes were found to target peptide bonds further away from the iodine incorporation, while proteolytic cleavages of nearby peptide bonds were inhibited. Di-iodinated amino acid residue containing peptides were found to be more susceptible to deiodination than the mono-iodinated derivative.

REFERENCES

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2. Abbruscato, T.J., et al., *Blood-brain barrier permeability and bioavailability of a highly potent and mu-selective opioid receptor antagonist, CTAP: Comparison with morphine.* Journal of Pharmacology and Experimental Therapeutics, 1997. **280**(1): p. 402-409.