

Receptor and blood-brain barrier characterization of opioid peptides in drug research & early development

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Introduction

The opioid receptors (ORs) are known to be distributed widely in the central nervous system (CNS) and in peripheral sensory and autonomic nerves. Activation of ORs by endogenous and exogenous ligands results in a multitude of physiological functions and behaviors, *e.g.* pain and analgesia, stress and social status, tolerance and dependence, learning and memory, eating and drinking, and many more [1]. Due to this widespread pharmacological profile of ORs, opioid peptides are becoming key players in the pharmaceutical industry, more specifically in research and development of pain modulating agents. Among the opioid receptor subtypes, the μ -opioid receptor subtype is the main target due to its essential contribution to control pain (*i.e.* narcotic analgesics used in clinic are all agonists of the μ -opioid receptor subtype) [2]. For analgesics to target ORs in the CNS and exert medical activity, opioid peptides should penetrate the blood–brain barrier (BBB), with limited efflux behavior, have a favorable receptor-subtype selectivity and sufficient metabolic stability.

Results and Discussion

The opioid receptor-subtype selectivity can be assessed not only by the classic radioligand binding methods, but also by novel techniques such as SAW (surface acoustic wave) measuring the binding kinetics. Pharmacokinetics include metabolic stability, brain influx and efflux characteristics, as well as brain capillary retention. Metabolic stability is evaluated *i.a.* by *in vitro* kinetic studies using different target tissues. Using *in vivo* mouse models, the influx transfer constant from serum into mouse brain is determined by multiple time regression, while the efflux kinetics are investigated with the intra-cerebroventricular injection technique. Furthermore, the brain parenchyma/capillary distribution is evaluated by the capillary depletion method. Finally, the *in vivo* antinociceptive activity can be quantified in a mouse model.

During these initial research and discovery phase, the peptide quality and its stability characteristics are often neglected, possibly leading to misinterpretation of biological results, and thus are important factors to avoid false functionality conclusions [3].

Results evaluating the requested and labeled (supplier's certificate of analysis) *versus* the experimentally determined quality of 46 peptides from one supplier were problematic. The quality of more than 30% of the evaluated peptides was below 90% compared to the requested 95% purity. This confirms a previous study where the quality of one peptide from different suppliers was also found to be problematic [7]. Moreover, these impurities do influence the functionality, as demonstrated by the observed baseline contraction of guinea pig ileum longitudinal smooth muscle in a tissue organ bath test which was due to the impurities and not to the peptide INSL6[151-161] itself [3].

The stability of peptides during *ex vivo* experiments was also evaluated, demonstrating that some remained stable but others were chemically and/or physically (adsorption to tissue/glass) unstable and thus unable to exert their full functionality.

In order to have a good antinociceptive activity, the BBB characteristics of opioid peptides should be favorable. Information about the BBB behavior of peptides, including the opioids, is scattered throughout the literature, with a wide variety of different study protocols being used. Therefore, the currently available BBB-data are collated in the database Brainpeps, which can *i.a.* be used for QSPR analyses [4]. Moreover, the CNS-functional drugability of a set of opioid peptides was comparatively scored using a Derringer's desirability function combining the different drugability requirements into a single figure-of-merit [5]. The overall *in vivo* antinociceptive effect of these opioid peptides was also investigated using a tail-flick mouse model [6]: the obtained *in vivo* results correlated well with the ranking from the desirability criterion.

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