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Characterization of the novel opioid and nociceptin peptides

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Opioid system is consists of μ, δ, κ and nociceptin (NOP) receptors and their respective endogenous neuropeptide ligands. In this study we have characterized novel non-mammalian opioid peptides Ile-enkephalin and Phe-enkephalin, and artificial NOP receptor partial agonist hexapeptide Ac-RYYRIR-ol.

Leu- and Met-enkephalin were the first endogenous opioid peptides identified in different mammalian species including the human. Comparative biochemical and bioinformatic evidence indicates that enkephalins are not limited to mammals. Lower vertebrate enkephalins were investigated with in vitro biochemical experiments using rat brain membrane preparations and turned out to be moderate affinity opioids with a definite preference for the δ-opioid receptor sites. Phe-enkephalin from the lungfish displayed low affinities toward the μ- and δ-opioid receptor, while exhibited moderate affinity toward the κ-opioid receptor. In [^{35}S]GTPγS binding studies, Met-enkephalin produced the highest stimulation followed by Leu-enkephalin, Ile-enkephalin from the clawed frog and Phe-enkephalin, was the least efficacious among these endogenous peptides (Bojnik et al. 2009a).

Some N/OFQ sequence unrelated hexapeptides can effectively bind to the NOP receptor and they were used as template for structure activity studies that lead to discovery of the new NOP selective ligands. The pharmacological profile of the novel hexapeptide Ac-RYYRIR-ol was investigated using various in vitro assays including receptor binding and G protein activation in rat brain membranes, mouse vas deferens, rat vas deferens, guinea pig ileum, mouse colon and calcium mobilization. In rat brain membranes Ac-RYYRIR-ol displaced [^{3}H]Ac-RYYRIK-ol (Bojnik et al. 2009b) with high affinity and stimulated [^{35}S]GTPγS binding with high potency. The stimulatory effect of Ac-RYYRIR-ol was antagonized by the selective NOP receptor antagonist UFP-101. In antagonist type experiments Ac-RYYRIR-ol inhibited the stimulatory effects induced by N/OFQ. In the electrically stimulated mouse vas deferens Ac-RYYRIR-ol displayed negligible agonist activity while antagonizing in a competitive manner the inhibitory effects of N/OFQ. In the mouse colon Ac-RYYRIR-ol produced concentration dependent contractile effects with similar potency and maximal effects as N/OFQ. Finally, in the Ca^{2+} mobilization assays Ac-RYYRIR-ol displayed lower potency and maximal effects compared with N/OFQ assays.

In conclusion, two novel, non-mammalian enkephalins were described and compared with those of the well-known Leu- and Met-enkephalin. Among the four structures tested, the 'mammalian type' Met-enkephalin exhibited the highest affinities in receptor binding assays and produced the most efficacious G-protein stimulation in brain membranes, while the newly identified 'lower vertebrate type' Ile- and Phe-enkephalins were found to be less effective. On the other hand, novel NOP receptor selective hexapeptide Ac-RYYRIR-ol has been shown to have fine selectivity, high potency, furthermore agonist and antagonist effects toward the NOP receptors were measured in various assays. This is likely due to its partial agonist pharmacological activity.

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Genetic modification of carotene producing Zygomycetes

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Carotenoids are terpenoid-type chemical compounds. These yellow to orange-red natural pigments are used in the food, pharmaceutical and cosmetic industry and as feed colour additives. Recently, they are attracting an increasing attention, due to their beneficial effects on health. In Zygomycetes fungi, β-carotene is the predominant carotenoid. Traditionally three species: *Blakeslea trispora*, *Phycomyces blakesleeanus* and *Mucor circinelloides* have been involved in the study of the carotene biosynthesis.

Mucor circinelloides has several characteristics advantageous for molecular genetic studies. For example, well functioning methods are available for the genetic transformation of this fungus based on autonomously replicating plasmids (Papp et al. 2008). However, integrative transformation methods are not well established and the fate of the transforming DNA has not yet been analyzed.

The aims of our work were (1) to investigate and compare the effect of overexpression of different isoprene biosynthesis genes for the carotene production; (2) to produce oxygenated β-carotene derivatives by heterologous expression of the *crtW* gene (encoding β-carotene ketolase) of the marine *Agrobacterium aurantiacum*; (3) to integrate the *crtW* gene into the *Mucor* genome by different methods; (4) and to reveal the carotenoid spectra and to characterize the carotenoid production of some Zygomycetes in order to determine new producer strains potentially applicable in further analysis.

Transformation of fungal protoplasts was carried out by the polyethylene glycol-mediated method. Three different isoprenoid genes were involved in the study. Expression vectors that contained one of these genes driven either by their own promoter or by the regulator