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Role of the sugar moiety on the opioid receptor binding and conformation of a series of enkephalin neoglycopeptides

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ABSTRACT

Glycosylation by simple sugars is a drug discovery alternative that has been explored with varying success for enhancing the potency and bioavailability of opioid peptides. Long ago we described two *O*-glycosides having either β-Glucose and β-Galactose of (D-Met^2, Pro^5)-enkephalinamide showing one of the highest antinociceptive activities known. Here, we report the resynthesis of these two analogs and the preparation of three novel neoglycopeptide derivatives (α-Mannose, β-Lactose and β-Cellobiose). Binding studies to cloned zebrafish opioid receptors showed very small differences of affinity between the parent compound and the five glycopeptides thus suggesting that the nature of the carbohydrate moiety plays a minor role in determining the binding mode. Indeed, NMR conformational studies, combined with molecular mechanics calculations, indicated that all glycopeptides present the same major conformation either in solution or membrane-like environment. The evidences provided here highlight the relevance for in vivo activity of the conjugating bond between the peptide and sugar moieties in opioid glycopeptides.

1. Introduction

Endogenous opioid neurotransmitters such as Met- and Leu-enkephalin represent one class of opioid receptor ligands that produce relatively non-selective agonist effects at both μ and δ receptors[1] but have inspired the design and development of many of both selective and non-selective μ/δ active peptides.[2] Although these peptides have played an important role in defining the pharmacological effects of combined μ/δ opioid receptor activation, natural and synthetic opioid peptides have historically been poor drug candidates, mainly because of their limited ability to cross the blood-brain barrier (BBB) that impairs their access to the corresponding central nervous system (CNS) sites of action after systemic administration.[3]

In spite of these hurdles, a number of structure-activity studies on opioid peptides suggest that glycosylation may facilitate biodistribution of these peptides across the BBB and enhance their potency after iv, ip or sc administration by producing centrally mediated behavioral effects.[4–6] A recent example is MMP2200, which is a glycosylated derivative of a Leu-enkephalin analog, that is approximately 10-fold more potent than the parent unglycosylated compound and twice as potent as morphine in producing antinociception in mice after systemic iv administration.[7–9]

Abbreviations: BBB, Blood brain barrier; CNS, Central nervous system; [3H]-DPN, [3H]-Diprenorphine; DMEM, Dulbecco's modified Eagle's medium; ENK, Enkephalin; HRMS, High resolution mass spectrometry; HPLC, High performance liquid chromatography; icv, intracerebroventricular administration; ip, intraperitoneal administration; it, intrathecal administration; iv, Intravenous administration; sc, Subcutaneous administration; Nx, Naloxone; NMR, Nuclear magnetic resonance; TSP, 2,2,3,3-Tetradeutero-3-trimethylsilylpropionic acid.

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However, when systemically administered in rhesus monkeys MMP2200 acted as peripheral μ/δ opioid receptor agonist with limited distribution to the central nervous system, suggesting the existence of species differences in the pharmacokinetics and BBB penetration of glycopeptides.[10]

These and other results on the opioid glycopeptide field are in good agreement with the more general realization that post-translational glycosylation of proteins and synthetic glycosylation of other peptides extend their half-life in serum,[11,12] enhance their potency,[13,14] and even influence their biodistribution,[15] including BBB penetration.[3,12–14,16–18]

Following this principle, a more than 20 years-old search for systemically active glycoopioids is now still under way and producing interesting compounds such as the above mentioned MMP2200. During this time, the initially difficult attempts to conjugate enkephalin analogs to sugar moieties have led to more amenable synthetic procedures to screen different glycosides of various complexities, most of them naturally occurring mono-, di- and trisaccharides. Also, different peptide to carbohydrate conjugation methods have been explored that range from native *O*- and *N*-glycosyl bond formation to chemical ligation at suitable functional groups on a rather limited number of enkephalin sequences. The analgesic potency of many of these compounds in mice is comparable to, or sometimes greater than, the potency of morphine after iv administration.[4] Regrettably, up to now, clear correlations between identity of the sugars, type and site of linkage to the peptide and enkephalin sequence with biological activity seem still far away.

We have contributed to these efforts by providing early examples of neoglycoenkephalins. In one case, we prepared a couple of neoglycopeptides by amide-linking a glucosyl-[19] and a galactosyl-amine residue[20] to the C-terminal of the μ-selective sequence H-Tyr-DMet-Gly-Phe-Pro-NH_2, that produces significant analgesic activity after systemic administration by acting upon both μ and δ receptors.[21] The Glc analog was about two orders of magnitude less potent by peripheral (ip) than by central (icv or it) administration in rats, but still about 2000 times more potent than morphine. By central administration (icv) the Gal analog was one order of magnitude more potent than the Glc analog and about 5000 times more potent than morphine. These data were obtained after experiments using the tail immersion analgesic test and were in fair agreement with the ones obtained on the paw pressure test.[19,20] In a second case, we synthesized a couple of positional analogs of the previous glycopeptides by *O*-glycosyl bond formation of Glc and Gal with the hydroxyl function of Hyp that substitutes Pro.[5] The most striking results were that the Gal analog was about 57000 times more potent than morphine, 1700 times more potent than the Glc analog and 10000 times more potent than the parent unglycosylated peptide, as assessed by the tail immersion analgesic test after icv administration in rats.[22] More recently, we have found that a α-mannoside of morphine is 100 fold more potent and twice long lasting as compared to morphine when ip injected to rats and assessed by the tail-flick and paw pressure analgesic tests.[23]

At the time of these early experiments with the glycopeptides, the molecular and structural characterization of opioid receptors have not been achieved yet, and cloning as well as the modern expression techniques were not available either. Thus, to shed light on the affinity and selectivity of these class of glycopeptides to bind to individual opioid receptor types and to study the role of the nature of the sugar moiety on their binding features, we have resynthesized the Glc and Gal *O*-linked Hyp[5] derivatives (**3** and **4**) above discussed and expanded these family with new compounds. Thus, owing to the improved pharmacological profile observed with our mannoside of morphine we have chosen to produce the enkephalin mannoside derivative **2**. Also, following other glycoopioid results that indicate that disaccharides seem to yield more dual δ and μ active compounds,[6] we have also prepared the corresponding *O*-linked glycopeptide analogs of lactose (β-Lac) **5** and cellobiose (β-Cel) **6**. The pharmacological properties of these new and the two old glycosylated derivatives (**3** and **4**) were assessed by radioligand binding assays on isolated μ and δ opioid receptors from zebrafish owing that this organism presents high receptor opioid homology respect to the human counterparts. A pilot study of the antinociceptive properties of the Man glycopeptide (**2**) was conducted on the tail-flick test, after ip administration in mice, to assess the potential permeability of this compound across the BBB. Finally, in an attempt to correlate their binding properties to their conformation in solution, NMR conformational studies combined with molecular mechanics calculations were also conducted.

2. Results and discussion

2.1. Chemistry

The five neoglycopeptides **2**–**6** depicted in Fig. 1 and their parent compound **1** were prepared by stepwise manual solid-phase peptide synthesis following standard Fmoc protocols from previously synthesized suitable glycosyl amino acid building blocks (Fig. 1).[24–27] The final products were purified (>98% by HPLC) and characterized by UPLC-TOF/MS. The analytical data from already reported products **3** and **4** which were previously prepared by solution phase methods was consistent with the new solid-phase synthesized materials.

2.2. Biological activity

As pointed out, CNS bioavailability is on the main focus on current glycoopioid studies, however, owing the mounting evidence of a functional interaction between μ and δ opioid receptors and a possible regulatory role for δ agonists, interests in opiod research

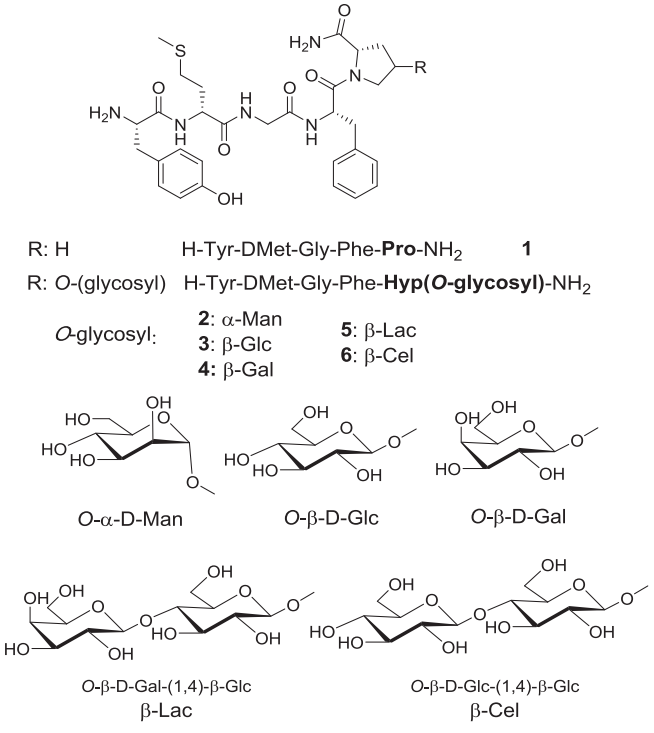

Fig. 1. Structures of the peptide (**1**) and glycopeptides (**2**–**6**) prepared in this work.