Antinociceptive Structure-Activity Studies with Enkephalin-Based Opioid Glycopeptides


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ABSTRACT

Development of opioid peptides as therapeutic agents has historically been limited due to pharmacokinetic issues including stability and blood-brain barrier (BBB) permeability. Glycosylation of opioid peptides can increase peptide serum stability and BBB penetration. To further define the requirements for optimizing in vivo antinociceptive potency following intravenous administration, we synthesized a series of enkephalin-based glycopeptides using solid phase 9-fluorenylmethyloxycarbamate methods. The compounds differed in the sixth and subsequent amino acid residues (Ser or Thr) and in the attached carbohydrate moiety. In vitro binding and functional smooth muscle bioassays indicated that the addition of mono- or disaccharides did not significantly affect the opioid receptor affinity or agonist activity of the glycopeptides compared with their unglycosylated parent peptides. All of the glycopeptides tested produced potent antinociceptive effects in male ICR mice following intracerebroventricular injection in the 55°C tail-flick test. The calculated A50 values for the Ser/Thr and monosaccharide combinations were all very similar with values ranging from 0.02 to 0.09 nmol/l. Selected compounds were administered to mice intravenously and tested for antinociception to indirectly assess serum stability and BBB penetration. All compounds tested produced full antinociceptive effects with calculated A50 values ranging from 2.2 to 46.4 µmol/kg with the disaccharides having potencies that equaled or exceeded that of morphine on a micromoles per kilogram basis. Substitution of a trisaccharide or bis- and tris-monosaccharides resulted in a decrease in antinociceptive potency. These results provide additional support for the utility of glycosylation to increase central nervous system bioavailability of small peptides and compliment our ongoing stability and blood-brain barrier penetration studies.

The addition of carbohydrate moieties to a protein (glycoprotein) or peptide (glycopeptide) produces changes in the molecular structure that, in turn, can have dramatic effects on the pharmacodynamic and/or pharmacokinetic properties of proteins and peptide hormones (Lis and Sharon, 1993). The glycopeptide follicle-stimulating hormone (FSH), for example, has multiple glycosylation sites and exists as a family of isoforms that differ with respect to their oligosaccharide structure (Ulloa-Aguirre et al., 1995; Stanton et al., 1996). The isoforms can differ substantially with respect to their in vitro and in vivo potency and efficacy (Barrios-De-Tomas et al., 2002). These observations have led to the modification of human FSH and other glycoproteins in an effort to improve pharmacokinetic properties while maintaining optimal activity. Site-directed mutations and the extension of the N terminus subunits with additional glycosylation sites are two approaches that have been used to increase the half-life of FSH (Perlman et al., 2003). This strategy has also been used to increase the half-life of erythropoietin, another glycoprotein, with at least one variant (darbepoetin-α) being approved for the treatment of anemia (Maedougall et al., 1999). Surprisingly, few studies have been done to explore glycosylation strategies in small peptides for therapeutic applications.

The development of neuropeptide drugs has been ham-

ABBREVIATIONS: FSH, follicle-stimulating hormone; BBB, blood-brain barrier; MVD, mouse vas deferens; GPI, guinea pig ileum.
pered by instability and poor BBB penetration. Several strategies have been used to overcome these obstacles, including substitution of unnatural amino acids (Hruby and Mosberg, 1982; Mosberg et al., 1982), the use of conformational constraints (Hruby, 1989), the design of highly potent peptide analogs (Handa et al., 1981; Horan et al., 1993), and the addition of lipophilic side groups or other transport vectors (Bodor et al., 1992; Rousselle et al., 2000). Although stability and binding issues have been successfully addressed, problems with transport and BBB penetration remain. Making the compound more lipophilic, for example, has proven to be self-limiting due to the aqueous environment of the transport medium (i.e., blood, cerebrospinal fluid) (Bodor et al., 1992). The addition of bulky transport vectors may also have adverse effects on peptide bioactivity, necessitating the incorporation of an enzymatic cleavage site into the transport vector to release the active peptide after transport (Rousselle et al., 2000).

It is reasonable to hypothesize that glycosylation of a small peptide will increase the hydrophilicity, stability, and bioavailability of peptides (Albert et al., 1993; Polt et al., 1994; Negri et al., 1998; Bilsky et al., 2000). In studies investigating peptides and proteins that had been glycosylated nonspecifically via the Amidori reaction, an increase in delivery to both the peripheral and central nervous system was observed (Poduslo and Curran, 1994). BBB penetration studies of glycopeptides have indicated up to a 3-fold increase in the rate of brain delivery of these compounds compared with the unglycosylated parent peptides (Bilsky et al., 2000; Egleton et al., 2000, 2001). Recent studies with glycopeptides in artificial membrane systems indicate that the amphipathicity of the glycopeptides may be an important factor in BBB penetration (Palian et al., 2003). In addition, there is evidence that suggests that the type of glycosylation can alter tissue distribution patterns (Susaki et al., 1999; Suzuki et al., 1999a,b), BBB penetration (Egleton et al., 2000; unpublished results), and peptide/receptor interactions (Gysin and Schwyzer, 1983; Sargent et al., 1988; Palian and Polt, 2001).

The subtle modifications of a glycopeptide may alter tissue and receptor specificity, potentially decreasing drug toxicity and side effects. In the opioid field, for example, there is considerable interest in developing compounds that have agonist actions at both δ- and μ-opioid receptors, with the optimal ratio of selectivity still needing to be determined in preclinical and clinical models (Gengo and Chang, 2004; Lipkowski et al., 2004). This prospect prompted our exploration of opioid glycopeptide chemistry and pharmacology using a linear hexapeptide (Tyr-$\delta$-Thr-Gly-Phe-Leu-Ser-CONH₂) that has affinity for δ and μ receptors. The current study is an extension of previous work showing that the glycosylation of the sixth residue of a linear opioid hexapeptide significantly increased the i.v. potency and in situ BBB permeability of the compound (Bilsky et al., 2000). We hypothesized that further modification of the attachment site and the carbohydrate moiety would alter pharmacokinetic and/or pharmacodynamic properties of the molecule. To test this hypothesis, we characterized a series of novel opioid glycopeptides (Fig. 1) in in vitro and in vivo bioassays.

**Materials and Methods**

**Animals.** Prior to any procedures, a total of 960 male, ICR mice (25–30 g) (Harlan Industries, Cleveland, OH) were housed in groups of five in Plexiglas chambers with food and water available ad libitum. Animals were maintained on a 12-h light/dark cycle (lights on at 7:00 AM) in a temperature- and humidity-controlled animal colony. All testing was carried out between 10:00 AM and 5:00 PM. Studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**Injections.** All compounds were dissolved in distilled water (i.c.v. injections) or physiological saline (i.v. injections). For i.c.v. injections, mice were lightly anesthetized with ether, and an incision was made in the scalp. Injections were performed using a 10-μl Hamilton microsyringe at a point 2-mm caudal and 2-mm lateral from the bregma. Compounds were injected at a depth of 3 mm in a volume of 5 μl. Intravenous (i.v.) injections were performed by restraining the mouse in a Plexiglas holder, dipping the tail for 10 s in 42°C warm water to dilate the tail vein, and subsequent injection into the vein with a 30-gauge needle.

**Antinociceptive Testing.** Antinociception was assessed using the 55°C warm water tail-flick test. The latency to the first sign of a rapid tail-flick was taken as the behavioral endpoint. Each mouse was tested for baseline latency in the warm water and recording the time to response. Mice not responding within 5 s were excluded from further testing (average latency = 1.95 ± 0.08 s). Mice were then administered the test compound and tested for antinociception at 10, 20, 30, 45, 60, 90, and 120 min postinjection. A maximum score was assigned (100%) to animals not responding within 10 s. This cutoff gives us almost identical calculated A₅₀ results compared with the 15-s cutoff when the data are expressed as percentage of antinociception while producing less tissue damage when animals are tested repeatedly. We ran separate groups of mice to assess the effects of repeated testing in the tail-flick assay to rule out any time-related changes in response latency that might affect antinociceptive potency determinations. From baseline through the 120-min time point, mean latencies were very consistent for un.injected controls (1.62–1.90 s), i.v. saline (1.62–1.70 s), and i.c.v. distilled water (1.72–2.02 s).

Antinociception was calculated by the following formula: % antinociception = 100 × (test latency-control latency)/(10-control latency).

**Synthesis and Purification of Glycopeptides.** The required acetate-protected glycosyl 9-fluorenylmethoxy carbamate amino acids were synthesized using published methods (Polt et al., 1994; Szabo et al., 1995; Mitchell et al., 2001). Deprotection of the Schiff base glycoside esters and reformation afforded the 9-fluorenylmethoxy carbamate amino acid glycosides in excellent yield. Peptide synthesis utilized deprotection with 30% piperidine in N,N-dimethylformamide and amino acids coupling with benzotriazole-1-y1-oxo-tris(dimethylamino)phosphonium hexafluoro-phosphate/N-hydroxybenzotriazole monohydrate in N,N-dimethylformamide. Couplings were sequential and proceeded in over 98% yield per step. The acetate-protecting groups from the carbohydrate were removed while on the resin via treatment with H₂N-NH₂/H₂O in MeOH. Cleavage from the resin was accomplished with 95% trifluoroacetic acid in CH₂Cl₂ and appropriate scavengers over 2 h. This also affected cleavage of the tert-butyl side chain protecting groups from the Tyr$^3$ and $\delta$-Thr$^4$ residues, but did not affect the glycosidic linkage. Precipitation of the crude peptide with ice-cold diethyl ether, followed by purification on a Vydak preparative C₁₈ reversed-phase high-pressure liquid chromatography column provided the pure compounds, which were characterized by $^1$H NMR, $^{13}$C NMR, correlation spectroscopy, total correlation spectroscopy, rotating frame nuclear Overhauser enhancement spectroscopy, and fast atom bombardment high resolution mass spectroscopy.
Radioligand Binding Studies. Membranes were prepared from whole brains taken from adult male Sprague-Dawley rats (250–300 g). All radioligand displacement experiments were run against the 3H-labeled ligands [D-Pen2,Phe(p-Cl)4,D-Pen5]enkephalin and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) as described previously (Hawkins et al., 1989; Vaughn et al., 1989). At least three experiments were done for each radioligand. Statistical comparisons between 1- and 2-site fits were made using the F-ratio test with a P value of 0.05 as the cut-off for significance (Shook et al., 1987). Data that were best fitted by a one-site model were reanalyzed with the logistic equation.

Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI) Bioassays. Electrically induced smooth muscle contractions of isolated MVD and strips of GPI longitudinal muscle myenteric plexus were used as a bioassay (Shook et al., 1987). Percentage of inhibition was calculated as the average contraction height for 1 min preceding the addition of the agonist divided by the contraction height 3 min after the addition of the agonist. IC50 values represent the mean of not less than four tissue samples. IC50 values, relative potency estimates, and their associated standard errors were determined by fitting the mean data to the Hill equation with a nonlinear least-squares method.

Results

Figure 1 summarizes the opioid receptor binding affinity and agonist potency of the compounds in the GPI and MVD bioassays, along with the i.c.v. and i.v. potency determinations in the 55°C tail-flick assay. The antinociceptive potencies are also represented graphically in Fig. 2 as a plot of the

<table>
<thead>
<tr>
<th>Compound</th>
<th>(Transport Sequence)</th>
<th>Delta Mu</th>
<th>MVD IC50</th>
<th>GPI IC50</th>
<th>ICR A50 (95% CI)</th>
<th>IV A50 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-Ser-CONH2</td>
<td>2.36</td>
<td>3.96</td>
<td>2.77 ± 0.48</td>
<td>27.9 ± 2.2</td>
<td>0.068 (0.054-0.086)</td>
</tr>
<tr>
<td>2</td>
<td>L-Thr-CONH2</td>
<td>9.71</td>
<td>11.7</td>
<td>2.32 ± 0.16</td>
<td>24.5 ± 5.0</td>
<td>0.038 (0.026-0.055)</td>
</tr>
<tr>
<td>3</td>
<td>L-Ser (β-Glc)-CONH2</td>
<td>3.35</td>
<td>8.16</td>
<td>1.56 ± 0.22</td>
<td>33.8 ± 6.1</td>
<td>0.023 (0.013-0.039)</td>
</tr>
<tr>
<td>4</td>
<td>L-Thr (β-Glc)-CONH2</td>
<td>16.8</td>
<td>39.8</td>
<td>0.73 ± 0.17</td>
<td>24.6 ± 7.6</td>
<td>0.022 (0.016-0.032)</td>
</tr>
<tr>
<td>5</td>
<td>L-Der (β-Glc)-CONH2</td>
<td>54.4</td>
<td>297.8</td>
<td>5.40 ± 1.05</td>
<td>34.4 ± 6.7</td>
<td>0.035 (0.024-0.050)</td>
</tr>
<tr>
<td>6</td>
<td>L-Der (β-Glc)-CONH2</td>
<td>24.5</td>
<td>31.8</td>
<td>2.20 ± 0.36</td>
<td>48.9 ± 7.2</td>
<td>0.040 (0.020-0.080)</td>
</tr>
<tr>
<td>7</td>
<td>L-Ser (α-Man)-CONH2</td>
<td>23.0</td>
<td>15.2</td>
<td>3.03 ± 0.36</td>
<td>23.3 ± 3.7</td>
<td>0.033 (0.025-0.043)</td>
</tr>
<tr>
<td>8</td>
<td>L-Ser (β-Xyl)-CONH2</td>
<td>46.0</td>
<td>65.8</td>
<td>1.94 ± 1.12</td>
<td>28.3 ± 3.9</td>
<td>0.092 (0.052-0.159)</td>
</tr>
<tr>
<td>9</td>
<td>L-Ser (β-Lactose)-CONH2</td>
<td>17.3</td>
<td>40.0</td>
<td>5.73 ± 1.24</td>
<td>34.8 ± 5.6</td>
<td>0.018 (0.011-0.031)</td>
</tr>
<tr>
<td>10</td>
<td>L-Ser (β-Maltose)-CONH2</td>
<td>9.86</td>
<td>30.8</td>
<td>1.71 ± 0.10</td>
<td>52.6 ± 5.8</td>
<td>0.062 (0.047-0.082)</td>
</tr>
<tr>
<td>11</td>
<td>L-Ser (β-Melibiose)-CONH2</td>
<td>6.48</td>
<td>41.9</td>
<td>6.06 ± 1.35</td>
<td>43.8 ± 8.3</td>
<td>0.034 (0.026-0.044)</td>
</tr>
<tr>
<td>12</td>
<td>L-Ser (β-Maltotriose)-CONH2</td>
<td>25.0</td>
<td>56.7</td>
<td>7.73 ± 1.00</td>
<td>71.7 ± 19.3</td>
<td>0.061 (0.042-0.113)</td>
</tr>
<tr>
<td>13</td>
<td>L-Ser (β-Glc), L-Ser (β-Glc)-CONH2</td>
<td>N</td>
<td>N</td>
<td>1.17 ± 0.22</td>
<td>53.5 ± 8.3</td>
<td>0.380 (0.185-0.793)</td>
</tr>
<tr>
<td>14</td>
<td>L-Ser (β-Glc), L-Thr (β-Glc)-CONH2</td>
<td>N</td>
<td>N</td>
<td>1.47 ± 0.29</td>
<td>107.6 ± 13.2</td>
<td>0.125 (0.082-0.165)</td>
</tr>
<tr>
<td>15</td>
<td>L-Thr (β-Glc), L-Ser (β-Glc)-CONH2</td>
<td>N</td>
<td>N</td>
<td>2.36 ± 0.27</td>
<td>160.8 ± 2.4</td>
<td>0.091 (0.069-0.122)</td>
</tr>
<tr>
<td>16</td>
<td>L-Thr (β-Glc), L-Thr (β-Glc)-CONH2</td>
<td>N</td>
<td>N</td>
<td>3.23 ± 1.00</td>
<td>185.0 ± 31.2</td>
<td>0.101 (0.048-0.220)</td>
</tr>
<tr>
<td>17</td>
<td>L-Ser (β-Glc), L-Ser (β-Glc), L-Ser (β-Glc)-CONH2</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>0.226 (0.154-0.329)</td>
<td>N</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structures of the tested glycopeptides and the unglycosylated parent peptides along with in vitro and in vivo bioassy data. The primary structure of the peptides is drawn at the top of the figure with the individual transport sequences drawn out and listed in the table below. Binding affinities at β- and μ-opioid receptors are listed for each compound along with IC50 values to inhibit electrically evoked smooth muscle contraction in the mouse vas deferens and guinea pig ileum bioassays (described in detail under Materials and Methods). The i.c.v. and i.v. A50 values (and 95% confidence intervals) are calculated from dose-response curves in the 55°C tail-flick assay. Footnotes for the i.c.v. and i.v. A50 values refer to the time of peak effect (1 = 10 min, 2 = 20 min, 3 = 30 min) for each individual compound.
and the parent peptides (1 (Ser versus Thr) did not affect the pharmacophore portion of evidence intervals.

/H9254 peptides had high affinity for affinity for statistical significance. Based on the results with the monosaccharide, several di- and trisaccharides were synthesized (compounds 9–11 and 12, respectively) to determine whether a larger carbohydrate group would further increase or decrease antinociceptive potency. Similar to the monosaccharides, all of the L-Ser attached disaccharides had similar binding affinities and IC_{50} potencies in the in vitro assays. The disaccharides were between 0.8- and 2.7-fold less potent than the monosaccharide 3 following i.c.v. administration. When administered i.v., all three disaccharides were significantly more potent than compound 3, with 11 (β-melibiose) being 5.3-fold more potent than 3 and 21.5-fold more potent than the unglycosylated parent peptide 1. A decrease in potency with the trisaccharide 12 was observed in the in vitro assays, and the compound was less potent compared with any of the disaccharides tested i.v.

A series of bis-monosaccharides (13–16) and one tris-monosaccharide (17) were synthesized to further explore the structure-activity relationships of the glycopeptides. These compounds maintained low nanomolar potency in the MVD bioassay, but had a decrease in potency in the GPI bioassay (2.0- to 6.6-fold less potent than 1). As a group, these compounds were 3.9 to 16.5 times less potent than the monosaccharide 3 following i.c.v. administration. The one bis-monosaccharide tested i.v. (13) was 12.4 times less potent than compound 3.

**Discussion**

In vivo glycosylation has been shown to alter the stability, intracellular transport, secretion, distribution, and overall activity of a variety of proteins (Hilgenfeldt, 1988; Mann et al., 1996; Gimenez-Roqueplo et al., 1998; Barrios-De-Tomasi et al., 2002). We, along with others, have hypothesized that glycosylation of smaller peptides may have desirable effects on the pharmacokinetic and/or pharmacodynamic properties of the parent peptide (Albert et al., 1993; Pelt et al., 1994; Negri et al., 1998; Susaki et al., 1999; Suzuki et al., 1999a,b; Bilsky et al., 2000). More specifically, we have shown that O-linked glycosylation of a potent opioid hexapeptide does not interfere with the pharmacophore portion of the molecule, if the carbohydrate moiety is placed on the sixth amino acid residue (current results and Bilsky et al., 2000). This observation is consistent with Schwzyer’s concept of message and address segments in opioid receptor activation (Schwzyer, 1986). The glycosylation does, however, increase pep-
tide stability, BBB penetration, and antinociceptive potency following i.v., s.c., and i.p. routes of administration (Bilsky et al., 2000). The current experiments further define the structure-activity relationship of a series of opioid glycopeptides with varied glyco-address segments with a constant pharmacophore region.

From the in vitro and in vivo data, the use of either an L-Ser or L-Thr amino acid in the sixth position on the C terminus appears to be a viable attachment point for carbohydrate groups. Glycopeptides with either amino acid displayed high affinity for \( \mu \) and \( \delta \) receptors, and potently inhibited electrically stimulated contractions in the MVD and GPI bioassays. Furthermore, the stereochiematic configuration of the sixth amino acid (L versus D) had minimal effects on the agonist potency of the unglycosylated and monoglycosylated compounds in smooth muscle bioassays and in the 55°C tail-flick assay following i.c.v. administration. These data further support the hypothesis that minor modifications of the address segment of opioid peptides have minimal effects on the pharmacophore message. With additional glycosylation sites (bis- and tris-monosaccharides) we observed a consistent and significant decrease in IC\(_{50}\) values and i.c.v. antinociceptive potency. This suggests multiple glycosylations of the parent peptide results in conformational changes that affect the pharmacophore (message segment). This hypothesis requires additional experimental studies and molecular modeling.

Substitution of \( \alpha \)-Man or \( \beta \)-Xyl for \( \beta \)-Glc decreased the affinity of the peptide at both \( \mu \) and \( \delta \) receptors, with a loss of \( \delta \) receptor selectivity. These changes were not observed in the smooth muscle bioassays, which may reflect differences in the membrane component of brain homogenate versus the complete membrane in intact tissues. The \( \beta \)-Xyl compound was approximately 4 times less potent than the \( \beta \)-Glc compound when administered i.c.v., although they were equipotent when administered i.v. Collectively, these data suggest...
that the carbohydrate group may have more profound effects on pharmacokinetics which ultimately plays a greater role in determining i.v. potency (see discussion below).

The disaccharides as a group were significantly more potent following i.v. administration than any of the monosaccharides tested. Based on these results, it was hypothesized that a greater carbohydrate surface area (increased hydrodynamic volume; Molineux, 2002) might be responsible for increased BBB transport and/or peptide half-life. To test this hypothesis further, a trisaccharide and a series of bis- and tris-monosaccharides were synthesized and tested. In general, we observed a decreased potency in vitro and a decrease in i.c.v. and i.v. potency.

From our data, we can conclude that disaccharides provide the optimal improvement in BBB transport and/or pharmacokinetic factors to maximize i.v. antinociceptive potency for hexapeptides. Although there were some variations in the disaccharides in terms of receptor affinity and bioassay IC\textsubscript{50} values, these cannot fully explain the higher antinociception induced by these peptides. There was no obvious correlation between the i.c.v. and i.v. potencies of these compounds, nor between the binding affinities, IC\textsubscript{50} values or selectivity ratios, and the measured i.v. potencies (data not shown). It is more likely that the changes in the carbohydrate moiety had effects on the stability and biodistribution of the compounds in vivo. Previous studies by our group have compared the metabolic stability and BBB penetration for a number of monosaccharide glycopeptides and their parent peptides. These studies showed an increase in metabolic stability in both brain and blood for glycopeptides (Egleton et al., 2001) coupled with an enhanced BBB penetration (Bilsky et al., 2000; Egleton et al., 2000, 2001). Studies investigating BBB penetration of compounds 1, 3, 10, and 12 indicate that a disaccharide (compound 10) had both the best brain delivery and metabolic stability, whereas the trisaccharide 12 had reduced BBB transport and stability compared with the dis-
accharide (manuscript in preparation). This decrease in both BBB penetration and stability of the trisaccharide is paralleled by the reduction in antinociceptive observed in this study. This indicates that the type of sugar used can modulate the bioavailability of opioid peptides to the brain.

Groups also have observed an effect of glycosylation on the distribution of peptides. Suzuki and colleagues investigated the effects of glycosylation on the pharmacokinetics of arginine-vasopressin and oxytocin analogs following i.v. administration (Suzuki et al., 1999; Suzuki et al., 1999a,b). Specific tissue uptake was dependent on both peptide sequence and the carbohydrate moiety added (Suzuki et al., 1999b). The uptake into the kidney was increased, whereas clearance via the liver was decreased (Suzuki et al., 1999b), indicating a shift from liver to renal clearance. Distribution studies of opioid peptides have shown a clearance predominantly via the liver-fecal route (Weber et al., 1992; Witt et al., 2001). Shifting the clearance from the liver-fecal route to the kidney-urine route of excretion has shown a good correlation in increasing both plasma half-lives and antinociception (Witt et al., 2001). Thus, it is possible that the addition of carbohydrates to our peptides lead to an increase in plasma half-life due to a change in excretion profile. This increase in plasma half-life then results in a higher uptake of peptide into the brain and, thus, an enhanced antinociceptive profile.

The glycosylation strategy, along with other approaches for improving the pharmacokinetic profile of peptides, has rekindled interest in developing peptide-based pharmacotherapies. Advances in synthetic peptide chemistry and manufacturing processes have driven down the costs associated with large-scale synthesis of these molecules. From our perspective, opioid peptides may offer several advantages over alkaloid-based compounds. The availability of a number of unique pharmacophores, based on naturally occurring neuropeptides that preferentially bind to δ-opioid receptors, may offer efficacy and tolerability advantages over μ-selective analgesics in both acute and chronic pain management (Bilsky et al., 2000; Gengo and Chang, 2004; Lipkowski et al., 2004). The probability of accumulation of active metabolites in renal and liver disease is also presumably less with peptide-based drugs versus alkaloids. The metabolites of the opioid glycopeptides, for example, would be small di- and tripeptides and sugars. Furthermore, the glycosylation approach may be applicable to any number of peptide molecules, although further testing will have to determine the effects of these modifications on a peptide by peptide basis (see, for example, Susaki et al., 1999; Suzuki et al., 1999a,b).

In conclusion, we have provided further evidence that glycosylation of a small opioid peptide can significantly increase i.v. antinociceptive potency of the compound compared with the parent peptide. These changes in i.v. potency are likely due to changes in pharmacokinetic factors rather than changes in binding affinity and μ/δ receptor selectivity. For the opioid peptide tested, the attachment of the carbohydrate groups can be accomplished with either a Ser or Thr in the sixth position, with the stereochemical configuration of that amino acid not being critical for activity. Finally, glycosylation with disaccharides resulted in the most potent compounds, with potencies on a micromolar per kilogram basis exceeding that of the prototypical opioid analgesic morphine.

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References


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