ABSTRACT
Neuropeptide pharmaceuticals have potential for the treatment of neurological disorders, but the blood-brain barrier (BBB) limits entry of peptides to the brain. Several strategies to improve brain delivery are currently under investigation, including glycosylation. In this study we investigated the effect of O-linked glycosylation on Ser6 of a linear opioid peptide amide Tyr-d-Thr-Gly-Phe-Leu-Ser-NH2 on metabolic stability, BBB transport, and analgesia. Peptide stability was studied in brain and serum from both rat and mouse by high-performance liquid chromatography. BBB transport properties were investigated by rat in situ perfusion. Tail-flick analgesia studies were performed on male ICR mice, injected i.v. with 100 μg of peptide ligand. Glycosylation of Ser6 of the peptide led to a significant increase in enzymatic stability in both serum and brain. Glycosylation significantly increased the BBB permeability of the peptide from a value of 1.0 ± 0.2 μl/min.1 g-1 to 2.2 ± 0.2 μl/min.1 g-1 (p < 0.05), without significantly altering the initial volume of distribution. Analgesia studies showed that the glycosylated peptide gave a significantly improved analgesia after i.v. administration compared with nonglycosylated peptide. The improved analgesia profile shown by the glycosylated peptide is due in part to an improvement in bioavailability to the central nervous system. The bioavailability is increased by improving stability and transport into the brain.

Bioavailability of peptide-based drugs to the brain is limited, due to poor metabolic stability, or inability to cross the blood-brain barrier (BBB). The BBB is located at the endothelial cells of the cerebrovascular capillary beds (Reese and Karnovsky, 1967). BBB endothelial cells are connected via tight cellular junctions that are characterized by high electrical resistance (Jones et al., 1992) and low paracellular diffusion. BBB endothelial cells have a small number of vesicles (Brightman and Reese, 1969), indicating low vesicular transport and are ensheathed by astrocytic end-feet, which provide factors to maintain BBB function (Arthur et al., 1987). The BBB is also a metabolic barrier, containing a number of enzymes, including aminopeptidase A, aminopeptidase M, and angiotensin-converting enzyme (Ailt and Lawrence, 2000). Inhibition of these enzymes in vitro results in increased penetration of opioid peptides (Brownson et al., 1994). Enzyme inhibition, however, is generally not a practical methodology to increase BBB entry of peptides, because of the large number (and concentration) of enzymes that degrade peptides. A number of other methodologies to increase BBB penetration have been investigated, such as improving lipophilicity by chemical modification (Witt et al., 2000b), targeting of known transport mechanisms (Pardridge et al., 1991; Abbruscato et al., 1997), glycosylation (Polt et al., 1994; Negri et al., 1998; Egleton et al., 2000), and/or coadministration with compounds to improve BBB entry (Witt et al., 2000a).

Glycosylation has proven to be a useful methodology for enhancing biodistribution to the brain. Improved analgesia has been reported for glycosylated deltorphin (Tomatis et al., 1997; Negri et al., 1999), cyclized met-enkephalin analogs (Polt et al., 1994; Egleton et al., 2000), and linear leu-enkephalin analogs (Bilsky et al., 2000). A number of different sugar moieties have been investigated, including glucose and xylose (Egleton et al., 2000). The improved analgesia exhibited by glycosylated-opioids may be due to increased bioavailability of glycopeptides via higher metabolic stability (Powell et al., 1993), reduced clearance (Fish et al., 1991), or improved BBB transport (Egleton et al., 2000).

Previously, we have shown that the leu-enkephalin analog Tyr-d-Thr-Gly-Phe-Leu-Ser-NH2 (when β-glycosylated on the Ser6) had a significantly improved analgesia compared with the nonglycosylated parent after administration by intravenous or subcutaneous routes (Bilsky et al., 2000). In this study we have investigated the bioavailability of Tyr-d-Thr-Gly-Phe-Leu-Ser-NH2 (SAM 995) and its O-linked Ser6-β-D-glucose analog (SAM 1095) (Table 1) to the brain, by using in vivo, in vitro, and in situ techniques and further characterized the analgesic profile.

ABBREVIATIONS: BBB, blood-brain barrier; BSA, bovine serum albumin; RAGE, receptor for advanced glycation end products.
Table 1
Structures of peptides investigated in this study
Receptor binding data from Bilsky et al. (2000). Guinea pig ileum (GPI) and mouse vas deferens (MVD) bioassays. Receptor binding for \( \mu \)- and \( \delta \)-opioid activity were carried out in isolated rat brain membranes.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>COD (nM)</th>
<th>MVD (( \delta )) IC(_{50}) (nM)</th>
<th>( \mu )-Binding IC(_{50}) (nM)</th>
<th>( \delta )-Binding IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM 995 Tyr-D-Thr-Gly-Phe-Leu-Ser-NH(_2)</td>
<td>25</td>
<td>2.7</td>
<td>4</td>
<td>2.4</td>
</tr>
<tr>
<td>SAM 1095 Tyr-D-Thr-Gly-Phe-Leu-Ser-NH(_2) (( \beta )-D-glucose)</td>
<td>33.8</td>
<td>1.6</td>
<td>8.2</td>
<td>3.4</td>
</tr>
</tbody>
</table>

\[ \% \text{ Protein binding} = \frac{(T - F)}{T} \times 100 \]

In Situ Perfusion. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Arizona. In situ brain perfusion studies were carried out in female Sprague-Dawley rats (250–300 g), based on the methods of Preston et al. (1995). Briefly, rats were anesthetized (acepromazine 0.6 mg \( \cdot \) ml\(^{-1}\), ketamine 3.1 mg \( \cdot \) ml\(^{-1}\), and xylazine 78.3 mg \( \cdot \) ml\(^{-1}\)) and heparinized (10,000 U \( \cdot \) kg\(^{-1}\)). The common carotids were exposed and cannulated with silicone tubing connected to a perfusion circuit. The perfusion fluid consisted of a protein containing mammalian Ringer’s solution [117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\)6H\(_2\)O, 10 mM D-glucose, 3.9% dextran (mol. wt. 70,000), and 1% bovine serum albumin, pH 7.4] at 37°C and ultrafiltered through a Centrifree microporation device (Amicon, Beverly, MA). Total concentration (T) of \(^{125}\)I-peptide introduced into the system and found in the ultrafiltrate (F) was determined by counting on a Beckman 5500 gamma counter (Beckman Coulter, Inc.). The percentage binding was then calculated as follows:

- Metabolic Stability. Brain extractions were performed on Sprague-Dawley rats (250–300 g) and ICR Mice (20–25 g) by using the method of Davis and Culling-Berglund (1985). Protein concentrations were determined by the Pierce bicinchoninic acid protein assay kit (Pierce, Rockford IL). Blood was collected from the abdominal aorta of both rats and mice and centrifuged at 4000g for 12 min. Plasma was separated and stored at \(-80^\circ\)C.

- To 180 \( \mu \)l of resuspended 15% brain homogenate or plasma was added SAM 995 or SAM 1095 to a final concentration of 100 \( \mu \)M. Incubations were carried out at 37°C for 0, 60, 120, 240, and 360 min in triplicate. Enzyme activity was terminated by addition of 200 \( \mu \)l of acetonitrile and 200 \( \mu \)l of 0.5% acetic acid and the samples were placed on ice. Samples were then centrifuged at 3000g for 12 min. Then 300 \( \mu \)l of the supernatant was taken and diluted to 25% acetonitrile before analysis by reverse phase-high-performance liquid chromatography.

- Iodination. Peptide SAM 995 and its glycosylated analog (SAM 1095) were mono-iodinated on the Tyr\(^+\) residue by using a standard chloramine-T procedure (Bolton, 1986). Purification of the iodinated peptides was carried out using a reverse phase PerkinElmer 250 HPLC gradient system (Davis and Culling-Berglund, 1985) and a Vydac 218TP5415 C18 column (4.6 \( \times \) 250 mm). The samples were eluted at 37°C by using a curvilinear gradient of 0.1% trifluoroacetic acid in acetonitrile before analysis by reverse phase-high-performance liquid chromatography.

- Octanol/Saline Distribution. Equal volumes of octanol and 0.05 M HEPES buffer in 0.1 M NaCl, pH 7.4, were mixed and allowed to equilibrate for 12 h. The layers were separated and stored at 4°C. The equilibrated 0.05 M HEPES buffer (500 \( \mu \)l) in 0.1 M NaCl, pH 7.4, buffer was added to 1 \( \mu \)Ci of iodinated peptide and mixed with 500 \( \mu \)l of octanol. The sample was centrifuged at 4000 rpm in a Beckman Coulter microcentrifuge for 1 min. The layers were separated and counted for radioactivity on a Beckman Coulter 5000 gamma counter (Beckman Coulter, Inc., Fullerton, CA). The octanol/saline distribution (D) was calculated as the ratio of peptide radioactivity in the octanol and saline phases.

- Protein Binding. The amount of iodinated peptide bound to bovine serum albumin (BSA) in perfusate was determined by ultrafiltration and centrifugal dialysis. Peptides were dissolved in a mammalian Ringer’s solution (117.0 mM NaCl, 4.7 mM KCl, 0.8 mM MgSO\(_4\)3H\(_2\)O, 24.8 mM NaHCO\(_3\), 1.2 mM KH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\)6H\(_2\)O, 10 mM D-glucose, 3.9% dextran (mol. wt. 70,000), and 1% bovine serum albumin, pH 7.4) at 37°C and ultrafiltered through a Centrifree microporation device (Amicon, Beverly, MA). Total concentration (T) of \(^{125}\)I-peptide introduced into the system and found in the ultrafiltrate (F) was determined by counting on a Beckman 5500 gamma counter (Beckman Coulter, Inc.).
where $C_{br}$ is the amount of test solute per unit mass of brain at time $T$, and $C_{pf}$ is the perfusion fluid concentration of test solute at time $T$. The value for $C_{pf}$ remains constant during these experiments, and a plot of $C_{br}/C_{pf}$ against time produces a straight line with slope $K_i$ (the initial unidirectional transfer constant) and an ordinate intercept of $V_i$ (the initial volume of distribution). Any departure from linearity indicates a back flux of solute from the brain to blood.

**Capillary Depletion.** The role of the vascular portion of brain uptake of the peptides was studied by capillary depletion (Triguero et al., 1990). After a 20-min in situ perfusion, the brain was removed and choroid plexi and meninges excised. The brain tissue was homogenized (Polytron homogenizer; Brinkman Instruments, Westbury, NY) in 1.5 ml of capillary depletion buffer [10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl$_2$, 1 mM MgSO$_4$, 1 mM Na$_2$PO$_4$, 10 mM D-glucose, pH 7.4] and kept on ice. Two milliliters of ice-cold 26% dextran (70,000) was added to the homogenate. Two aliquots of homogenate were taken and centrifuged at 5400 $g$ for 15 min in a Microfuge (Beckman Coulter, Inc.). The capillary-depleted supernatant was separated from the vascular pellet. Homogenization procedures were all carried out within a 2-min time span. The homogenate, supernatant, and pellet were then counted for radioactivity as described above.

**Analgesia Studies.** Six male ICR mice (20–30 g) were used for each peptide in this study. The mice were housed in groups of four in Plexiglas cages in a light-and temperature-controlled environment with food and water ad libitum. The peptides were administered i.v. at an injection concentration equal to 15 $\mu$M in 100 $\mu$l of 0.9% saline. Antinociception was measured by a radiant heat, tail-flick analgesia meter (ITTC, Woodland Hills, CA). Baseline latency response for each mouse was established before peptide administration.

Antinociception was calculated as the percentage of maximum possible effect (%MPE):

$$\% \text{MPE} = \frac{\text{recorded time} - \text{baseline time}}{\text{maximum time (15 s)} - \text{baseline time}} \times 100$$

Differences between area under the curve were determined by one-way analysis of variance with the Newman-Keuls post hoc test by using the PCS statistical package (Tallarida and Murray, 1987).

**Results**

**Metabolic Stability.** Metabolic stability of SAM 995 and SAM 1095 was assessed using two biological matrices (brain and serum) in two rodent species (rat and mouse). For both matrices and species glycosylation improved the metabolic half-life of the peptide (Table 2). Stability in brain was increased by greater than 1.5-fold for both species. In contrast, serum stability in mouse was increased greater than 4-fold compared with 1.8-fold in rat serum.

**Physiochemical Data.** Octanol/saline distribution studies show that glycosylated analog SAM 1095 has a greater than 10-fold decrease in lipophilicity, with a $p$ value of 2.82 ± 0.22 for the parent SAM 995 compared with 0.25 ± 0.05 for the analog.

Binding of the peptide to 1% BSA was not significantly altered by glycosylation (Table 2).

**In Situ Perfusion.** In situ studies at 20 min (Fig. 1) showed that both SAM 995 and SAM 1095 had significantly higher association with the brain than the nonpermeable marker [¹⁴C]sucrose. Multiple time uptake studies of the two peptides (Fig. 1) show that SAM 1095 has a significantly higher rate of brain uptake than SAM 995 (Table 3, with $K_i$ values of 2.2 ± 0.3 $\mu$M · min$^{-1}$ · g$^{-1}$ compared with 1.0 ± 0.2 $\mu$M · min$^{-1}$ · g$^{-1}$, $p < 0.01$). However, initial volumes of distribution, $V_i$ (intercepts of linear regression) were not significantly different from each other (Table 3). Capillary depletion analysis at 20 min shows more SAM 1095 reached the brain parenchyma compared with SAM 995 (Table 3). Self-inhibition studies showed that the passage of $^{125}$I-SAM 1095 across the BBB was reduced by the addition of 1 to 10 $\mu$M nonradioactive SAM 1095 to the perfusate (Fig. 2). In contrast, addition of 10 $\mu$M SAM 995 did not significantly affect the uptake of $^{125}$I-SAM 1095 (Fig. 2).

**Antinociception.** Data derived from Fig. 3A provides evidence SAM 1095 has improved analgesia compared with SAM 995. SAM 1095 gives an above-baseline antinociceptive response for 120 min compared with SAM 995, which was at baseline, by 60 min. The area under the curve derived from Fig. 3A shows that SAM 1095 response was significantly ($p < 0.01$) larger than SAM 995 by ~6-fold (Fig. 3B).

**Discussion**

In previous studies we showed that glycosylation of Ser$^6$ on the linear peptide Tyr-D-Thr-Gly-Phe-Leu-Ser-NH$_2$ improved its analgesic potency by ~4-fold after peripheral administration (Bilsky et al., 2000). In the present study we have investigated the effect of glycosylation of Tyr-D-Thr-Gly-Phe-Leu-Ser-NH$_2$ on its antinociceptive properties. The results presented here indicate that glycosylation of Tyr-D-Thr-Gly-Phe-Leu-Ser-NH$_2$ enhances its analgesic potency by ~6-fold across both species of mice. The metabolic stability of the analogs was assessed using two biological matrices (brain and serum) in two rodent species (rat and mouse). For both matrices and species glycosylation improved the metabolic half-life of the peptide (Table 2). Stability in brain was increased by greater than 1.5-fold for both species. In contrast, serum stability in mouse was increased greater than 4-fold compared with 1.8-fold in rat serum.
BBB in situ characteristics of SAM 995 and SAM 1095 uptake into the rat brain

$K_m$ and $V_i$ are calculated from the linear regressions of the data from Fig. 1, with $K_m$ representing the gradient and $V_i$ the intercept.

<table>
<thead>
<tr>
<th></th>
<th>SAM 995</th>
<th>SAM 1095</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ ($\mu\text{M} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)</td>
<td>1.0 ± 0.2</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>$V_i$ ($\mu\text{g} \cdot \text{g}^{-1}$)</td>
<td>9.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Capillary fraction at 20 min (pellet)</td>
<td>0.91 (32%)</td>
<td>0.66 (12%)</td>
</tr>
<tr>
<td>Parenchyma fraction at 20 min (supernatant)</td>
<td>1.92 (68%)</td>
<td>4.81 (88%)</td>
</tr>
</tbody>
</table>

Fig. 2. Self-inhibition studies for $^{125}$I-SAM 1095 by using in situ perfusion. Each column represents the mean and S.E. of four animals. Nonlabeled SAM 1095 (1–10 $\mu$M) inhibits the uptake of $^{125}$I-SAM 1095 into rat brain in a dose-dependent manner (*, $p < 0.05$; **, $p < 0.01$ versus zero nonlabeled SAM 1095; ANOVA followed by Newman-Keuls analysis); however, nonlabeled SAM 995 (10 $\mu$M) does not inhibit $^{125}$I-SAM 1095 BBB passage.

Fig. 3. Analgesia data from i.v. injection of 15 $\mu$M of either SAM 995 or SAM 1095. A, each line represents the mean and S.E. analgesia profile from six male ICR mice. B, mean and S.E. area under the curve calculated from data in A (**, $p < 0.01$; Student’s $t$ test).

Ser-NH$_2$ on bioavailability, metabolism, and transport to the brain.

One of the major determinants of bioavailability to target organs is the metabolic stability of the drug. This is of particular importance for peptide pharmaceuticals because of the presence of peptidases in the serum, on capillary endothelial cells and on the target organ. For brain-targeted delivery, the presence of the BBB as a metabolic barrier can reduce the ability of enzymatically labile peptides such as met-enkephalin from entering the brain (Brownson et al., 1994). We investigated the stability of SAM 995 and SAM 1095 in serum and brain of both rat and mouse. Glycosylation universally increased the metabolic half-life of the peptide in both biological media (Table 2). Interestingly, in mice the stability was increased greater than 4-fold in serum compared with only 1.8-fold in the serum of rats. This could be due to different levels or types of peptidases within the serum of the two species. Previous studies have shown different half-lives of degradation by using both peptide and nonpeptide drugs in rat versus mouse (Takenaga et al., 1999; Hollenberg, 2000). The topoisomerase inhibitor NB-506 is transformed to its major metabolite considerably faster in rat than mouse (Takenaga et al., 1999). There is also evidence for species differences in the renin-angiotensin system (Hollenberg, 2000). Angiotensin-converting enzyme is known to be important in opioid peptide metabolism (Brownson et al., 1994). It must also be remembered that this stability study was carried out in vitro under saturation conditions [i.e., the substrate was in excess (500 $\mu$M) compared with the enzymes]. Thus, this is a good tool for investigating relative stability of peptides. However, in vivo metabolic stability under nonsaturation conditions will be considerably reduced.

Other factors that affect bioavailability to the brain include serum protein binding and lipophilicity. Binding of both SAM 995 and SAM 1095 to 1% BSA was investigated. No significant difference in binding to BSA was noted (Table 2). Lipophilicity is one of the major determinants of passive membrane diffusion. Addition of a glucose group to the peptide significantly reduced the lipophilicity (measured by octanol saline distribution) of the peptide by ~10-fold (Table 2).

BBB penetration of the two peptides was assessed using a rat in situ perfusion methodology. Both peptides entered the brain with a 2-fold higher uptake of SAM 1095 compared with SAM 995 ($K_m$ values of 1.0 ± 0.2 $\mu$M $\cdot$ min$^{-1}$ $\cdot$ g$^{-1}$ and 2.2 ± 0.3 $\mu$M $\cdot$ min$^{-1}$ $\cdot$ g$^{-1}$ for SAM 995 and SAM 1095, respectively). The $V_i$ values for the two peptides were not significantly different (Table 3). The $V_i$ represents distribution space at time zero for the two peptides. The values are 9.7 and 14.3 $\mu$M $\cdot$ g$^{-1}$ for SAM 995 and SAM 1095, respectively. These levels were similar to values previously reported for brain vascular space ranging from 3 to 18 $\mu$M $\cdot$ g$^{-1}$ (Heisey, 1968; Williams et al., 1996a; Egleton et al., 2000). The $K_m$ values in this study were also similar to previous studies of centrally active peptides, both glycosylated (Egleton et al., 2000) and nonglycosylated (Williams et al., 1996a; Abbruscato et al., 1997) ranging from 0.57 to 2.57 $\mu$M $\cdot$ g$^{-1}$.

To ensure that the peptides were entering the brain and not accumulating in the capillary endothelial cells, capillary depletion studies were performed. Studies reveal that ~2.5-fold greater accumulation of SAM 1095 occurred within the brain parenchyma (thus available for receptor binding) than SAM 995 (Table 3).

We also investigated the potential mechanism by which SAM 1095 was crossing the BBB (i.e., was the transport via a nonsaturable or saturable route). Lipophilicity has been shown to be a major determinant for the ability of a drug to diffuse across a membrane and remains one of the better determinants for in vivo peptide permeability (Banks and Kastin, 1985), although this is highly dependent on the peptides studied (Buchwald and Bodor, 1998). In this study octanol/saline distribution studies indicated that the addition of the glucose group significantly reduced lipophilicity by ~10-fold. This was similar to previously reported reductions...
in lipophilicity by glycosylation (Egleton et al., 2000), indicating that the improved uptake was not due to an increase in diffusion.

Self-saturation studies were performed in situ, and 1 and 10 μM SAM 1095 inhibited the uptake of 125I-SAM 1095 by 25 and 58%, respectively. Interestingly, 10 μM SAM 995 in the perfusion media had no effect on the uptake of 125I-SAM 1095, indicating that the two peptides do not share a common saturable transport mechanism. There have been reported several saturable transport mechanisms at the BBB that peptides use to enter the brain, including the large neutral amino acid transporter (Abbruscato et al., 1997) and the receptor for advanced glycation end products (RAGE) (Mackie et al., 1998) and transporters for enkephalins (Zlokovic et al., 1988). The glucose, Glut-1, carrier mediated-transporter is the primary route for glucose entry to the brain. However, it is unlikely that SAM 1095 uses the Glut-1 glucose transporter to enter the brain. Previous studies have shown that glycosylated peptides of a similar size do not use Glut-1 (Williams et al., 1996b), and furthermore the presence of 10 mM glucose in our perfusate would saturate the transporter. Other saturable glucose transporters have previously been reported to transport small glycopeptides, specifically Glut-2 (Nomoto et al., 1998); however, this transporter is only found in specific brain nuclei at very low levels (Leloup et al., 1994).

Adsortive and receptor-mediated mechanisms can also transport glycosylated peptides and proteins at the BBB. The human immunodeficiency virus-1 coat protein gp-120 crosses the BBB by an adsorptive endocytotic mechanism (Banks et al., 1997), and this is a potential route for human immunodeficiency virus entry to the brain. Other large glycoproteins such as wheat germ agglutinin (Broadwell et al., 1988) also use this mechanism. A number of peptides use receptor-mediated endocytosis to cross the BBB. The receptor for advanced glycation end products (RAGE) is expressed in numerous tissues (Brett et al., 1993), including brain endothelium (Mackie et al., 1998). The RAGE receptor has been shown to transport numerous peptides and proteins that have been glycosylated by the Amidori reaction; furthermore the RAGE receptor also transports β-amyloid across the BBB. This is a likely candidate for the transport of SAM 1095. In this study we showed that the increase in transport is glycosylation-dependent because the nonglycosylated parent failed to inhibit the uptake of SAM 1095. Previous studies have shown transporters for several small peptides, including Leu-enkephalin (Zlokovic et al., 1988) and [d-Pen², d-Pen⁵]-enkephalin (Williams et al., 1996a). Although the exact mechanism of transport is unknown, there is some evidence that the organic anion-transporting peptide transporter may be involved in the transport of [d-Pen², d-Pen⁵]-enkephalin (Gao et al., 2000). This transporter could be a potential route for the transport of SAM 1095.

In previous studies with these peptides, we showed that SAM 1095 gave considerably better analgesia after peripheral administration (i.v. and s.c.) than SAM 995, by using a single time point analysis. In this study, we investigated the effect of glycosylation on the time course and duration of analgesia. SAM 1095 led to significantly better analgesia than SAM 995 (Fig. 3). The duration of measurable analgesia for an i.v. dose of 15 μM SAM 1095 was 120 min compared with 60 min for SAM 995. Thus, glycosylation not only improves the maximal analgesia but also improves duration of analgesia.

The improved analgesia seen after glycosylation of Tyr-D-Thr-Gly-Phe-Leu-Ser-NH₂ (SAM 995) is due to a number of factors that combine to improve bioavailability to the brain. Receptor binding studies (Table 1) showed only a slight decrease in SAM 1095 binding to both μ- and δ-opioid receptors (Bilsky et al., 2000). However, analgesia after central (i.c.v.) administration was significantly better for SAM 1095 compared with SAM 995 (Bilsky et al., 2000). This improved analgesia may be due to the improved stability of the peptide within the brain, leading to an increased concentration at receptor binding sites, or an improved distribution within the brain. From Table 2, it is apparent that the stability of the peptide is increased by the addition of a glucose moiety. It must however be remembered that these stability studies were carried out in vitro by using saturating concentrations of peptides (500 μM). Thus, they represent relative stabilities compared with each other rather than half-lives expected within the in vivo situation. In vivo it is likely that the half-lives will be considerably shorter. The improved analgesia of SAM 1095 compared with SAM 995 on peripheral administration can be accounted for by improved BBB transport and increased half-life within the serum. Serum half-life was increased by increased stability and also possibly by reduced clearance. Glycosylation has been shown previously to reduce clearance and shift excretion from the liver to the kidney (Fisher et al., 1991). The increased bioavailability means more peptide available to cross the BBB via the improved transport mechanism.

In conclusion, glycosylation of the linear peptide Tyr-D-Thr-Gly-Phe-Leu-Ser-NH₂ improves analgesia by increasing the bioavailability of the peptide to the brain. The bioavailability is improved by a combination of increased plasma half-life (metabolically and possibly clearance), improved entry into the brain, and improved brain stability.

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