Novel Vasoconstrictor Formulation to Enhance Intranasal Targeting of Neuropeptide Therapeutics to the Central Nervous System

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ABSTRACT

The intranasal route of drug administration is noninvasive, convenient, and rapidly targets therapeutics to the central nervous system (CNS) using olfactory and trigeminal neural pathways connecting the nasal passages to the brain. The purpose of this research was to enhance intranasal drug targeting to the CNS by incorporating a vasoconstrictor [phenylephrine (PHE)] into nasal formulations containing therapeutic neuropeptides [hypocretin-1 (HC) or the dipeptide L-Tyr-D-Arg (D-KTP)]. Concentrations in CNS tissues, peripheral tissues, and blood were determined at 30 min following intravenous or intranasal administration of 125I-labeled neuropeptides with and without PHE. Compared with intranasal controls, inclusion of 1% PHE in nasal formulations significantly reduced absorption into the blood for HC (65% reduction) and D-KTP (39% reduction) and in most remaining brain regions by ~50% for both. The dramatic reduction in blood concentrations with PHE contributed to brain-to-blood concentration ratios that were significantly increased for HC throughout the brain (1.6–6.8-fold) compared with intranasal controls. For D-KTP, 1% PHE significantly increased ratios only in the olfactory bulbs (5.3-fold). With a 5% PHE formulation, D-KTP ratios were significantly increased to additional brain areas (1.5–16-fold). Vasoconstrictor nasal formulations may have particular relevance for CNS therapeutics with adverse side effects where it would be advantageous to limit systemic exposure.

It has been reported that greater than 98% of small-molecule and nearly 100% of large-molecule central nervous system (CNS) drugs developed by the pharmaceutical industry do not cross the blood-brain barrier (Pardridge, 2005). Intracerebroventricular or intraparenchymal drug administration can directly deliver therapeutics to the brain; however, these methods are invasive, inconvenient, and impractical for the numbers of individuals requiring therapeutic interventions for treating CNS disorders. Intranasal drug administration is a noninvasive and convenient means to rapidly target therapeutics of varying physical and chemical properties to the CNS. Although the exact mechanisms underlying intranasal delivery to the CNS are not well understood, the olfactory and trigeminal neural pathways connecting the nasal passages to the CNS are clearly involved (Thorne et al., 2004; Dhanda et al., 2005). In addition to these neural pathways, perivascular pathways, and pathways involving the cerebrospinal fluid (CSF) or nasal lymphatics may play a central role in the distribution of therapeutics from the nasal cavity to the CNS (Thorne et al., 2004). Numerous therapeutics have been delivered to the CNS following intranasal administration and have demonstrated pharmacological effects in animals and in humans (Dhanda et al., 2005), with clinical investigations currently underway for intranasal treatment of Alzheimer’s disease (Gozes and Divinski, 2007; Reger et al., 2008) and obesity (Hallschmid et al., 2008).

The intranasal method of drug delivery holds great promise as an alternative to more invasive routes; however, several factors limit the efficiency of intranasal delivery to the CNS. Several factors limit the efficiency of intranasal delivery to the CNS: the short distance that therapeutic formulations can travel from the nasal cavity to the brain, the absorption of formulations into the nasal mucosa, and the rapid clearance into the nasopharynx. In this study, a 1% PHE formulation was included in nasal formulations containing therapeutic neuropeptides [hypocretin-1 (HC) or the dipeptide L-Tyr-D-Arg (D-KTP)]. Concentrations in CNS tissues, peripheral tissues, and blood were determined at 30 min following intravenous or intranasal administration of 125I-labeled neuropeptides with and without PHE. Compared with intranasal controls, inclusion of 1% PHE in nasal formulations significantly reduced absorption into the blood for HC (65% reduction) and D-KTP (39% reduction) and in most remaining brain regions by ~50% for both. The dramatic reduction in blood concentrations with PHE contributed to brain-to-blood concentration ratios that were significantly increased for HC throughout the brain (1.6–6.8-fold) compared with intranasal controls. For D-KTP, 1% PHE significantly increased ratios only in the olfactory bulbs (5.3-fold). With a 5% PHE formulation, D-KTP ratios were significantly increased to additional brain areas (1.5–16-fold). Vasoconstrictor nasal formulations may have particular relevance for CNS therapeutics with adverse side effects where it would be advantageous to limit systemic exposure.

ABBREVIATIONS: CNS, central nervous system; CSF, cerebrospinal fluid; PHE, phenylephrine; HC, hypocretin-1; D-KTP, L-Tyr-D-Arg, a structural analog of kyotorphin; PBS, phosphate-buffered saline; AUC, area under the blood concentration-time curve.
CNS. Absorption of intranasally applied drugs into the capillary network in the nasal mucosa can decrease the amount of drug available for direct transport into the CNS. Additional factors within the nasal cavity, including the presence of nasal mucociliary clearance mechanisms, metabolizing enzymes, efflux transporters, and nasal congestion can also reduce the efficiency of delivery into the CNS (Vyas et al., 2006).

We investigated the effect of including a vasoconstrictor in neuropeptide nasal formulations. We hypothesized that a vasoconstrictor would enhance intranasal drug targeting to the CNS by limiting absorption into the systemic circulation and increasing the amount of neuropeptide available for direct transport into the CNS. Vasoconstrictors are commonly administered to reduce nasal congestion by reducing blood vessel diameter, reducing blood flow, and increasing blood pressure. Vasoconstrictors have frequently been used in combination with other drugs in nasal formulations to prevent adverse systemic effects by reducing systemic absorption (Urtti and Kyyrönen, 1989; Kyyrönen and Urtti, 1990a,b; Luo et al., 1991; Järvinen and Urtti, 1992) or to prolong the duration of action by reducing clearance from the delivery site (Adams et al., 1976; Liu et al., 1995). Vasodilators have also been used to enhance systemic bioavailability of drugs (Olanoff et al., 1987; Urtti and Kyyrönen, 1989). We hypothesized that reduced systemic absorption with a vasoconstrictor would increase residence time and increase deposition in the nasal epithelium. Increased deposition in the nasal epithelium could facilitate CNS delivery along several pathways other than the blood, such as along olfactory and trigeminal neural pathways, perivascular pathways, or pathways involving the CSF or nasal lymphatics, providing additional insight into the mechanisms of intranasal drug delivery to the CNS.

The objective of this research was to evaluate the effect of a short-acting vasoconstrictor on intranasal drug targeting to the CNS of two different neuropeptides. The vasoconstrictor selected was phenylephrine hydrochloride (PHE), which is a nasal decongestant with a rapid onset and short duration of action when given topically (O’Donnell, 1995). The neuropeptides evaluated were hypocretin-1 (HC, mol. wt. 3562), a 33-amino acid peptide involved in appetite and sleep regulation; and the dipeptide L-Tyr-D-Arg (D-KTP; mol wt. 337), an enzymatically stable structural analog of the endogenous neuropeptide kyotorphin (L-Tyr-L-Arg) demonstrating neuroprotective effects in rats (Tengamnuay and Mitra, 1990; Tengamnuay et al., 2000). In addition, there is one report of intranasal administration of L-Tyr-L-Arg demonstrating neuroprotective effects in rats (Nazarenko et al., 1999).

**Materials and Methods**

**Experimental Design.** This study was conducted in two parts. The first part of the study investigated the biodistribution of HC following intranasal administration with and without 1% PHE (n = 23–28). Intranasal drug targeting of HC to the CNS relative to intravenous administration was assessed previously in our laboratory (Dhuria et al., 2008); therefore, these experiments were not repeated. Preliminary experiments indicated that the time interval between intranasal application of 1% PHE and the onset of intranasal delivery of HC with 1% PHE did not significantly affect concentrations of HC in CNS tissues of blood (data not shown), demonstrating that the effect of topically applied PHE is rapid. As a result, all subsequent experiments were conducted without intranasal pretreatment with the vasoconstrictor (i.e., neuropeptide and PHE were administered together). A separate group of animals (n = 6) was used to evaluate the distribution of HC into CSF following intranasal administration in the presence and absence of 1% PHE.

The second part of the study investigated intranasal drug targeting of D-KTP to the CNS. First, to assess intranasal drug targeting of D-KTP to the CNS relative to intravenous administration, we compared the biodistribution of D-KTP following both routes of administration (n = 6–7). Next, to assess whether PHE enhances intranasal drug targeting of D-KTP to the CNS, we investigated the biodistribution of D-KTP following intranasal administration with 1% PHE (n = 8). A higher concentration of PHE (5%) was also evaluated (n = 6) to determine whether the effect of the vasoconstrictor was dose-dependent. Finally, CSF was sampled from a separate group of animals (n = 4–6) after intravenous administration and intranasal administration of D-KTP in the presence and absence of 1% PHE.

For all experiments, a mixture of unlabeled and 125I-labeled neuropeptide (10 nmol of HC or D-KTP) was administered to anesthetized rats. CNS tissues, peripheral tissues, and blood were sampled following perfusion and fixation of animals approximately 30 min after the onset of drug delivery. Concentrations were determined based on radioactivity measured in tissues and blood by gamma counting. CNS tissue concentrations were normalized to blood concentrations at 30 min, providing an assessment of intranasal drug targeting to the CNS relative to the blood. Comparisons of concentrations and tissue-to-blood concentration ratios were made between different groups.

**Materials.** HC (American Peptide Co., Inc., Sunnyvale, CA) and D-KTP (H-Tyr-D-Arg-OH acetate salt; Bachem California, Torrance, CA) were custom 125I-labeled with the lactoperoxidase method (GE Healthcare, Woburn, MA). Solutions contained less than 1% unbound 125I as determined by thin layer chromatography and less than 15% acetonitrile. The radiolabeled HC and D-KTP had an average specific activity of 7.4 × 104 GBq/mmol at the reference date and were used within 30 days of synthesis. Phosphate-buffered saline (PBS; 10× concentrate) and phenylephrine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO).

**Animals.** Adult male Sprague-Dawley rats (200–300 g; Harlan, Indianapolis, IN) were housed under a 12-h light/dark cycle with food and water provided ad libitum. Animals were cared for in accordance with institutional guidelines, and all experiments were approved by HealthPartners Research Foundation Animal Care and Use Committee, Regions Hospital.

**Animal Surgeries.** Animals were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.; Abbott Laboratories, Abbott Park, IL). Body temperature was maintained at 37°C by a rectal probe connected to a temperature controller and heating pad (Fine
Science Tools, Inc., Foster City, CA). For intranasal and intravenous experiments, the descending aorta was cannulated for blood sampling and perfusion using a 20-gauge, 1.25-inch catheter (Jelco; Johnson and Johnson Medical Inc., Arlington, TX) connected to a three-way stopcock (B. Braun Medical Inc., Bethlehem, PA). In addition, for intravenous experiments, the femoral vein was cannulated for drug administration using a 25-gauge, 0.75-inch catheter (BD Biosciences, Franklin Lakes, NJ) connected to tubing and a three-way stopcock (B. Braun Medical Inc.).

Preparation of Formulations. Intranasal and intravenous dose solutions contained a mixture of unlabeled and $^{125}$I-labeled neuropeptide (10 nmol; 50–55 μCi) dissolved in PBS (10 mM sodium phosphate and 154 mM sodium chloride, pH 7.4) to a final volume of 48 and 500 μl, respectively. For intranasal experiments with vasoconstrictor, 10% PHE (w/v) or 50% PHE (w/v) stock solutions were prepared and added to dose solutions containing neuropeptide to make a final concentration of 1 or 5% PHE, respectively. Dose solution aliquots for each experiment were stored at −20°C until the day of the experiment.

Drug Administration. Intranasal administration was performed with animals lying on their backs and rolled gauze (1.25 cm diameter) placed under the neck to maintain rat head position, which prevented drainage of the dose solution into the trachea and esophagus. A pipette (P20) was used to intranasally administer 48 μl of dose solution containing 10 nmol of neuropeptide over 14 min. Eight 6-μl nose drops were given noninvasively to alternating nares every 2 min while occluding the opposite naris. The drop was placed at the opening allowing the animal to snort the drop into the nasal cavity. Intravenous administration through the femoral vein was performed with animals lying on their backs. Using an infusion pump (Harvard Apparatus Inc., Holliston, MA), 500 μl of a solution containing an equivalent dose was administered over 14 min.

Tissue and Fluid Sampling. Blood samples (0.1 ml) were obtained via the descending aorta cannula at 5, 10, 15, 20, and 30 min after the onset of drug delivery. After every other blood draw, 0.9% sodium chloride (0.35 ml) was replaced to maintain blood volume during the experiment. Peripheral and CNS tissues were obtained at 30 min after the onset of drug delivery. Animals were euthanized under anesthesia by perfusion and fixation through the descending aorta cannula with 60 ml of 0.9% sodium chloride and 360 ml of 4% paraformaldehyde in 0.1 M Sorenson’s phosphate buffer using an infusion pump (15 ml/min; Harvard Apparatus Inc.). A gross dissection of major peripheral organs (muscle, liver, kidney, spleen, and heart) was performed as well as dissection of the superficial and deep cervical lymph nodes and the axillary lymph nodes. The brain was removed and olfactory bulbs were dissected. Serial (2-mm) coronal sections of the brain were made using a rat brain matrix (Braintree Scientific, Braintree, MA). Microdissection of specific brain regions was performed on coronal sections using a rat brain atlas as a reference (Paxinos and Watson, 1997). A posterior portion of the trigeminal nerve was dissected from the base of the cranial cavity from the anterior lacerated foramen to the point at which the nerve enters the pons. This tissue sample contained the trigeminal ganglion and portions of the ophthalmic (V1) and maxillary (V2) branches of the trigeminal nerve. Dura from the spinal cord was removed and sampled before dissecting the spinal cord into cervical, thoracic, and lumbar sections. The left and right common carotid arteries were dissected from surrounding tissues with the aid of a dissection microscope. Each tissue sample was placed into a preweighed 5-ml tube, and the wet tissue weight was determined using a microbalance (MC210S; Sartorius, Goettingen, Germany).

CSF was sampled via cisternal puncture at 30 min after the onset of drug delivery in a separate group of animals. Animals were placed on their ventral side over a rolled towel to position the head at a 45° angle. A 20-gauge needle attached to 30-cm-long polyethylene 90 tubing was inserted into the cisterna magna. CSF was collected (~50 μl) into the tubing until flow stopped or until blood was observed. The tubing was immediately clamped if blood was observed to avoid contamination due to blood-derived radioactivity. Only CSF samples containing clear fluid were included in the analysis.

Sample and Data Analysis. Radioactivity in each tissue sample was determined by gamma counting in a Cobra II auto gamma counter (PerkinElmer Life and Analytical Sciences, Waltham, MA). Assuming minimal degradation of the $^{125}$I-labeled neuropeptides, concentrations were calculated based on the specific activity of standards sampled from the dose solution (fmol/CPM), counts per minute measured in the tissue (CPM), and tissue weight (g).

Dose-normalized concentrations in blood, CNS tissues, and peripheral tissues from intranasal and intravenous experiments at 30 min were calculated and expressed in nanomoles per liter (assuming a density of 1 g/ml) as mean ± S.E. Outliers were identified using the Grubbs statistical test for outliers and visually using box plots. The area under the blood concentration-time curve (AUC) from 0 to 30 min was calculated using the trapezoidal method without extrapolation to infinity. Because the concentrations observed in the CNS after intranasal delivery could be due to absorption from the nasal vasculature and diffusion or receptor-mediated transport across the blood-brain barrier, CNS tissue concentrations were normalized to blood concentrations at 30 min to assess direct transport across the CNS blood-brain barrier. CNS tissue concentrations were normalized to blood concentrations at 30 min to assess direct transport across the CNS blood-brain barrier. CNS tissue concentrations were normalized to blood concentrations at 30 min to assess direct transport across the CNS blood-brain barrier.

Results

HC Biodistribution with and without 1% PHE. Intranasal administration of HC with 1% PHE significantly reduced absorption of HC into the blood at all time points measured compared with intranasal HC controls (Fig. 1). Intranasal administration of HC over 14 min resulted in a gradual increase in blood concentration, ranging from 0.4 nM at 5 min to 3.4 nM at 30 min, which generated a blood AUC of 56.20 nmol · min/l (Fig. 1). PHE (1%) significantly re-
duced the HC blood concentration at 30 min to 1.2 nM (65% reduction), and the blood AUC to 16.89 nmol · min/l (70% reduction).

PHE (1%) significantly increased HC concentrations in the olfactory bulbs, while reducing concentrations to most other CNS tissues (Table 1). Olfactory bulb concentrations significantly doubled from 2.7 to 5.6 nM in the presence of 1% PHE (Table 1). Concentrations of HC in the brain (excluding the olfactory bulbs) ranged from 0.6 to 1.3 nM for intranasal HC controls. With 1% PHE, no significant differences were observed in HC concentrations in the anterior olfactory nucleus, frontal cortex, caudate/putamen, and septal nucleus, although tissue concentrations were slightly reduced. 1% PHE significantly reduced concentrations by half in remaining brain regions (Table 1). A decreasing concentration gradient was observed in the rostral-caudal direction of the spinal cord after intranasal administration. PHE (1%) significantly reduced concentrations in the thoracic and lumbar segments of the spinal cord from 0.4 to 0.2 nM (Table 1). CSF distribution of HC was significantly increased from 0.2 to 0.3 nM with 1% PHE, and aside from the olfactory bulbs, this was the only other CNS sample in which HC concentrations were increased in the presence of the vasoconstrictor (Table 1). PHE (1%) significantly decreased HC concentrations in the dorsal dura from 2.7 to 1.5 nM, whereas concentrations in the ventral dura and spinal dura were unaffected (Table 1).

In the nasal cavity, 1% PHE significantly reduced concentrations of HC in the respiratory epithelium and significantly increased concentrations in the olfactory epithelium. Superficial and deep cervical lymph nodes, which are drainage sites from nasal lymphatic vessels, contained significantly greater concentrations of HC in the presence of 1% PHE (Table 1). In fact, one of the highest concentrations observed outside of the CNS after intranasal delivery was found in the cervical lymph nodes (18.3 nM), and with 1% PHE this concentration was doubled. The concentration of HC in the trigeminal nerve, which innervates the nasal cavity and enters the CNS at the level of the brainstem, was significantly reduced by 2.9-fold with 1% PHE from 4.9 to 1.7 nM (Table 1). Concentrations of HC in the walls of the carotid artery following perfusion with saline and fixative were increased with 1% PHE from 83 to 256 nM; however, these differences were not found to be significantly different (p = 0.31).

In peripheral tissues, 1% PHE significantly reduced HC concentrations in the spleen and heart, but it had no significant effect on concentrations in the muscle, liver, and kidneys (Table 1). The greatest concentration of HC in peripheral tissues was observed in the kidneys both with (2.8 nM) and without 1% PHE (3.0 nM).

**TABLE 1**

Concentrations following intranasal administration of HC (10 nmol) with and without 1% PHE

<table>
<thead>
<tr>
<th></th>
<th>HC Control (n = 28)</th>
<th>HC + 1% PHE (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain and spinal cord</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory bulbs</td>
<td>2.68 ± 0.33</td>
<td>5.60 ± 0.49*</td>
</tr>
<tr>
<td>Anterior olfactory nucleus</td>
<td>1.11 ± 0.14</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.93 ± 0.09</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>Caudate/putamen</td>
<td>0.58 ± 0.08</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Septal nucleus</td>
<td>0.88 ± 0.32</td>
<td>0.54 ± 0.12</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.67 ± 0.07</td>
<td>0.39 ± 0.04*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.61 ± 0.06</td>
<td>0.32 ± 0.02*</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.60 ± 0.05</td>
<td>0.30 ± 0.02*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.04 ± 0.11</td>
<td>0.68 ± 0.06*</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.65 ± 0.06</td>
<td>0.38 ± 0.03*</td>
</tr>
<tr>
<td>Pons</td>
<td>0.93 ± 0.14</td>
<td>0.44 ± 0.04*</td>
</tr>
<tr>
<td>Medulla</td>
<td>1.26 ± 0.22</td>
<td>0.76 ± 0.11*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.67 ± 0.07</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>Cervical spinal cord</td>
<td>0.80 ± 0.12</td>
<td>0.96 ± 0.27</td>
</tr>
<tr>
<td>Thoracic spinal cord</td>
<td>0.35 ± 0.03</td>
<td>0.19 ± 0.03*</td>
</tr>
<tr>
<td>Lumbar spinal cord</td>
<td>0.35 ± 0.02</td>
<td>0.17 ± 0.01*</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>0.17 ± 0.02</td>
<td>0.28 ± 0.04*</td>
</tr>
<tr>
<td>Meninges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal meninges</td>
<td>2.71 ± 0.33</td>
<td>1.51 ± 0.19*</td>
</tr>
<tr>
<td>Ventral meninges</td>
<td>7.47 ± 1.19</td>
<td>7.54 ± 1.29</td>
</tr>
<tr>
<td>Spinal meninges</td>
<td>2.66 ± 0.59</td>
<td>4.58 ± 1.47</td>
</tr>
<tr>
<td>Nasal epithelia</td>
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<tr>
<td>Respiratory epithelium</td>
<td>19,921 ± 1758</td>
<td>11,457 ± 1348*</td>
</tr>
<tr>
<td>Olfactory epithelium</td>
<td>4241 ± 628</td>
<td>13,330 ± 905*</td>
</tr>
<tr>
<td>Lymph nodes</td>
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<tr>
<td>Superficial cervical</td>
<td>3.56 ± 0.25</td>
<td>6.50 ± 0.69*</td>
</tr>
<tr>
<td>Deep cervical</td>
<td>18.29 ± 3.94</td>
<td>35.58 ± 3.54*</td>
</tr>
<tr>
<td>Trigeminal nerve</td>
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<td></td>
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<tr>
<td>Trigeminal nerve</td>
<td>4.93 ± 0.70</td>
<td>1.71 ± 0.15*</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>82.70 ± 13.13</td>
<td>256 ± 135</td>
</tr>
<tr>
<td>Blood</td>
<td>3.38 ± 0.16</td>
<td>1.19 ± 0.08*</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.53 ± 0.05</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>0.76 ± 0.05</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.00 ± 0.30</td>
<td>2.75 ± 0.47</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.89 ± 0.06</td>
<td>0.50 ± 0.04*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.37 ± 0.06</td>
<td>0.18 ± 0.02*</td>
</tr>
</tbody>
</table>

* p < 0.05, unpaired t test comparing to intranasal HC controls.

Data expressed as mean concentration (nanomole/L) ± S.E. at 30 min.

Cerebrospinal fluid obtained from a separate group of animals (n = 6).
significantly increased to the cervical (2.4-fold), thoracic (1.6-fold), and lumbar segments (1.3-fold) (Fig. 2). Significantly greater drug targeting to the CSF was observed with 1% PHE (2.7-fold), whereas no significant differences in ratios were observed in the trigeminal nerve (Fig. 2). PHE (1%) also significantly increased drug targeting to the superficial and deep cervical lymph nodes (5.7-fold for both; data not shown). Inclusion of 1% PHE in the nasal formulation also significantly enhanced targeting of HC to the ventral meninges (2.9-fold) and to the dorsal meninges (1.7-fold) (data not shown).

**D-KTP Biodistribution following Intranasal and Intravenous Delivery.** Intranasal drug targeting of D-KTP to the CNS was confirmed by comparing intranasal and intravenous drug delivery. Intranasal compared with intravenous administration of D-KTP resulted in significantly lower concentrations in the blood at all time points measured (Fig. 3). Intranasal administration of D-KTP over 14 min resulted in a gradual increase in blood concentration, with a peak concentration of 11.7 nM at 30 min, whereas intravenous infusion resulted in a peak concentration of 83 nM at 10 min, which steadily declined to 55 nM at 30 min. The resulting D-KTP blood AUC was significantly less following intranasal administration (145.30 versus 1708.83 nmol · min/l).

Intranasal compared with intravenous administration resulted in D-KTP brain and spinal cord concentrations that were significantly lower (~3-fold); however, the intravenous route was accompanied by 5-fold greater blood concentrations (Table 2). D-KTP brain concentrations after intranasal administration ranged from 1.8 to 4.3 nM, with the highest concentration in the olfactory bulbs. Intravenous brain concentrations ranged from 5.0 nM in the pons to 7.5 nM in the caudate/putamen. In the spinal cord, intranasal D-KTP resulted in a decreasing concentration gradient from the rostral to caudal direction, whereas intravenous delivery resulted in the highest concentration in the lumbar segment of the spinal cord (Table 2). Distribution into the CSF and dorsal dura were significantly greater with intravenous compared with intranasal administration (Table 2).

In the nasal cavity, the respiratory and olfactory epithelia contained very high levels of D-KTP following intranasal compared with intravenous administration (Table 2). Superficial cervical lymph node concentrations were significantly greater with intravenous delivery, whereas deep cervical lymph node concentrations of D-KTP were significantly greater with intranasal delivery. No statistically significant differences were noted in trigeminal nerve concentrations (p = 0.41), although D-KTP levels were slightly elevated in the intranasal group (Table 2). Additionally, no statistically significant differences were observed in concentrations in the carotid artery walls following perfusion with saline and fixative; however, concentrations were higher with intranasal delivery (p = 0.13) (Table 2).

In peripheral tissues, intranasal delivery of D-KTP resulted in significantly lower concentrations compared with intravenous administration (Table 2). The kidneys contained the highest peripheral tissue concentration of D-KTP, regardless of the route of administration.

**D-KTP Biodistribution with and without PHE.** Inclusion of PHE in intranasal formulations reduced absorption of D-KTP into the blood compared with intranasal D-KTP controls (Fig. 3). PHE (1%) significantly reduced the D-KTP blood concentration at 30 min to 5.1 nM (56% reduction) and the blood AUC to 71.48 nmol · min/l (51% reduction). With 5% PHE, D-KTP blood concentration at 30 min was further reduced to 4.0 nM (66% reduction), and the D-KTP blood AUC was further reduced to 45.65 nmol · min/l (69% reduction) compared with intranasal D-KTP controls (Fig. 3).

**Fig. 3.** Blood concentration-time profiles of D-KTP following intranasal administration, intranasal administration without PHE, and intranasal administration with 1% PHE or 5% PHE (mean concentration ± S.E.; n = 6–8). Intranasal administration of D-KTP (10 nmol) resulted in significantly less absorption into the blood compared with intravenous administration, and including 1% PHE or 5% PHE in the nasal formulation further reduced blood concentrations compared with intranasal D-KTP controls over the course of 30 min (*, p < 0.05, unpaired t test, compared with intranasal D-KTP controls). PHE dose-dependently increased concentrations of D-KTP in the olfactory bulbs to levels higher than those achieved with intravenous delivery, while reducing concentrations in most of the remaining brain regions (Table 2). PHE (1%) did not significantly affect concentrations of D-KTP in the anterior olfactory nucleus, but the presence of the vasconstrictor significantly reduced concentrations by half to all remaining brain regions as well as to the spinal cord (Table 2). Similar trends were observed with 5% PHE, except fewer CNS tissues were significantly different from intranasal D-KTP controls (Table 2). PHE (1%) reduced D-KTP concentrations in the CSF from 0.5 to 0.3 nM, but these differences were not significant (p = 0.09) (Table 2). The effect of 5% PHE on CSF distribution of D-KTP was not evaluated. CSF concentrations of D-KTP were relatively low in comparison with concentrations in the brain, regardless of the route of drug administration. No significant effects on D-KTP concentrations in the dura were noted with PHE.

In the nasal cavity, PHE dose-dependently increased deposition in the olfactory epithelium (Table 2). D-KTP olfactory epithelium concentrations were found to be predictive of olfactory bulb concentrations, with a positive correlation coefficient of 0.99 (data not shown). PHE (1%) significantly increased D-KTP concentrations in the respiratory epithelium, whereas 5% PHE had no significant effect (Table 2). PHE significantly increased D-KTP concentrations in superficial cervical lymph nodes from 6.5 to 21 nM with 1% PHE and to 13 nM with 5% PHE. D-KTP concentrations in the deep cervical lymph nodes were slightly elevated with PHE; however, differences were not significant (Table 2). Cervical lymph node concentrations were among the highest observed outside of the CNS following intranasal administration. No statistically significant differences were noted in trigeminal
nerve concentrations with PHE; however, these values were slightly reduced in the presence of vasoconstrictor (Table 2). Additionally, no significant differences were observed in concentrations of D-KTP in the walls of the carotid artery, although 1% PHE reduced concentrations, whereas 5% PHE had little effect (Table 2).

PHE significantly reduced exposure of D-KTP to all peripheral tissues sampled (except the heart with 5% PHE) (Table 2). Similar reductions in peripheral tissue concentrations were observed with 1% PHE and 5% PHE, with the greatest reduction in the kidney and liver.

**D-KTP Drug Targeting to the CNS and Lymphatics with and without PHE.** Intranasal compared with intravenous administration of D-KTP resulted in significantly greater brain tissue-to-blood concentration ratios, and 5% PHE, but not 1% PHE, significantly enhanced intranasal drug targeting of D-KTP to the brain and to the trigeminal nerve (Fig. 4). The intranasal route of administration targeted D-KTP to the CNS compared with intravenous delivery, with the greatest tissue-to-blood concentration ratios in the trigeminal nerve and the olfactory bulbs, whereas intravenous administration resulted in relatively uniform ratios throughout the CNS. PHE (1%) significantly increased olfactory bulb ratios (5.3-fold increase) compared with intranasal D-KTP controls. No other significant differences in D-KTP drug targeting were observed with 1% PHE (Fig. 4). With 5% PHE, intranasal drug targeting of D-KTP was increased to many more CNS tissues (Fig. 4). Compared with controls, 5% PHE significantly increased D-KTP targeting to the superficial (5.1- and 4.6-fold, respectively) and cervical (2.3-fold) lymph nodes compared with intranasal D-KTP controls (data not shown). PHE (1%) or 5% PHE also significantly increased to the trigeminal nerve with 5% PHE (16.1-fold), anterior olfactory nucleus (3.2-fold), frontal cortex (2.3-fold), or hippocampus (1.5-fold), hypothalamus (3.8-fold), and cerebellum (2.1-fold). In the spinal cord, drug targeting to the cervical spinal cord was increased with 5% PHE but not significantly (p = 0.07). Intranasal drug targeting was also significantly increased to the trigeminal nerve with 5% PHE (2.2-fold) (Fig. 4). Inclusion of 1% PHE or 5% PHE in nasal formulations also significantly enhanced targeting to the superficial (5.1- and 4.6-fold, respectively) and cervical (3.0- and 4.8-fold, respectively) lymph nodes compared with intranasal D-KTP controls (data not shown). PHE (1%) or 5% PHE also significantly enhanced targeting of D-KTP to the meninges, with slightly greater targeting to the ventral portion (3.6- and 3.4-fold, respectively) compared with the dorsal portion (2.3- and 3.2-fold, respectively).
We hypothesized that inclusion of a vasoconstrictor in nasal formulations would reduce absorption into the blood, increase the residence time of the drug in the nasal epithelium, and facilitate intranasal delivery into the brain along pathways involving the olfactory nerves, trigeminal nerves, CSF, or nasal lymphatic channels. Our results indicate that over a 30-min period, inclusion of a vasoconstrictor in the nasal formulation drastically reduced blood concentrations and enhanced intranasal delivery to the CNS along olfactory pathways, while reducing transport along trigeminal pathways. PHE dose-dependently increased concentrations of HC and D-KTP in the olfactory epithelium and olfactory bulbs, consistent with delivery along olfactory nerves through the cribriform plate, suggesting that olfactory epithelium deposition is critical for efficient delivery of intranasally applied drugs to rostral brain regions. Unexpectedly, concentrations in the trigeminal nerve and in remaining brain regions were either unchanged or reduced in the presence of PHE. Intranasal drug targeting, assessed by tissue-to-blood concentration ratios, was enhanced with 1% PHE throughout the brain for HC and to the olfactory bulbs for D-KTP, mainly due to the reduction in blood concentrations observed in the presence of the vasoconstrictor. Increasing the vasoconstrictor concentration to 5% PHE increased D-KTP targeting to additional brain areas. These findings indicate that, at least for two neuropeptides with different molecular weights, inclusion of a vasoconstrictor in nasal formulations can enhance intranasal drug targeting to the brain. Inclusion of PHE in the nasal formulation also enhanced drug targeting of HC and D-KTP to the lymphatic system and to the meningeal membranes surrounding the brain.

The reason for reduced concentrations in the trigeminal nerve following intranasal administration with PHE is not entirely clear. We speculated that trigeminal nerve concentrations were reduced because drug concentration in the respiratory epithelium, which is innervated by this nerve, was also reduced with PHE. However, this was only observed for HC and therefore did not completely explain our findings. We also considered the possibility that reduced blood concentrations observed with PHE could have led to reduced transport within or along the trigeminal nerve and to brain tissues innervated by this nerve. There is evidence that blood vessels within the trigeminal nerve are permeable to certain molecules (Malmgren and Olsson, 1980). Because the present findings did not allow us to distinguish between blood-mediated versus nonblood-mediated transport into the CNS, the implications of these results in understanding intranasal drug delivery mechanisms were limited.

We expected the vasoconstrictor effect would be more pronounced for a smaller peptide that would more readily enter the nasal vasculature; however, blood AUC over 30 min was reduced by 70% for HC and by 51% for D-KTP. This observation may be explained by the presence of the proton-coupled peptide transporter, PEPT2, which is involved in the clearance of di- and tripeptides into the blood (Teuscher et al., 2001; Shu et al., 2002; Bahadduri et al., 2005). PEPT2 transporters in the nasal vasculature could overcome the ability of the vasoconstrictor to reduce absorption into the blood. When a different peptide similar in molecular weight to D-KTP that has not been reported to interact with transporters was evaluated, an 83% reduction in blood concentration was observed with 1% PHE in the nasal formulation (unpublished observations). The use of a PEPT2 inhibitor in combination with the vasoconstrictor and D-KTP could provide additional insight into mechanisms of intranasal delivery and could be another formulation strategy to enhance intranasal drug targeting to the CNS.

The effect of PHE on the absorption of D-KTP and HC into the blood is in agreement with previously published data that demonstrated significant reductions in systemic absorption of nasally applied drugs in the presence of vasoconstrictors (Urtti and Kyyro¨nen, 1989; Kyyro¨nen and Urtti, 1990b; Lee et al., 1991; Luo et al., 1991; Ja¨rvinen and Urtti, 1992). However, these findings are not consistent with a recent study by Charlton et al. (2007), which demonstrated that inclusion of 1% ephedrine in a nasal formulation of an angiotensin-II antagonist increased blood concentrations (Charlton et al., 2007). To account for this unexpected finding, they noted that the ephedrine was coadministered with the drug in their study, whereas vasoconstrictors were applied before initiating drug administration in other studies (Kyyro¨nen and Urtti, 1990a; Ja¨rvinen and Urtti, 1992). It would be interesting to evaluate whether this is explained by the differences in formulation. In our preliminary experiments, no differences were observed between PHE coadministration and pretreatment of the nasal cavity with PHE before beginning intranasal drug administration (data not shown). Furthermore, our findings of increased delivery to the olfactory bulbs in the rostral brain and reduced delivery to most remaining brain areas following intranasal administration with a vasoconstrictor are not consistent with the Charlton et al. (2007) study, in which enhanced delivery to all brain regions was observed with 1% ephedrine. A confounding factor in that study was that animals were not perfused to remove blood from the cerebral vasculature before brain dissection, possibly resulting in increased brain concentrations due to the presence of blood-derived drug.
Our findings show that inclusion of a vasoconstrictor in the nasal formulation facilitated transport of HC, but not D-KTP, along pathways involving the CSF and the lymphatic system. Consistent with published findings showing increased CSF distribution after intranasal administration with decreasing molecular weights (Sakane et al., 1995), CSF concentrations were higher for D-KTP than for HC. Concentrations within the CSF were not sufficiently higher than brain concentrations to suggest that brain concentrations were due to entry into the CSF followed by distribution into the brain. Intranasal administration with 1% PHE had opposite effects on entry into the CSF, with increased uptake for HC and decreased uptake for D-KTP. In the lymphatic system, we expected that PHE would increase transport into the cervical lymph nodes due to drainage pathways leading from the nasal cavity to the lymph nodes of the neck (Weller et al., 1992; Kida et al., 1993; Walter et al., 2006). Increased cervical lymph node concentrations were found for HC, but not for D-KTP, which is consistent with studies evaluating lymphatic uptake of drugs, where absorption into the lymphatic system was higher for larger molecular weight compounds after drug administration (Supersaxo et al., 1990).

High concentrations of HC and D-KTP in the walls of the carotid artery, even after removal of blood with saline perfusion, suggest that intranasally applied neuropeptides have access to perivascular spaces. Concentrations in the walls of the carotid artery were much higher than blood concentrations following intranasal administration, such that the level of drug present could not be explained by diffusion from blood. Instead, the neuropeptides may have entered the blood vessel walls from the nasal epithelium where high concentrations of drug were present. When PHE was added to the nasal formulation, it is possible that the vasoconstrictor could have entered the brain and reduced cerebral blood flow, which could have affected the distribution of HC and D-KTP within the CNS. Several researchers have demonstrated an important role of arterial pulsations in the distribution of solutes within the CNS, suggesting that reduced blood flow would result in diminished perivascular transport and hence reduced CNS concentrations (Rennels et al., 1985; Hadaczek et al., 2006). Additional experiments evaluating the effect of vasoconstrictors on cerebral blood flow or drug distribution within perivascular spaces could improve our understanding of intranasal drug delivery mechanisms.

The current study was limited by the fact that a single time point after intranasal administration was evaluated, suggesting the need for future studies to characterize the pharmacokinetic profile of vasoconstrictor effects on intranasal drug delivery to the CNS. Additionally, because measurements in this study were based on radioactivity, they may not necessarily reflect intact peptide. In support of this work, we previously demonstrated that intact 125I-HC was detected by necessarily reflect intact peptide. In support of this work, we previously demonstrated that intact 125I-HC was detected by nuclear imaging techniques in patient populations with nasal congestion due to colds or allergies.

In conclusion, we demonstrated for the first time that inclusion of a short-acting vasoconstrictor in nasal formulations can enhance intranasal drug targeting to the brain, lymphatics, and meninges, while significantly reducing absorption into the blood. This novel strategy for enhancing intranasal targeting to the CNS using vasoconstrictors may be most suitable for potent CNS therapeutics that have adverse effects in the blood or peripheral tissues, are rapidly degraded by enzymes in the blood or gastrointestinal tract, or are extensively bound by tissue or plasma proteins. Vasoconstrictor nasal formulations containing CNS therapeutics could be used to target brain tumors or to treat pain disorders, avoiding undesirable side effects that often accompany traditional routes of drug administration. Inclusion of vasoconstrictors in nasal formulations can result in enhanced drug targeting to multiple brain areas, the lymphatic system, and the meninges, which may hold relevance for the treatment of various neurological disorders, autoimmune disorders, or meningitis.

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