Amiloride Peptide Conjugates: Prodrugs for Sodium-Proton Exchange Inhibition

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ABSTRACT

Inhibition of the sodium-proton exchanger (NHE) plays an important role in reducing tissue damage during ischemic reperfusion injury; however, pharmacological inhibitors of NHE have restricted access to acutely ischemic tissues because of severely compromised tissue perfusion. We describe the syntheses, characterization, and NHE inhibitory activities of a novel class of amiloride derivatives where peptides are conjugated to the amiloride C(5) amino group. These new peptide-C(5)-amiloride conjugates are inactive; however, peptide residues were chosen such that selective cleavage by neutral endopeptidase 24.11 (enkephalinase) liberates an amino acid-C(5)-amiloride conjugate that inhibits NHE in a glial cell line. These results confirm the feasibility of using peptide-amiloride conjugates as NHE inhibitor prodrugs. We envision the design of analogous peptide-amiloride prodrugs that can be administered prior to ischemic events and subsequently activated by endopeptidases selectively expressed by ischemic tissues.

The sodium-proton exchanger (NHE) represents a family of sodium-dependent transport proteins that participate in various cellular functions (Orlowski and Grinstein, 1997). Seven isoforms (NHE1–7) have been identified (Numata and Orlowski, 2001; Brett et al., 2002; Slepkov and Fliegel, 2002). NHE1 and NHE5 to 7 are particularly important in maintaining the intracellular pH (pHi) in human heart and brain. Additionally, increased NHE1 activity has been shown to maintain an alkaliotic pHi in several human cancer cell types, including transformed astrocytes, i.e., malignant glioma cells (McLean et al., 2000; Hegde et al., 2004). Inhibition of NHE1 in glioma cells causes a reduction of intracellular sodium and associated cytotoxic edema (F. A. Gorin, R. Sriram, W. Harley, C. Floyd, A. Marshall, B. Lyeth, and T. Jue, unpublished data).

There is a shift from oxidative to nonoxidative glycolysis during ischemia with increased intracellular acidosis. This reduction in pHi activates NHE, which increases intracellular Na⁺/H⁺ levels (step 2, Fig. 1) (Orlowski and Grinstein, 1997). The specifics of normalizing increased [Na⁺], remain unclear but include regulation by Na⁺/K⁺ ATPase and sodium-dependent calcium influx (reverse mode) by the sodium-calcium exchanger (NCX) (Satoh et al., 2003). Persistent activation of the reverse mode of NCX during vascular perfusion further increases intracellular Ca²⁺ ([Ca²⁺]), which is believed to initiate the irreversible cellular damage observed during ischemia-reperfusion (I/R) (Piper et al., 1996). This sequence of physiological events leading to I/R injury is initiated by NHE activation; consequently, the controlled inhibition of NHE is an area of intense research (Maseer et al., 2003) (Fig. 1).

The significance of NHE participation in I/R injury in animals has been shown by demonstrating that NHE inhibitors, such as cariporide (Fig. 2), are effective in preventing cellular damage resulting from cerebral and myocardial ischemia when administered prior to the ischemic event (Klein et al., 1995; Scholz et al., 1995; Gumina et al., 1999; Suzuki et al., 2002). In a recent human clinical study (Tardif et al., 2002)...

ABBREVIATIONS: NHE, sodium proton exchanger; NCX, sodium-calcium exchanger; I/R, ischemia-reperfusion; EIPA, ethylisopropylamiloride; SAR, structure-activity relationship; Am, amiloride; LC, liquid chromatography; MS, mass spectrometry; BGE CF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein.
Cariporide was administered intravenously prior to coronary artery bypass graft surgery. Its cardioprotective effects were shown to be greatest when the drug was already present in the myocardial tissue prior to reperfusion. A major therapeutic challenge in delivery of NHE inhibitors to tissues during the early phases of ischemia is the lack of adequate perfusion. A potential solution to these challenges would be to devise a biologically inactive NHE inhibitor prodrug that would reside in tissues and be transformed into an active NHE inhibitor (Gorin and Nantz, 2004) during an ischemic event. For example, cellular endopeptidases are activated in the early stages of ischemia, which subsequently initiate apoptotic cell death (Denault and Salvesen, 2002). Activation of an extant prodrug by peptidases eliminates drug delivery concerns, whereas NHE inhibition is selective and occurs immediately subsequent to the ischemic event. We describe herein a proof of concept of such an approach by preparing an inactive prodrug and demonstrating its enzymatic activation to yield a potent NHE inhibitor (Fig. 2).

As depicted in Fig. 2, amiloride, a potassium-sparing diuretic approved for human use (Cragoe, 1992), has been modified to enhance NHE and NCX inhibitory activities (Simchowitz et al., 1992). Derivatization of the C(2) acyl guanidine side chain increased NCX inhibition (cf. amiloride versus dichlorobenzamil), and C(5)-amino group alkylation increased NHE inhibition [cf. amiloride versus ethylisopropylamiloride (EIPA)] (Simchowitz et al., 1992). Given the extensive structure-activity relationship (SAR) data available for amiloride derivatization, we chose to initially modify amiloride such that inactive prodrugs would be capable of generating relatively specific NHE inhibitors.

Central to our prodrug strategy (Fig. 3) is the conjugation of an extant prodrug by peptidases to eliminate drug delivery concerns, whereas NHE inhibition is selective and occurs immediately subsequent to the ischemic event. We describe herein a proof of concept of such an approach by preparing an inactive prodrug and demonstrating its enzymatic activation to yield a potent NHE inhibitor (Fig. 2).

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of amiloride to a peptide sequence. Since modification of the amiloride C(5)-amino group increased NHE inhibition in a number of examples (Simchowitz et al., 1992), we selected this position as the site of peptide attachment. The incorporation of an amino acid side chain imparts considerable flexibility in the design of a prodrug for an ischemia-induced conversion to an amiloride-based NHE inhibitor. Specifically, a peptide-conjugate strategy offers two distinct advantages: specific peptide sequences permit selective cleavage by peptideases for site-specific tissue activation of prodrugs, and the hydrophilic nature of a peptide-amiloride conjugate (e.g., peptide-C(5)-Am, Fig. 3) could be employed to deter intracellular permeation and limit the conjugate’s action to cell surface transporters. It is useful that the peptide-Am conjugate be inactive or at least weakly active. Peptide side chain cleavage by resident enzymes present in ischemic tissue, then, by design, would liberate an amiloride analog inhibitor of NHE [e.g., Gly-C(5)-Am]. In this report, peptide side chains were initially selected to resemble portions of \(5\text{[Leu]}\)-enkephalin peptide analogs because of the longstanding SAR information pertaining to the cleavage of these opioid peptides by neutral endopeptidase 24.11 (enkephalinase) (Roques et al., 1993). Here, we describe the preparation, enzymatic cleavage, and NHE-inhibitory activities of novel peptide-C(5)-Am conjugates (Fig. 3).

Materials and Methods

**Chemistry**

Experimental details and spectral characterization data for compounds 3a to d and 4a to d are provided in the Supplemental Data. Peptides were purchased from Genetel Laboratories (Madison, WI) as trifluoroacetic acid salts. The peptide sequences in conjugates 4b to d were designed to mimic analogs of \(5\text{[Leu]}\)-enkephalin (see Discussion; Gorin et al., 1980). N-(3-amino-5,6-dichloro-pyrazine-2-carbonyl)-guanidine hydrochloride (2) was prepared according to a literature procedure (Cragoe et al., 1967). NMR spectra were recorded with a Varian Inova spectrometer (\(1^H\) at 400 MHz and \(13^C\) at 100 MHz). Mass spectral analyses (LC-MS-electrospray ionization) were performed at University of California Molecular Structure Facility (Davis) using a Thermo Finnigan LCQ fitted to an electrospray source and ion trap mass analyzer with an ABI 120A high-performance liquid chromatography. Samples were injected onto a Vydac (Davis) using a Thermo Finnigan LCQ fitted to an electrospray detector. The detector was an Orca II-ER CCD digital camera (Hamamatsu Corporation, Bridgewater, NJ), which is controlled by C-Imaging Simple PCI software (Compix, Cranberry Township, PA).

**Materials and Methods**

**Intracellular pH Measurements.** U87 glial cells were grown on glass coverslips and incubated for 30 min at 37°C with the pH-sensitive dye, 2',7'-bис[(2-carboxyethyl)-5(6)-carboxyfluorescein]-AM (BCECF-AM; Molecular Probes, Eugene, OR). Cells were rinsed with HEPES-Ringer buffer twice and maintained at 37°C for an additional 15 min to permit intracellular hydrolysis of the AM ester, trapping BCECF within the cells. Cells on coverslips were placed in a Hitachi F2000 fluorescent spectrophotometer and excited at 440 and 507 nm with fluorescent emissions recorded at 535 nm (Hegde et al., 2004).

**Fluorescence Microscopy.** Visualization of intracellular fluorescent amiloride derivatives was conducted using high-speed imaging, epifluorescence microscopy. Excitation light was provided by a xenon arc lamp coupled to the Polychrome IV scanning monochromator (Till Photonics, Graefelfing, Germany) that alternately excites with different wavelengths. Excitation light was delivered by fiber optics to cells through the epifluorescence port of a Nikon E600 microscope coupled to a Nikon Fluo 60× water immersion lens. The detector was an Orca II-ER CCD digital camera (Hamamatsu Corporation, Bridgewater, NJ), which is controlled by C-Imaging Simple PCI software (Compix, Cranberry Township, PA).

Intracellular emission intensities were collected in regions of interest from an average of four to eight cells per field using Simple PCI imaging software. Emission intensities were subtracted from mean intracellular intensities measured in glialoma cells prior to the addition of the fluorescent amiloride derivatives. Statistical significance was calculated using the Wilcoxon rank sum test (Sigma Statview version 3.0; SPSS Inc., Chicago, IL) as previously described (Vali et al., 2000). Images were captured as TIFF files (C-Imaging Simple PCI software), and light and fluorescent images of the same field were imported in Photoshop 6.0 and vector graphics added using Illustrator 9.0.1 (Adobe Systems, Mountain View, CA).

**Results**

**Synthesis of Peptide-C(5)-Am Conjugates.** Although amino acid conjugation to amiloride has been disclosed in a single report (Pató et al., 1999), no examples of direct peptide conjugation to the C(5)-amino group have been reported. Consequently, we have developed an approach (Fig. 4) that closely follows the pioneering efforts of Cragoe et al. (1967) for synthesis of C(5)-amino, alkoxyl, and thio analogs of amiloride (Fig. 4).

The reaction of C-terminus benzyl-protected amino acids 1a to d with dichloropyrazine 2 proceeded in the presence of base to regioselectively deliver peptide-C(5)-Am conjugates (Fig. 4). Hydrogenolysis removed the benzyl protecting group and afforded conjugates 4a to d in 28 to 47% overall yields. The structural integrity of the conjugates was confirmed by spectroscopic analyses (\(1^H\) and \(13^C\) NMR) and mass spectral analysis (LC-MS). To unequivocally assign C(5) as the position of amino acid attachment, an X-ray crystal structure of adduct 3a was obtained (X-ray crystallographic data for compound 3a are contained in the Supplemental Data). The data clearly show that the amino acid moiety resides para to the guanidine side chain.

**Enzyme Digestion Study.** To determine their susceptibilities toward enzymatic cleavage, we incubated the peptide-C(5)-Am conjugates 4a to d at 25°C with 10 units of enkephalinase (endopeptidase 24.11, Calbiochem). Reaction aliquots were taken following a 24-h incubation period and analyzed directly by LC-MS (electrospray; Thermo Finnigan, San Jose, CA) for the presence of starting peptide-C(5)-Am conjugates and the targeted cleavage product, Gly-C(5)-Am (4a). The data show that conjugate 4d released 4a following endopeptidase digestion (Table 1). Since there are no basic amino acid residues in conjugates 4a to d, trypsin was employed as a...
potential negative control to ensure that any observed cleavage products could be ascribed only to endopeptidase-specific interactions (i.e., the conjugates are devoid of the requisite Arg and Lys residues for trypsin-mediated digestion) (Jackson, 1999). Trypsin digestion did not cleave any of the amiloride conjugates with the exception of 4d (Table 1).

**Evaluation of NHE Inhibition by Peptide-C(5)-Am Conjugates.** NHE inhibition by peptide-C(5)-Am conjugates was evaluated using pH$_i$ measurements in the U87 human glial cell line (Hegde et al., 2004). Cells loaded with BCECF were then acidified using the ammonium chloride prepulse method (Roos and Boron, 1981). The subsequent sodium-dependent recovery of pH$_i$ by these cells in the absence of bicarbonate was monitored using a spectrofluorometer. Sodium-dependent proton extrusion in U87 glial cells and in primary astrocytes is mediated by NHE1 (McLean et al., 2000; Hegde et al., 2004) and was monitored in the presence and absence of the novel amiloride peptide (4d) and amino acid (4a) conjugates. Concentrations of these conjugates capable of inhibiting 50% of NHE1 activity (IC$_{50}$) were deter-

<table>
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<tr>
<th>Entry</th>
<th>Conjugate</th>
<th>Peptidase</th>
<th>LC-MS Fragments*</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4a</td>
<td>Enkeph</td>
<td>254.1 (MH$^+$-Cl)</td>
<td>Enkephalinase-resistant</td>
</tr>
<tr>
<td>2</td>
<td>4a</td>
<td>Trypsin</td>
<td>254.1 (MH$^+$-Cl)</td>
<td>Trypsin-resistant</td>
</tr>
<tr>
<td>3</td>
<td>4b</td>
<td>Enkeph</td>
<td>676.3 (parent, MH$^+$)</td>
<td>Enkephalinase-resistant</td>
</tr>
<tr>
<td>4</td>
<td>4b</td>
<td>Trypsin</td>
<td>676.3 (parent, MH$^+$)</td>
<td>Trypsin-resistant</td>
</tr>
<tr>
<td>5</td>
<td>4c</td>
<td>Enkeph</td>
<td>628.4 (MH$^+$-Cl)</td>
<td>Enkephalinase-resistant</td>
</tr>
<tr>
<td>6</td>
<td>4c</td>
<td>Trypsin</td>
<td>628.4 (MH$^+$-Cl)</td>
<td>Trypsin-resistant</td>
</tr>
<tr>
<td>7</td>
<td>4d</td>
<td>Enkeph</td>
<td>288.1 not observed</td>
<td>Enkephalinase-mediated release of 4a</td>
</tr>
<tr>
<td>8</td>
<td>4d</td>
<td>Trypsin</td>
<td>215.4$^b$</td>
<td>Trypsin-mediated cleavage of the Gly-Am linkage</td>
</tr>
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* Principal LC-MS fragments observed on direct injection of an aliquot sampled from the digestion at 24 h.

$^b$ Fragment corresponding to the pyrazine product obtained on cleavage of the peptide from the Am ring.

**Table 1**

Enzymatic digestion of peptide-C(5)-Am conjugates

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**Fig. 4.** Synthesis of peptide-amiloride conjugates.
mined and compared with the known NHE inhibitors, amiloride and cariporide (Table 2). The IC₅₀ of amiloride depends upon external \([\text{Na}^+]\), and its value in the U87 glial cells (50 \(\mu\text{M}\)) is modestly higher than those described in the U118 glial cell line (17 \(\mu\text{M}\)) and in primary rat astrocytes (6 \(\mu\text{M}\)) (McLean et al., 2000). The IC₅₀ of cariporide (74 nM) is comparable with values published using CHO cell lines (30 nM) (Kawamoto et al., 2001) (Table 2).

Conjugate 4d did not inhibit NHE1 in U87 glial cells, and concentrations exceeding 100 \(\mu\text{M}\) produced interfering fluorescent background. In contrast, compound 4a was at least 4-fold more potent than the parent compound, amiloride, but less active than the benzoylguanidine derivative, cariporide. The inhibition of NHE1 was rapidly reversed following the removal of 4a, in contrast to the slow and incomplete recovery observed with amiloride and EIPA (data not shown). This suggested that intracellular permeation by the more hydrophilic 4a differed significantly from that of more hydrophobic amiloride and the \(\text{Am}-\text{C(5)}\) alkyl homolog, EIPA.

**Intracellular Translocation of Amiloride Congeners.**

The intracellular translocation of compound 4a into U87 cells was compared with amiloride and EIPA (Fig. 2). These three amiloride derivatives are intrinsically fluorescent when excited at 380 nm with 510-nm emission, and their presence inside cells can easily be visualized using a variable wavelength, quantitative fluorescent microscopy system (Kraut et al., 1993). Their relative molar absorptivity constants measured with a spectrofluorometer are quite comparable,¹ so that intracellular accumulation of amiloride, EIPA, and 4a could be visualized using fluorescent microscopy.

U87 cells were incubated with 50 \(\mu\text{M}\) of compound 4a, amiloride, or EIPA for 90, 180, and 360 min, washed twice at 22°C with isotonic phosphate buffer, and intracellular fluorescence then was visualized by 380-nm excitation and 510-nm emission (Kraut et al., 1993). These studies were repeated three times for each compound with the same results. Within 90 min, there was intracellular accumulation of amiloride and EIPA that was associated with the endoplasmic reticulum (Fig. 5, A and B). In contrast, no intracellular fluorescence above mean intracellular background could be detected in intact glioma cells with compound 4a following incubations of either 90 or 180 min. Compound 4a did demonstrate significant intracellular fluorescence only in the rare dying and dead cells having increased membrane permeabilities. These dying and dead cells were identified by their costaining with trypan blue, a visible dye that is excluded by viable cells (Fig. 5, C and D; Hegde et al., 2004). The intracellular detection of Gly-C(5)-Am (4a) fluorescence only in trypan blue positive (i.e., dead) cells further verified that 4a remains excluded from viable U87 cells after 180 min, relative to amiloride and EIPA (Fig. 5).

**Discussion**

There is great interest in the development of inhibitors of NHE and NCX to limit ischemic tissue damage produced during vascular reperfusion. A fundamental impediment to the field has been the delivery of these compounds to poorly vascularized tissues during the early phases of ischemic injury when NHE/NCX inhibition would be most beneficial.

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TABLE 2

<table>
<thead>
<tr>
<th>NHE Inhibitor Drug</th>
<th>IC₅₀ ((\mu\text{M})) ± S.D. (NHE Inhibition)</th>
<th>Relative Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>50 ± 27</td>
<td>1</td>
</tr>
<tr>
<td>Cariporide</td>
<td>0.074 ± 0.072</td>
<td>676</td>
</tr>
<tr>
<td>Gly-C(5)-Am (4a)</td>
<td>13 ± 7</td>
<td>4</td>
</tr>
<tr>
<td>Leu-Phe-Gly-Gly-Gly-C(5)-Am (4d)</td>
<td>No inhibition ≤ 100 (\mu\text{M})</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

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¹ The relative molar absorptivity constants measured with a spectrofluorometer are quite comparable, indicating similar intracellular accumulation of amiloride, EIPA, and 4a.
(Masereel et al., 2003; Tardif et al., 2004). In this report, we describe a novel strategy utilizing inactive produgs that can be enzymatically activated to generate inhibitors of NHE. This strategy can be applied to address several of the limitations of the current therapies for I/R cytotoxicity. By design, an inactive peptide prodrug can reside in tissues prior to the onset of ischemia. Peptidases generated during early phases of ischemia cleave the prodrug and unmask an NHE inhibitor molecule. The advantages of this approach are selective inhibition of NHE in ischemic tissues with prodrug activation during a critical phase where early NHE inhibition can limit the cell death caused by sodium-mediated Ca\(^{2+}\) overload (Satoh et al., 2003). Additionally, inactive peptide produgs can be converted to small, water-soluble molecules whose biological activities are restricted to cell surface exchangers, thereby limiting unintended intracellular toxicity (Numata and Orlowski, 2001).

The present work describes a method for preparing peptide-amiloride conjugates. Amiloride was chosen because of the extensive SAR information (Simchowitz et al., 1992) pertaining to its inhibition of sodium-mediated transport proteins, notably NHE and NCX. Initially, the peptide sequences in conjugates 4b to d were selected to mimic analogs of \(^{5}\)[Leu]-enkephalin, a member of the opioid neuropeptide family, which has the amino acid sequence Tyr-Gly-Gly-Phe-Leu-OMe (Gorin et al., 1980). Amiloride serves as a \(^{1}\)[Tyr] surrogate in the conjugate panel. The enzymatic cleavage of enkephalin by enkephalinase (e.g., neutral endopeptidase 24.11) is well characterized and known to occur selectively between adjacent Gly residues (Roques et al., 1993). Conjugate 4b was designed as a negative control because it contains two D-amino acids in the peptide sequence instead of a Gly-Gly motif to ensure resistance to enkephalinase-mediated cleavage (Gorin et al., 1980). Enzyme-mediated cleavage of 4c was designed to afford Gly conjugate 4a provided that the amiloride substitution for \(^{5}\)[Leu] does not alter the Gly-Gly recognition by enkephalinase. The possibility that the Tyr-to-amiloride substitution could alter Gly-Gly substrate recognition by enkephalinase led to the insertion of an additional Gly in the peptide sequence, and thus, the rationale for conjugate 4d.

The enzyme digestion data (Table 1) show that 4a is stable and persists in the presence of both enkephalinase and trypsin (entries 1 and 2). As predicted, 4b was resistant to peptidase cleavage by either enzyme because of the D-amino acid substitutions. Digestion of \(^{5}\)[Leu]-enkephalin amiloride analog 4c with enkephalinase did not liberate 4a, likely because of steric interference by the amiloride residue (entry 5) (Roques et al., 1993). However, the inclusion of the additional Gly in peptide conjugate 4d resulted in a complete digestion by enkephalinase to furnish 4a (entry 7). The additional Gly in 4d appears to ameliorate the adverse effect of the Tyr-to-amiloride substitution in conjugate 4c, thereby restoring enkephalinase specificity to release 4a.

The enzymatic specificity for release of 4a by enkephalinase is supported by the observation that none of the conjugates released 4a when digested with trypsin. The digestion of conjugate 4d by trypsin did not release 4a, suggesting that the C(2) guanidine moiety of the amiloride terminus in 4d mimics L-arginine. In addition, the hexapeptide 4d may be capable of forming the requisite peptide loop intermediate for trypsin cleavage (Jackson, 1999), unlike the shorter pentapeptide homolog 4c. These enzymatic studies illustrate the feasibility of using a targeted enzyme-mediated strategy to release 4a from peptide-amiloride precursors.

With 4d and 4a as a potential prodrug-drug pair, we assayed the biological efficacies of these compounds as NHE inhibitors in a human glial cell line. The IC\(_{50}\) values determined in the glial cell study (Table 2) clearly show that conjugate 4a is a more potent NHE inhibitor than amiloride. In contrast, conjugate 4d was essentially inactive. The relative IC\(_{50}\) values for 4a and 4d confirm the ideal activity difference between an inactive peptide prodrug and its corresponding active drug form, in this case a new, potent NHE inhibitor.

During the course of the IC\(_{50}\) determinations, we observed that washout of 4a restored the steady-state pH of U87 cells in less than 1 min. In contrast, amiloride washout was associated with a prolonged and incomplete recovery of steady-state pH. These observations suggested that 4a is more effectively removed from the NHE protein than its more hydrophobic parent compound, amiloride. This effect is predicted by their respective cLogP values,\(^{2}\) which also predicts that 4a would be less likely to permeate the cells unlike amiloride or EIPA (Kraut et al., 1993). The intrinsic fluorescence of amiloride derivatives permitted the use of a quantitative fluorescent microscopy system to detect their intracellular accumulation (Kraut et al., 1993). Fluorescent microscopy failed to detect the intracellular accumulation of 4a after 180 min, in contrast to the rapid cell permeation observed with amiloride and EIPA (Fig. 5). The permeation properties of the more polar 4a hopefully restrict its activity to cell surface exchanger proteins while limiting nonspecific intracellular toxicity, which we have observed with the use of amiloride and EIPA.

In conclusion, we have shown that an inactive peptide-amiloride conjugate can be transformed into a potent NHE inhibitor under enzyme-specific conditions. To our knowledge, compound 4a is the first amino acid analog of amiloride that appears to have several desirable pharmacological and chemical properties for an NHE inhibitor. This peptide prodrug strategy may find general application as a new therapeutic approach for limiting the damage produced by ischemic-reperfusion injury. Enkephalinase is present in the brain’s cerebrospinal fluid and has been shown to degrade exogenously administered opioid peptides (Molineaux and Ayala, 1990). In the future, we anticipate coupling peptide sequences to the C(5) position of amiloride to generate active prodrugs that are substrates for endopeptidases specifically activated during early stages of brain I/R. Furthermore, the selective activation of an NHE inhibitor prodrug by glioma-specific endopeptidases could assist with the regional treatment of intracellular edema associated with these aggressive intracerebral tumors (Gorin et al., 2004; Hegde et al., 2004).

Recently, we have adapted this drug design strategy to synthesize C(2) amino acid and peptide conjugates of amiloride, which demonstrate dual NHE and NCX inhibitory activities (Gorin and Nantz, 2004). We are hopeful that these peptide-C(2)-amiloride derivatives will complement the novel C(5) peptide conjugates described in this report to even more effectively limit I/R tissue damage.
Acknowledgments

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References


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