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Report

The Use of α-Aminoboronic Acid Derivatives to Stabilize Peptide Drugs During Their Intranasal Absorption

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α-Aminoboronic acid derivatives, potent and reversible inhibitors of aminopeptidases, were tested nasally *in situ* in rats for stabilizing externally administered peptides. These inhibitors, at nanomolar concentrations, were found to inhibit greatly the degradation of the model peptide, leucine-enkephalin (Leu-enk), in the nasal perfusate. Enzyme inhibition was greater with boroleucine and borovaline than that observed with boroalanine. Boroleucine was more than 100 times more effective in enzyme inhibition than bestatin and more than 1000 times more effective than puromycin.

KEY WORDS: α-aminoboronic acid derivatives; transition-state analogue inhibitors; peptides; aminopeptidases; nasal absorption; leucine-enkephalin.

INTRODUCTION

A large number of biologically active peptides have been isolated and characterized. As their oral bioavailability is very low due to poor absorption and/or degradation in the gastrointestinal tract and the liver, the use of these peptides as medicinal agents has been limited to the intravenous route of administration. In order to circumvent this limitation, alternative routes of administration of these compounds, such as the nasal (1,2), buccal (3), rectal (4,5), and vaginal (6), have been sought. Among these the nasal route has been the route most investigated for peptide delivery. Peptides have generally shown a relatively low nasal bioavailability. For example, studies with insulin and the luteinizing hormone-releasing hormone (LHRH) analogue, nafarelin acetate, indicated that much larger nasal than parenteral doses are required to produce similar effects (1,7–9). Studies performed in monkeys using 270-μg nasal and 5-μg subcutaneous doses showed that the nasal bioavailability of nafarelin acetate was only ~2%, and the area under the plasma drug level (AUC) vs the time after nasal administration increased in a nonlinear fashion as a function of the dose administered (1). Saturable enzymatic degradation or absorption pathways were suggested as feasible explanations of the data. Therefore, one of the major problems in the intranasal route of administration is the rapid degradation of peptides at the site of administration by proteolytic enzymes (10), especially at low peptide doses. The major component of the enzymes responsible for peptide degradation has been identified as aminopeptidases (11). Effective proteolytic inhibitors may offer improved nasal peptide bioavailability.

The design of transition-state analogue inhibitors is based on the hypothesis that molecules which resemble a substrate in its transition-state geometry have a much higher affinity for the active site of an enzyme than the substrate itself. The α-aminoboronic acid derivatives (Fig. 1), in which the boron atom has a trigonal geometry, can form a tetrahedral boronate ion. These tetrahedral boronates resemble the transition state of peptides during their hydrolysis by proteases and would be expected to act as transition-state analogues for proteases (12,13). These α-aminoboronic acid derivatives were previously shown to be effective inhibitors of human enkephalin degrading aminopeptidase, microsomal leucine aminopeptidase, and cytosolic leucine aminopeptidase (14).

We report herein our investigation dealing with the potential use of these aminopeptidase inhibitors to stabilize peptides during their residence in the nasal mucosa. Also, a comparison of the aminopeptidase inhibition activity with different aminoboronic acid derivatives and with known aminopeptidases inhibitors, such as puromycin and bestatin, was made. Leucine-enkephalin (Leu-enk), a pentapeptide previously reported to undergo extensive hydrolysis in the nasal cavity (10), was used as a model peptide.

EXPERIMENTAL

Materials

Leucine enkephalin (Tyr-Gly-Gly-Phe-Leu), Des-Tyr-Leu-enk (Gly-Gly-Phe-Leu), bestatin, and puromycin were obtained from Sigma Chemical Company. Sodium pentobarbital injection (Nembutal) was purchased from Abbott Laboratories. The α-aminoboronic acid derivatives, 3-methyl-1(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)-

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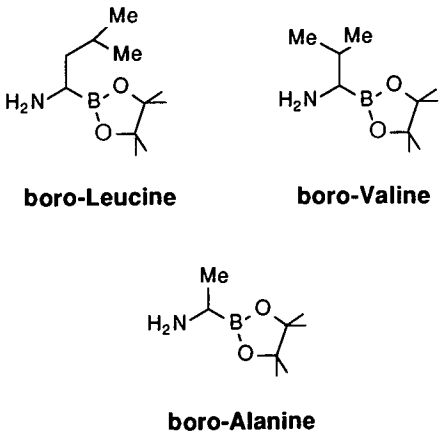

Fig. 1. Structures of the α-aminoboronic acid derivatives.

1-butamine, trifluoroacetic acid salt (abbreviated boroleucine), 4,4,5,5-tetramethyl-α-(1-methylethyl)-1,3,2-dioxaborolane-2-methanamine, trifluoroacetic acid salt (abbreviated borovaline), and α,4,4,5,5-pentamethyl-1,3,2-dioxaborolane-2-methanamine, trifluoroacetic acid salt (abbreviated as boroalanine), were prepared as previously described (14). (For convenience, the α-aminoboronic acid derivatives have been abbreviated by the prefix "boro" and the corresponding amino acids.) All other materials were reagent or analytical grade and used as received.

Nasal Perfusion Studies in Rats and Analytical Method

The *in situ* nasal perfusion studies and analytical methods were similar to those described previously (10). Briefly, male Lewis rats weighing ~300 g each were anesthetized with sodium pentobarbital (50 mg/kg). An incision was made in the neck and the trachea was cannulated with a polyethylene tube (PE 240). A second tube, which served to introduce the perfusing solution, was inserted through the esophagus to the posterior part of the nasal cavity. The nasopalatine was closed with an adhesive to prevent drainage of the perfusing solution from the nasal to the oral cavity. Concentrated stock solutions of Leu-enk, bestatin, and puromycin were prepared in 0.05% acetic acid and were used within 3 days. Concentrated stock solutions of the aminoboronic acid derivatives were prepared daily in phosphate buffer (0.1 *M*, pH 7.4).

Leu-enk solution (60 μg/ml, 10 ml) in phosphate buffer (0.1 *M*, pH 7.4), in the absence and presence of different aminoboronic acid derivatives, bestatin, puromycin, and, in particular, different concentrations of boroleucine, was placed in a water-jacketed container, maintained at 37°C, and circulated through the nasal cavity of anesthetized rats using a peristaltic pump. The perfusing solution was passed through the nasal cavity, then out of the nostrils through a funnel, and returned to the water-jacketed container. Aliquots (100 μl) of the perfusing solution were periodically removed, diluted with 200 μl of 0.1 *M* citric acid (pH 2.3) to quench hydrolysis, and subjected to analysis. The concentrations of the parent enkephalin and its metabolite, Des-Tyr-Leu-enk (Gly-Gly-Phe-Leu), in the perfusing solution were determined as a function of time using a high-pressure liquid chromatographic (HPLC) assay (10).

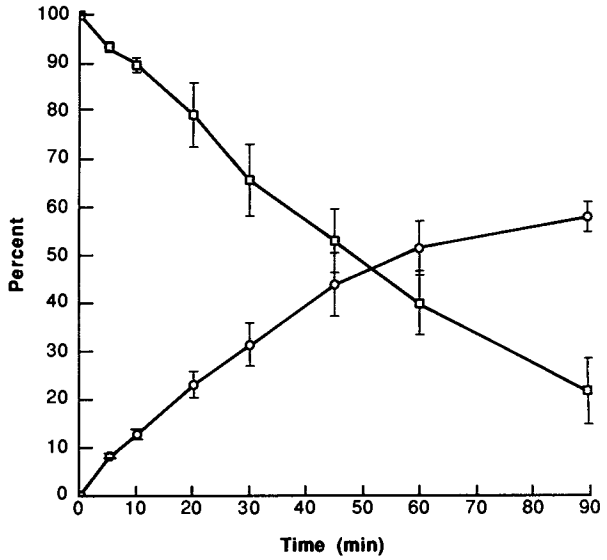

Fig. 2. *In situ* nasal disappearance of 0.1 m*M* leucine-enkephalin (□) and appearance of Des-Tyr-leucine-enkephalin (○) in the nasal perfusate (10 ml). Symbols represent the means ± SD (N = 4).

RESULTS AND DISCUSSION

The α-aminoboronic acid derivatives (Fig. 1), potent and reversible inhibitors of aminopeptidases, were tested nasally *in situ* in rats for inhibiting the degradation of Leu-enk. In the absence of inhibitors, the degradation of Leu-enk in the nasal perfusate and the appearance of the metabolite (Des-Tyr-Leu-enk) are shown in Fig. 2. Fifty percent of the parent peptide was hydrolyzed in 45 min, in agreement with

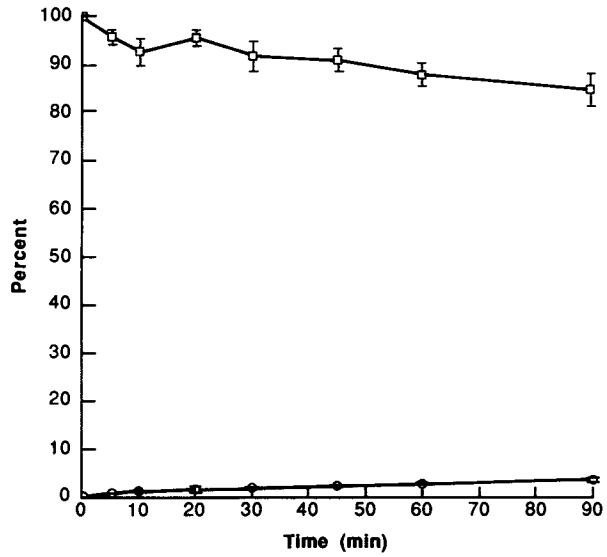

Fig. 3. *In situ* nasal disappearance of 0.1 m*M* leucine-enkephalin (□) and appearance of Des-Tyr-leucine-enkephalin (○) in the nasal perfusate (10 ml) in the presence of 0.1 μ*M* boroleucine. Symbols represent the means ± SD (N = 4).

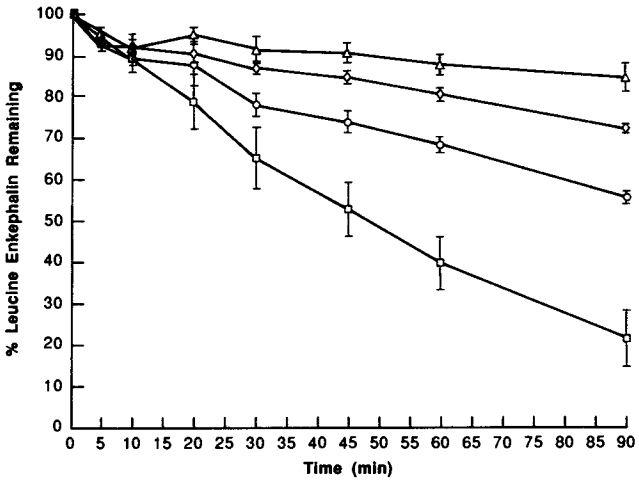

Fig. 4. *In situ* nasal disappearance of 0.1 m*M* leucine-enkephalin in the nasal perfusate (10 ml) in the absence of inhibitor (□) and in the presence of 0.01 (○), 0.03 (◇), and 0.1 (△) μ*M* boroleucine. Symbols represent the means ± SD (N = 4).

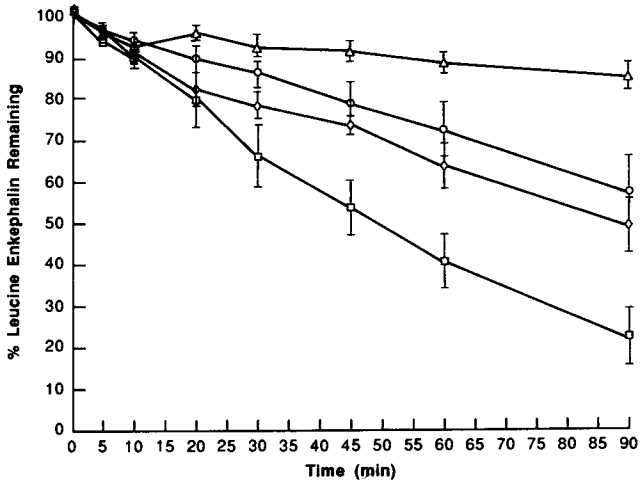

Fig. 6. *In situ* nasal disappearance of 0.1 m*M* leucine-enkephalin in the nasal perfusate (10 ml) in the absence of inhibitor (□) and in the presence of 100 μ*M* puromycin (◇), 10 μ*M* bestatin (○), and 0.1 μ*M* boroleucine (△). Symbols represent the means ± SD (N = 4).

previously published data (10). However, in the presence of 0.1 μ*M* boroleucine, more than 90% of the parent peptide was still intact in the nasal perfusate after 45 min and the concentration of the metabolite was <4% even after 90 min (Fig. 3).

Figure 4 shows the inhibition of degradation of Leu-enk in the nasal perfusate at different concentrations of boroleucine compared to degradation in the absence of inhibitor. In the presence of 0.01, 0.03, and 0.1 μ*M*, the percentages of Leu-enk found intact in the nasal perfusate after 90 min were 56, 73, and 86, respectively. However, in the absence of inhibitor, only 22% was found intact after 90 min.

At equimolar concentrations (0.1 μ*M*), boroleucine, borovaline, and boroalanine each inhibited the degradation of Leu-enk; however, boroalanine was not as effective as boroleucine or borovaline (Fig. 5). This specificity was previously shown for cytosolic leucine aminopeptidase, where boroleucine was two to three orders of magnitude better an inhibitor than boroalanine (14). The result presented here is consistent with this observation, suggesting the similarity in the specificity of the cytosolic leucine aminopeptidase from porcine kidney and the aminopeptidases from the nasal cavity of rats. It should be noted that the difference in the inhibition of human enkephalin degrading aminopeptidase by boroleucine and boroalanine is minimal (14). Other known peptidase inhibitors, bestatin and puromycin, were tested in this model and compared to the aminoboronic acid derivatives. Bestatin [a reported inhibitor of leucine aminopeptidase, aminopeptidase B, and aminopeptidase N (15)] and puromycin [an inhibitor of aminopeptidase B and N but not of leucine aminopeptidase (16)] were less effective than boroleucine, even at concentrations 100 and 1000 times higher, respectively (Fig. 6). While in the presence of 0.1 μ*M* boroleucine, 85% of the Leu-enk was intact after 90 min; only 57 and 49% of the peptide were intact after the same time in the presence of 10 and 100 μ*M* bestatin and puromycin, respectively.

In summary, the α-aminoboronic acid derivatives are excellent inhibitors of the degradation of peptides in the nasal cavity by aminopeptidases.

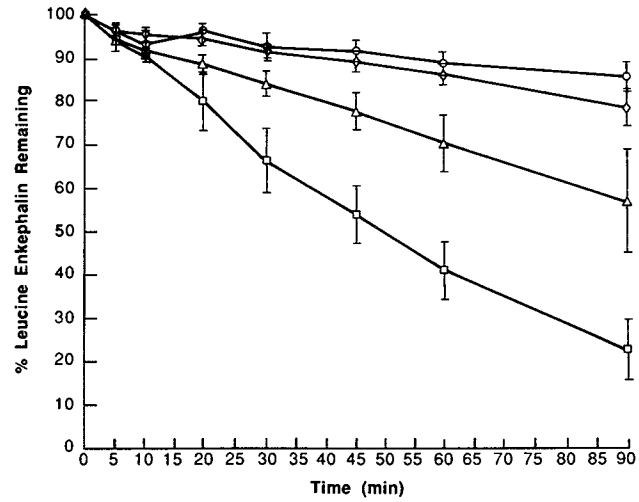

Fig. 5. *In situ* nasal disappearance of 0.1 m*M* leucine-enkephalin in the nasal perfusate (10 ml) in the absence of inhibitor (□) and in the presence of 0.1 μ*M* boroalanine (△), 0.1 μ*M* borovaline (◇), and 0.1 μ*M* boroleucine (○). Symbols represent the means ± SD (N = 4).

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