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Nasal mucosal metabolism of an LH-RH fragment and inhibition with boroleucine

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Abstract

This study is a continuation of investigations of the metabolism of peptides by the rat nasal mucosa, and methods to inhibit metabolism. LH-RH_{3-10} was rapidly metabolized by aminopeptidases when perfused through the isolated rat nasal cavity. Metabolism was almost completely inhibited by co-perfusing a very low concentration of boroleucine, an aminopeptidase inhibitor. LH-RH_{3-10} is different from peptides previously evaluated in similar studies, in that non-polar amino acids are adjacent to the site of peptidase cleavage. This study shows that such peptides are metabolized by nasal aminopeptidases, and that metabolism can be inhibited by boroleucine.

Key words: Peptidase; Absorption; Nasal; Leutininzing hormone releasing hormone; Boroleucine

1. Introduction

There is great interest in delivering peptide and protein drugs by non-injection routes. However, peptide drugs are commonly metabolized at the absorption site, resulting in incomplete bioavailability. This is particularly true when peptides are administered orally, the most preferred route of drug administration. The nasal, buccal, and rectal mucosal membranes are alternative absorption sites, and these have the advantage of often providing much greater bioavailability of peptide and protein drugs than does the oral route (Aungst et al., 1988). However, these sites are also capable of rapidly metabolizing peptides (Lee and Yamamoto, 1990). Metabolism of peptide drugs at potential mucosal absorption sites is an area only beginning to be characterized. For example, although it is known that the nasal mucosal membrane has aminopeptidase activity, the types of aminopeptidases present and the types of substrates cleaved have not been clearly defined. Information on the activities of peptidases at mucosal absorption sites and their substrate specificities will be useful for future mucosal drug delivery projects.

The bioavailability of peptides administered by any of these routes might be improved by inhibiting metabolism at the absorption site, and so the study of metabolism inhibitors is also very important. Aminoboronic acid derivatives, such as boroleucine, have been shown to be extremely potent inhibitors of aminopeptidases (Shenvi,

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1986), and to inhibit the rapid metabolism of peptides by rat nasal and intestinal mucosal membranes (Hussain et al., 1989, 1990a; Aungst et al., 1991). Boroleucine has so far been shown to inhibit the mucosal membrane metabolism of leucine enkephalin (Tyr-Gly-Gly-Phe-Leu) and thymopentin (Arg-Lys-Asp-Val-Tyr). For these peptides the amino acids adjacent to the site of aminopeptidase cleavage are polar. We now extend these studies of mucosal membrane metabolism and the effects of boroleucine, to a peptide with non-polar amino acids at the N-terminus. The peptide studied is LH-RH_{3-10} (Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), a fragment of leutinizing hormone releasing hormone (LH-RH). LH-RH_{3-10} is also different from previously evaluated peptides in the number of component amino acids.

2. Materials and methods

LH-RH_{3-10} and LH-RH_{4-10} were obtained from Sigma Chemical Co. Boroleucine [3-methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)-1-butamine] was prepared as a trifluoroacetic acid salt as described previously (Shenvi, 1986).

Experiments were performed using both CD and Lewis male rats (Charles River), weighing approx. 300 g. Experiments were carried out with the rats maintained under pentobarbital anesthesia. The nasal cavity was isolated using a surgical procedure wherein the trachea was cannulated, and a second cannula was passed through the esophagus to the posterior nasal cavity and sealed in place. Perfusate was passed through the esophageal cannula into the nasal cavity, out the nares, and into a reservoir maintained at 37°C. The perfusate was 0.1 M phosphate buffer, pH 7.4. The initial volume of perfusate was 10 ml, and 0.1 ml samples were removed from the reservoir over a 2 h interval. The perfusate was recirculated, and the flow rate was 1.5 ml/min. Experiments were performed with perfusates containing LH-RH_{3-10} at 50 μg/ml, alone or with 0.15 μg/ml boroleucine. LH-RH_{3-10} disappearance and LH-RH_{4-10} appearance were characterized. In a separate study the perfusate initially contained LH-RH_{4-10}. Perfusate samples were mixed with 0.2 ml of 0.1 M citric acid, and assayed by HPLC. The mobile phase consisted of 19% acetonitrile and 0.5% phosphoric acid in 0.1 M monobasic sodium phosphate. A C8 column and UV absorbance at 210 nm were used. There were three or four rats in each group, and data represent the mean ± SD.

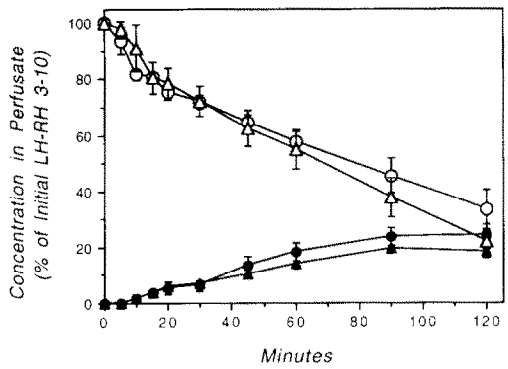

Fig. 1. Disappearance of LH-RH_{3-10} (○, △) and appearance of LH-RH_{4-10} (●, ▲), in perfusates of nasal cavities of Lewis (circles) and Sprague-Dawley (triangles) rats.

3. Results and discussion

LH-RH_{3-10} concentrations in a perfusate solution decreased as it was perfused through the isolated rat nasal cavity (Fig. 1). Concentrations of the metabolite, LH-RH_{4-10}, increased in conjunction with LH-RH_{3-10} disappearance. These data indicate that LH-RH_{3-10} is readily metabolized by aminopeptidases of the rat nasal mucosal membrane. This study was performed using two strains of rats, and the results were almost identical. Perfusion of LH-RH_{3-10} (50 μg/ml) with a very low proportional concentration (0.15 μg/ml) of the aminopeptidase inhibitor, boroleucine, almost completely inhibited LH-RH_{3-10} metabolism, as shown in Fig. 2. LH-RH_{4-10} was not detected in the presence of boroleucine. Since LH-RH_{3-10} perfusate concentrations hardly decreased when metabolism was inhibited, there was apparently little absorption of LH-RH_{3-10} under the conditions of this study. This could be because only a small percentage of the perfusate

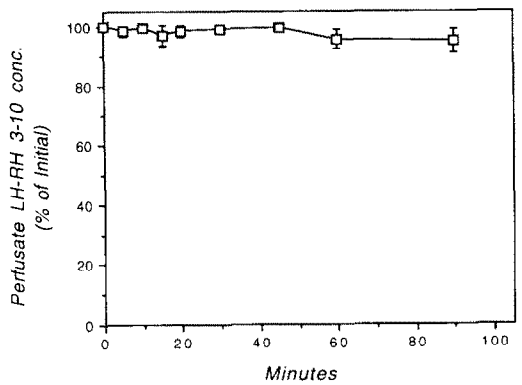

Fig. 2. LH-RH_{3-10} perfusate concentrations when perfused with boroleucine.

is exposed to the nasal membrane at any time, since the volume of perfusate is much greater than the capacity of the nasal cavity. LH-RH_{4-10} was also separately perfused through the rat nasal cavity, and its concentration decreased with time (Fig. 3). LH-RH_{4-10} disappearance probably also reflects metabolism, although we did not attempt to characterize the profiles of other additional metabolites. There was not mass balance of LH-RH_{3-10} and LH-RH_{4-10} concentrations in the results given in Fig. 1, because of subsequent metabolism of LH-RH_{4-10}.

Previous studies of nasal metabolism by aminopeptidases and their inhibition by aminoboronic acid derivatives have been performed only with peptides with polar amino acids at the cleavage site. This study provides new information that the relatively non-polar N-terminus of LH-RH_{3-10} is susceptible to metabolism by aminopeptidases of the rat nasal mucosa, and that this metabolism is inhibited by boroleucine, a representative aminoboronic acid derivative aminopeptidase inhibitor. This is evidence that the rat nasal mucosa has either multiple forms of aminopeptidases or non-specific aminopeptidases. This is consistent with the results of Stratford and Lee (1986), who measured hydrolysis rates of amino acid-methoxynaphthylamide substrates in rabbit nasal mucosa homogenates. Substrates with both non-polar and polar amino acids were hydrolyzed, with Ala > Leu > Glu > Arg.

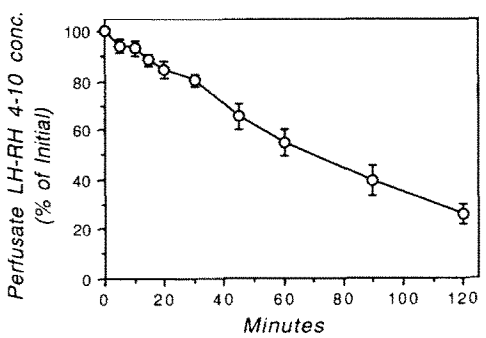

Fig. 3. LH-RH_{4-10} concentrations when separately perfused through rat nasal cavities.

It is important to emphasize that boroleucine, and other aminoboronic acid-derived aminopeptidase inhibitors, inhibit the aminopeptidase-mediated hydrolyses of various substrates. Further studies are continuing this evaluation. These enzyme inhibitors are useful for stabilizing peptides at transmucosal absorption sites, as tools to study metabolism or to improve peptide bioavailability. In this perfusion model, compound disappearance from the perfusate is generally due to metabolism rather than absorption. However, in a non-perfusion model the relative rates of absorption and metabolism of a nasal dose of leucine enkephalin were estimated to be similar, so the inhibition of metabolism at the absorption site should make more peptide available for absorption (Hussain and Aungst, 1992). The aminoboronic acid derivatives are effective aminopeptidase inhibitors at very low concentrations. At inhibitory concentrations boroleucine caused less membrane protein release, an indicator of membrane damage, than other peptidase inhibitors or sodium glycocholate, a permeation enhancer (Hussain et al., 1990b). Also, the nasal mucosal aminopeptidase inhibition was reversed when boroleucine was removed (Hussain et al., 1990b).

4. Acknowledgments

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5. References

Aungst, B.J., Blake, J.A. and Hussain, M.A., An in vitro evaluation of metabolism and poor membrane permeation impeding intestinal absorption of leucine enkephalin, and methods to increase absorption. *J. Pharmacol. Exp. Ther.*, 259 (1991) 139–145.

Aungst, B.J., Rogers, N.J. and Shefter, E., Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of a bile salt absorption promoter. *J. Pharmacol. Exp. Ther.*, 244 (1988) 23–27.

Hussain, M.A. and Aungst, B.J., Nasal absorption of leucine enkephalin in rats and the effects of aminopeptidase inhibition, as determined from the percentage of the dose unabsorbed. *Pharm. Res.*, 9 (1992) 1362–1364.

Hussain, M.A., Koval, C.A., Shenvi, A.B. and Aungst, B.J., An aminoboronic acid derivative inhibits thymopentin metabolism by mucosal membrane aminopeptidases. *Life Sci.*, 47 (1990b) 227–231.

Hussain, M.A., Koval, C.A., Shenvi, A.B. and Aungst, B.J., Recovery of rat nasal mucosa from the effects of aminopeptidase inhibitors. *J. Pharm. Sci.*, 79 (1990a) 398–400.

Hussain, M.A., Shenvi, A.B., Rowe, S.M. and Shefter, E., The use of α-aminoboronic acid derivatives to stabilize peptide drugs during their intranasal absorption. *Pharm. Res.*, 6 (1989) 186–189.

Lee, V.H.L. and Yamamoto, A., Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Del. Rev.*, 4 (1990) 171–207.

Shenvi, A.B., α-Aminoboronic acid derivatives: effective inhibitors of aminopeptidases. *Biochemistry*, 25 (1986) 1286–1291.

Stratford, R.E. and Lee, V.H.L., Aminopeptidase activity in homogenates of various absorptive mucosae in the albino rabbit: implications in peptide delivery. *Int. J. Pharm.*, 30 (1986) 73–82.