Nasal Absorption of Leucine Enkephalin in Rats and the Effects of Aminopeptidase Inhibition, as Determined from the Percentage of the Dose Unabsorbed

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INTRODUCTION

Coadministered peptidase inhibitors could be useful for increasing the bioavailability of peptide drugs that are rapidly metabolized at the absorption site. Certain aminoboronic acid derivatives are known to be very potent aminopeptidase inhibitors (1). They have been shown to inhibit the metabolism of YGGFL (leucine enkephalin) (2) and thymopentin (3) in rat nasal perfusates and, also, inhibited the metabolism of YGGFL by the rat small intestine *in vitro* (4). However, inhibition of the metabolism of peptides does not necessarily ensure increased absorption, since several *in vitro* studies have shown that peptides and other hydrophilic compounds with molecular weights greater than ≈700 were poorly absorbed through mucosal membranes, even in the absence of metabolism (4–6). Even though aminoboronic acid peptidase inhibitors have clearly been shown to inhibit peptide metabolism at mucosal absorption sites, their effects on the absorption and bioavailability of these peptides have not yet been clearly shown. Some reasons for this lack of bioavailability data are that analyzing very low plasma concentrations of peptides is often difficult, and many of these compounds have very short systemic half-lives. An alternative way to evaluate how inhibiting metabolism affects peptide absorption is to measure the rate of peptide disappearance from the absorption site. For this method to be valid, the contribution of metabolism to peptide disappearance must be determined (e.g., by measuring the metabolites). It is then assumed that any disappearance from the absorption site not due to metabolism is due to absorption, where absorption is defined as membrane permeation. In the rat, nasal mucosal metabolism of YGGFL can be completely accounted for in the appearance of GGFL (des tyrosyl leucine enkephalin), the product of aminopeptidase action (2,7). Recovery of YGGFL and GGFL from the nasal cavity can therefore be used to evaluate the rate of absorption (8). We have evaluated nasal YGGFL metabolism and absorption rates, and in addition, we evaluated the effect of boroleucine, an aminoboronic acid peptidase inhibitor.

MATERIALS AND METHODS

YGGFL and GGFL were obtained as the acetate salts (Sigma Chemical Co.). Boroleucine was prepared as the pinacol ester and as the trifluoroacetic acid salt, as described previously (1). It is 3-methyl-1-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)-1-butamine.

Male Lewis rats were anesthetized with sodium pentobarbital and underwent surgery to cannulate the trachea and close off the nasal cavity, as described previously (2,3,7). Rats remained anesthetized and kept on their back throughout the experiment. A 50-μl volume of a dosing solution containing 2 mg/ml YGGFL in 0.1 *M*, pH 7.4, phosphate buffer was administered into one of the nostrils with a microliter syringe. At a selected time after dosing, the nasal cavity was flushed by infusing 10 ml of pH 3.8 phosphate buffer into the nostril opposite the dosing site, and this was collected from the other nostril. The amount of YGGFL recovered in the flush was measured. The amounts of YGGFL converted to GGFL were also determined at the various times after dosing. Each rat was used for only one data point. The effects of boroleucine were studied under the same experimental conditions, with boroleucine added to the YGGFL dosing solution. The ratio of YGGFL and boroleucine doses was 177/1 (on a molar basis).

The concentrations of YGGFL and GGFL were determined by HPLC, as described previously (2). Concentrations were converted to the percentage of the dose recovered.

RESULTS AND DISCUSSION

A plot of the time course of the percentage of the YGGFL dose recovered from the nasal dosing site by flushing the site is shown in Fig. 1a. Shown in Fig. 1b are the percentages of the dose recovered as the metabolite, GGFL. Previous studies (2,7) have shown that YGGFL metabolism by rat nasal mucosa is essentially completely through GGFL formation. This conclusion was based on mass balance when measuring GGFL formation and the almost-complete inhibition of YGGFL disappearance from a nasal perfusate in the presence of aminopeptidase inhibition. Little absorption was apparent in those perfusion studies, because only a small fraction of the perfusate is exposed to the membrane at any time.

YGGFL disposition after nasal dosing can be represented by the following scheme:

$$\begin{array}{l} \text{YGGFL} \xrightarrow{k_1} \text{GGFL} \rightarrow \text{Absorbed GGFL} \\ \quad\downarrow k_2 \qquad\qquad\quad \text{Metabolized GGFL} \\ \text{Absorbed YGGFL} \end{array} \qquad (1)$$

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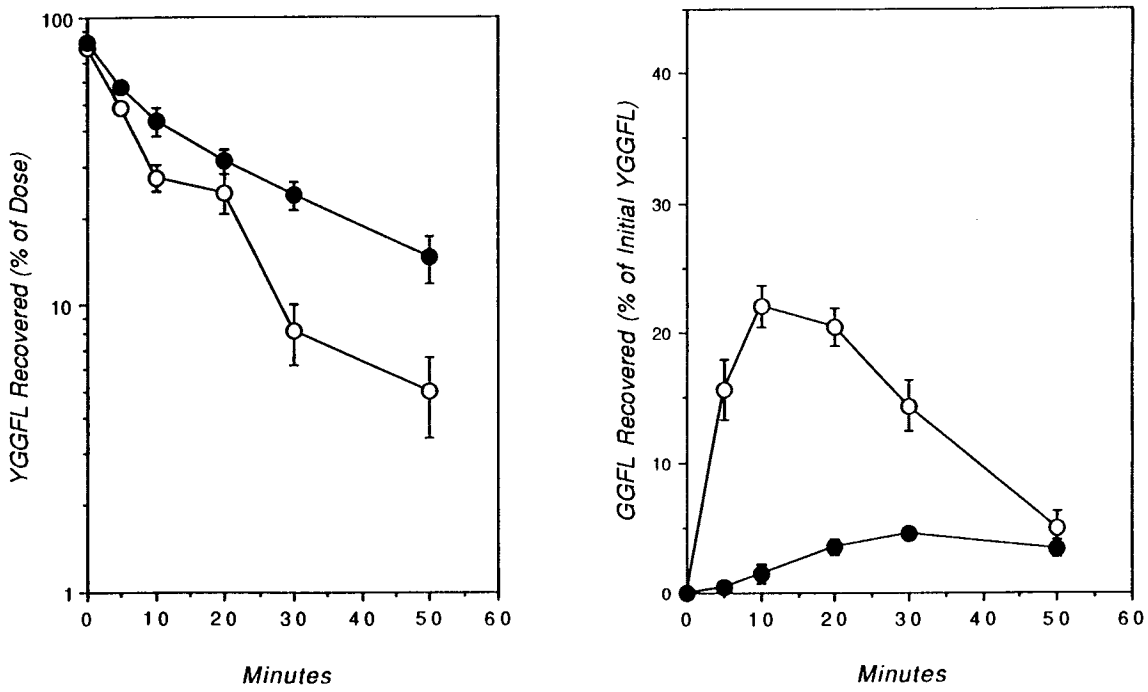

Fig. 1. YGGFL (a) and GGFL (b) recovery from the nasal cavity after dosing with 50 μl of a 2 mg/ml solution alone (○) or with boroleucine (●). Each point is the mean ± SE of four rats.

YGGFL disappearance from the nasal cavity is due to the sum of metabolism by aminopeptidases (rate constant = k_1) and absorption (rate constant = k_2). In this context, absorption means penetration of the epithelial membrane.

$$k_1 + k_2 = k_T \quad (2)$$

Therefore, k_T is the YGGFL total disappearance rate constant. From the semilog plot of YGGFL disappearance (Fig. 1a), a first-order k_T was estimated to be 0.068 min^{-1} ($t_{1/2}$ = 10.1 min).

Boroleucine slowed YGGFL disappearance from the nasal cavity (Fig. 1a) and almost completely inhibited GGFL formation (Fig. 1b). If we assume that in the presence of boroleucine,

$$k_2 \gg k_1, \quad \text{then} \quad k_T \approx k_2 \quad (3)$$

YGGFL disappearance in the presence of boroleucine had a first-order rate constant of 0.039 min^{-1} ($t_{1/2}$ = 17.7 min). According to Eq. (3), this approximates k_2, the absorption rate constant. Then k_1 can be calculated from Eq. (4) for rats not dosed with boroleucine.

$$k_1 = k_T - k_2 \quad (4)$$

We estimate k_1 to be 0.029 min^{-1} ($t_{1/2}$ = 23.9 min).

In this study absorption is defined as penetration of the epithelial membrane, rather than entrance into the systemic circulation. Lee (9) has shown that YGGFL metabolism by the nasal mucosa is due primarily to membrane-associated aminopeptidases, as in the ileum, where metabolism is associated with the brush border. We also assumed that boroleucine had no effect on YGGFL absorption or membrane permeability. This is not an unreasonable assumption, based on our previous work showing that boroleucine caused no protein release when perfused through the nasal cavity, whereas bestatin, puromycin, and sodium glycocholate did (10).

We conclude that YGGFL, a model peptide, is absorbed nasally, in the absence of absorption-promoting adjuvants. Absorption and metabolism are competing processes with similar rates. Boroleucine reduced the rate of YGGFL metabolism, and this approach should improve the possibility of systemic bioavailability.

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REFERENCES

1. A. B. Shenvi. α-Aminoboronic acid derivatives: Effective inhibitors of aminopeptidases. *Biochemistry* 25:1286–1291 (1986).
2. M. A. Hussain, A. B. Shenvi, S. M. Rowe, and E. Shefter. The use of α-aminoboronic acid derivatives to stabilize peptide drugs during their intranasal absorption. *Pharm. Res.* 6:186–189 (1989).
3. M. A. Hussain, C. A. Koval, A. B. Shenvi, and B. J. Aungst. An aminoboronic acid derivative inhibits thymopentin metabolism by mucosal membrane aminopeptidases. *Life Sci.* 47:227–231 (1990).
4. B. J. Aungst, J. A. Blake, and M. A. Hussain. 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.* 259:139–145 (1991).

5. B. Matuszewska, G. G. Liversidge, F. Ryan, J. Dent, and P. L. Smith. In vitro study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogues. *Int. J. Pharm.* **46**:111–120 (1988).
6. M. D. Donovan, G. L. Flynn, and G. L. Amidon. Absorption of polyethylene glycols 600 through 2000: The molecular weight dependence of gastrointestinal and nasal absorption. *Pharm. Res.* 7:863–868 (1990).
7. A. Hussain, J. Faraj, Y. Aramaki, and J. E. Truelove. Hydrolysis of leucine enkephalin in the nasal cavity of the rat—a possible factor in the low bioavailability of nasally administered peptides. *Biochem. Biophys. Res. Commun.* **133**:923–928 (1985).
8. J. A. Faraj, A. A. Hussain, Y. Aramaki, K. Iseki, M. Kagoshima, and L. W. Dittert. Mechanism of nasal absorption of drugs. III. Nasal absorption of leucine enkephalin. *J. Pharm. Sci.* **79**:698–702 (1990).
9. V. H. L. Lee. Peptidase activities in absorptive mucosae. *Biochem. Soc. Trans.* **17**:937–940 (1989).
10. M. A. Hussain, C. A. Koval, A. B. Shenvi, and B. J. Aungst. Recovery of rat nasal mucosa from the effects of aminopeptidase inhibitors. *J. Pharm. Sci.* **79**:398–400 (1990).