NASAL ABSORPTION OF ENKEPHALINS IN RATS

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INTRODUCTION

In recent years, the possibility that the intranasal administration route might be useful for many compounds which are not absorbed orally has received a great deal of attention. For instance, the β-blocker propranolol (Hussain et al., 1979, 1980 a,b), the contraceptive agent progesterone (David et al., 1981; Hussain et al., 1981) and the anti-arrhythmic compound clofilium tosylate (Su et al., 1984) have been shown to be effectively absorbed via the intranasal route when compared to oral administration. These compounds undergo extensive degradation due to first-pass hepatic metabolism which can be minimized after nasal administration. For drugs which are poorly absorbed by the oral route such as sulbenicillin, cefazolin, and cephacetrile, it was demonstrated that the percent dose excreted in urine after nasal administration was nearly one-half of that after intramuscular administration (Hirai et al., 1981). The absorption of low molecular weight polypeptides, luteinising hormone-releasing hormone (LH-RH) and its analogues used as a contraceptive agent, was evaluated by the nasal route (Fink et al., 1974; Berquist et al., 1979; Gennser and Liedholm, 1974; London et al., 1973; Anik et al., 1984). Although the absorption efficiency by the nasal route was lower than the I.V. route for these polypeptides, the absorption was reproducible, and the advantage of non-parenteral route for such a compound was an important factor. Research has also been carried out on the nasal absorption of high molecular weight polypeptides such as insulin (Moses et al., 1983; Hirai et al., 1978, 1981 a,b), interferon (Greenberg et al., 1978; Harmon et al., 1976; 1977; Johnson et al., 1976) and growth hormone releasing factor (Evans et al., 1983).
More recently, analogues of the naturally occurring enkephalins have been evaluated for their analgesic activity (Frederickson, 1970; Motta, 1980; Gesellchen et al., 1979, 1980; Leander and Wood, 1982). These polypeptides have to be administered parenterally to have measurable activity. This paper describes (1) the nasal absorption of analogues of enkephalin, (2) the effect of surfactant sodium glycocholate on nasal absorption, and (3) the histological examination of the nasal mucosa of rats after nasal administration of enkephalins.

EXPERIMENTAL

Materials - Two polypeptides were chosen for the nasal absorption studies: (1) \([^{3}H]\)-Tyr*-D-Ala-Gly-L-Phe-D-Leu-OH\(^{1}\) (\([^{3}H]\)DADLE), molecular weight = 569.7 and (2) metkephamid\(^{2}\), Tyr-D-Ala-Gly-Phe-N-Me-Met-NH\(_2\)·CH\(_3\)COOH, molecular weight = 660.78. These two pentapeptides, tyrosine\(^{3}\), Tyr-D-Ala-Gly (TDAG)\(^{4}\), unlabeled DADLE\(^{5}\) and PCS liquid scintillation fluid\(^{6}\) were used as received. Distilled deionized water was used for preparation of the HPLC mobile phase. All other reagents were analytical grade. Stock solutions of DADLE and metkephamid were prepared fresh daily.

Equipment - For the assay of \([^{3}H]\)-DADLE, the high-performance liquid chromatography (HPLC) analyses were performed using a gradient liquid chromatography system\(^{7}\) with variable UV wavelength absorbance detector\(^{8}\). The system consisted of two solvent delivery pumps\(^{9}\), a guard column packed with pellicular ODS media\(^{10}\), a 250 x 4.6-mm reverse phase C-18 Spheri-5 \(\mu\)m column\(^{11}\), and an OmniScribe linear recorder\(^{12}\).

For the assay of metkephamid, the HPLC system consisted of a solvent delivery pump\(^{13}\), a guard column packed with pellicular ODS packing\(^{14}\), a 250 x 4.6-mm reverse phase C-18 5-6 \(\mu\)m column\(^{15}\), a recorder\(^{16}\), a power supply\(^{17}\), and a fluorescent detector\(^{18}\).

Sample Preparation and Analysis - The relative absorption of \([^{3}H]\)-DADLE, expressed as total radioactive equivalents absorbed by the intranasal and subcutaneous routes, was measured as previously reported (Su et al., 1984). For the quantitative analyses of absorption of \([^{3}H]\)-DADLE, the procedure is described as follows. Serum samples from treated rats were stored at -20°C overnight and thawed just prior to HPLC analysis. The \([^{3}H]\)-DADLE stock solution and serum samples were chromatographed on a C-18 reverse phase HPLC column for separation and identification of

\(^{1}\)New England Nuclear, Boston, MA.
\(^{2}\)Synthesized by Lilly Research Laboratories, Indpls., IN.
\(^{3}\)Ajinomoto Company, Tokyo, Japan.
\(^{4}\)Synthesized by Lilly Research Laboratories, Indpls., IN.
\(^{5}\)Synthesized by Lilly Research Laboratories, Indpls., IN.
\(^{6}\)Amersham, Arlington Heights, IL.
\(^{7}\)Beckman Instruments, Inc., Fullerton, CA.
\(^{8}\)Model LDC Spectromonitor III, Laboratory Data Control, Riviera Beach, FL.
\(^{9}\)Beckman Instruments, Inc., Fullerton, CA.
\(^{10}\)Whatman, Inc., Clifton, NJ.
\(^{11}\)Brownlee Labs, Inc., Santa Clara, CA.
\(^{12}\)Texas Instruments, Austin, TX.
\(^{13}\)Altex Model 110A, Rainin Instrument Co., Inc., Emeryville, CA.
\(^{14}\)Brownlee Labs, Inc., Santa Clara, CA.
\(^{15}\)Zorbax ODS, DuPont Instruments, Wilmington, DE.
\(^{16}\)Linear Instruments Corp., Irvine, CA.
\(^{17}\)Model 150 Xenon Power Supply, Perkin Elmer, Norwalk, CT.
\(^{18}\)Model 650-10S, Perkin Elmer, Norwalk, CT.