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Buccal drug absorption. I. Comparative levels of esterase and peptidase activities in rat and hamster buccal and intestinal homogenates

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Summary

Esterase, aminopeptidase, carboxypeptidase, and endopeptidase activities were quantitated in homogenates of rat and hamster buccal and intestinal tissues. Whole homogenates (cytosol + cell membranes) of both tissues from the rat had higher esterase activity than supernatants of these homogenates. In both species intestinal homogenates showed significantly higher esterase and aminopeptidase activities than buccal homogenates, while carboxypeptidase activity in buccal homogenates was significantly higher than intestinal homogenates. Although hamster buccal and intestinal homogenates had similar levels of endopeptidase activity, rat intestinal homogenates had significantly higher endopeptidase activity than buccal homogenates. The results suggest that the utility of the buccal mucosa as a delivery route for some drugs may be compromised – as with the intestinal mucosa – by the presence of drug-degrading enzymes in the mucosa.

Introduction

Absorption from the gastrointestinal tract is frequently a problem for many drugs. This may be due to unfavorable physicochemical properties (Atkinson et al., 1962) or to degradation of the drug (Nelson, 1962). Degradation may be either enzymatic or non-enzymatic, and may take place in the gastric lumen (Renwick, 1982), the brush-border membrane (Adibi and Kim, 1981), or during the first-pass through the liver after absorption (Benet, 1979). Many alternate routes of delivery have been employed to circumvent these problems. These include: parenteral (Boylan and Fites, 1979), rectal (Feldman, 1975; Nishihata et al., 1983), vaginal (Okada et al., 1982), nasal (Su et al., 1985), ophthalmic (Lee, 1987), dermal (Parker et al., 1984), and buccal (Gibaldi and Kanig, 1965; Sanghera et al., 1986) routes, all of which have been studied to varying extents.

Until recently (Ishida et al., 1981; Nagai and Machida, 1985; Bardgett et al., 1984; Abrams,

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1983; Anders et al., 1983), the buccal route of drug delivery had received relatively little attention, despite the fact that it provides some attractive features. These include: (1) reduction of drug degradation possible with gastrointestinal administration; (2) ease of administration and removal of drug dose; (3) potential for controlled drug delivery rate with a suitable device; (4) elimination of direct gastric irritation; (5) ability to alter the site of drug application to avoid mucosal irritation; and (6) avoidance of variable gastrointestinal transit time and chemical environment as factors in absorption.

A knowledge of the enzymatic profile of the buccal mucosa may be helpful in optimizing drug delivery by this route. The purpose of this study was to determine some of the differences between enzymatic activities in buccal and intestinal tissues from different species. Esterase, aminopeptidase, carboxypeptidase and endopeptidase activities in homogenates of rat and hamster buccal and intestinal tissues were studied.

Materials and Methods

Materials

p-Nitroaniline (PNA), L-leucine-*p*-nitroanilide (LPNA), hippuric acid (HA), *p*-nitrophenol (NP), *N*-1-naphthylethylenediamine · 2 HCl (NED), hippuryl-L-phenylalanine (HPA), and [D-Ala^2]-Met-enkephalinamide were obtained from Sigma (St. Louis, MO). *p*-Nitrophenylacetate (NPA) was obtained from Aldrich (Milwaukee, WI). Hippuryl-L-phenylalanineamide (HPAA) was obtained from K&K Labs (Plainview, NY). All other compounds were reagent grade and used as received.

Instruments

Absorbance measurements were made with a recording spectrophotometer (Perkin-Elmer, Model 555) equipped with a digital temperature-controller (Perkin-Elmer, Model 5700701). The temperature and pH for all experiments was maintained at 37 °C and 7.40, respectively. The HPLC system consisted of a Water's Model 6000A pump and Model 440 UV detector. The HPLC column (4.6 × 150 mm) used was packed according to a literature procedure (Bristow et al., 1977) with a stationary phase of Hypersil-ODS (Shandon, C-18, 5μ particles). Quantitation of compounds was accomplished by manual peak height measurements.

Methods

Preparation of tissue homogenates

Male albino rats (250–350 g) and male golden hamsters (90 g) were used for these studies. The animals were allowed free access to food and water prior to the experiments. At the time of the experiments the animals were anesthetized (i.p. pentobarbital) and the tissues of the oral cavity were exsanguinated using a method similar to that employed for exsanguination of brain tissue (Munger et al., 1978; Patel, 1980). The method involved intra-cardial infusion of buffer (isotonic Sorensen's phosphate, pH = 7.40) subsequent to severing the jugular vein to allow drainage of blood from the head. The infusion was continued until the drainage from the jugular vein was nearly colorless.

Duodenal and buccal tissues were excised and rinsed with distilled water. The tissues were blotted dry, weighed and placed in a ground glass homogenizing tube with the appropriate volume of ice-cold buffer to make either 5 or 10% (w/v) homogenates. The tissues were homogenized with a ground glass pestle driven by a 1/5 horsepower drill motor (Skil, Model 456). The homogenates were kept on ice and used within 4 h of preparation. For determination of supernatant activity the homogenates were centrifuged (Beckman, Model J-21C) at 11,400 *g* at 4 °C for 15 min and an aliquot of the supernatant was assayed for enzyme activity.

Assay methods

Esterase activity was determined by monitoring the change in absorbance at 405 nm resulting from the cleavage of *p*-nitrophenylacetate (NPA) to produce *p*-nitrophenol (NP). Buffer (3.0 ml) was pre-incubated at 37 °C in both reference and sample cells of the spectrophotometer. An aliquot of an NPA stock solution was added to both cells to give the desired concentration. Whole homogenate

or supernatant (25 μl) was added to the sample cell and mixed by inverting 2 or 3 times. The change in absorbance at 405 nm was monitored for 2 min. Activity (expressed as μmol NP formed/min/g tissue) was calculated using a molar absorptivity of 1.22×10^4 AU/M for NP. Molar absorptivity was determined from a standard curve.

Aminopeptidase activity was determined by utilizing a modified version of the procedure of Goldbarg and Rutenberg (1958) as described in a commercial assay kit (Sigma, 1983). L-Leucine-*p*-nitroanilide (LPNA) was used as the substrate. The assay involved diazotization of *p*-nitroaniline (PNA) released from LPNA, and subsequent coupling with *N*-1-naphthylethylenediamine (NED) to produce a highly colored azo dye. Quantitation was accomplished by measuring the absorbance at 550 nm.

A volume of LPNA solution (0.475 ml for all except hamster intestinal homogenates, which used 0.490 ml) was pre-incubated in a small test tube. An aliquot of homogenate (25 μl for all except hamster intestine, which was 10 μl) was added, and mixed by vortexing. After 5 min the reaction was stopped by addition of HCl (2 N, 0.25 ml), followed by addition of sodium nitrite solution (0.2% w/v, 0.25 ml) with vortex mixing. After 3 min ammonium sulfamate solution (0.5% w/v, 0.50 ml) was added and mixed by vortexing. After 3 more minutes NED solution (0.5 mg/ml in ethanol, 1.0 ml) was added and mixed by vortexing. The magenta color was allowed to develop for 30–40 min before measurement of the absorbance at 550 nm. Activity (expressed as μmol PNA formed/min/g tissue) was calculated based on a standard curve constructed using the same assay method with known concentrations of PNA.

Carboxypeptidase activity was quantitated by measuring the production of hippuric acid (HA) from hippuryl-L-phenylalanine (HPA) (Folk and Schirmer, 1963). An aliquot of HPA solution (2.0 ml) was placed in a small test tube and pre-incubated at 37°C. Tissue homogenate (50 μl, 5% w/v) was added and mixed well. After 10 min a sample was removed and added to an appropriate volume of acetonitrile to give a final acetonitrile concentration of 25%. The samples were mixed well and placed on ice until all samples were collected. Samples were centrifuged and HA was quantitated by HPLC using the previously described system with a mobile phase of 15 : 85 (CH_3CN : 0.05 M NaH_2PO_4, pH = 3.0) at a flow rate of 1.0 ml/min, which gave a retention volume of ~ 5.0 ml. Activity (expressed as μmol HA formed/min/g tissue) was calculated from a standard curve for HA.

Endopeptidase activity was determined using either hippuryl-L-phenyl-alanineamide (HPAA) or [D-Ala^2]-Met-enkephalinamide, an enkephalin analogue with the primary structure Tyr-D-Ala-Gly-Phe-MetNH_2 (TAGPM) as substrates. The method using HPAA as the substrate was as follows. An HPAA solution (0.50 ml, 5.00×10^{-4} M) was placed in a small test tube and pre-incubated at 37°C. An aliquot of tissue homogenate (25 μl, 5% w/v) was added. After 15 min the reaction was stopped by the addition of acetonitrile (167 μl). Samples were centrifuged and kept on ice until time of assay. HA was quantitated by HPLC as described above.

The method using TAGPM as the substrate involved pre-incubating an aliquot of TAGPM solution (2.5 mM, 40 μl) at 37°C after which an aliquot of tissue homogenate (10% w/v, 10 μl) was added. The samples were incubated for 30 min (5 min for rat intestinal homogenates) and the reaction was terminated by the addition of acetonitrile (75 μl). Remaining TAGPM was quantitated by HPLC using the previously described system with a mobile phase of 30 : 70 (CH_3CN : 0.1% H_3PO_4 in 0.1 M $NaClO_4$) at a flow rate of 1.0 ml/min, which gave a retention volume of ~ 3.5 ml. Activity (expressed as μmol TAGPM lost/min/g tissue) was calculated from a standard curve for TAGPM.

Results and Discussion

Esterase activity in whole homogenates and supernatants

For the purposes of this study a total value for enzyme activity in the tissues was desired and the use of whole homogenates (cytosol + cell membranes) appeared to be most appropriate for this

TABLE 1

Comparison of esterase activities in rat tissue homogenates and homogenate supernatants as determined by the rate of hydrolysis of p-nitrophenyl acetate (NPA) to p-nitrophenol (NP) at 37°C and pH = 7.40

Tissue	Esterase activity [a]		
	Whole	Supernatant	membrane-assoc. [b]
Buccal	19.1 ± 11.7	10.1 ± 3.91	9.00
Intestinal	131 ± 49.2	36.0 ± 12.4	95.0

[a] μmol NP formed/min/g tissue ($\bar{x} \pm$ S.D., $n = 8$).
[b] Calculated as the difference between supernatant and whole values.

purpose. Whole homogenates would likely contain several enzymes, any number of which may be capable of catalyzing the degradation of a particular substrate. Although the terms esterase, aminopeptidase, carboxypeptidase and endopeptidase imply specific enzymes, their use in this discussion refers to the total enzyme-catalyzed hydrolysis of a particular substrate.

Whole homogenates would be most representative of the intact tissue and provide the best indication of the enzyme activity a substrate would encounter in that tissue since whole homogenates contain both cell membranes and cellular contents. Accordingly, any membrane-associated enzymes would be present in the membrane environment. The difference between the activity in the whole homogenates and in the supernatants or cytosolic fractions should give an indication of the activity in the cell membranes.

Esterase activities in whole homogenates and supernatants from rat buccal and intestinal tissues were determined and the data are summarized in Table 1. The data showed that there were significant levels of membrane-associated esterase activity (calculated as the difference between the values for whole homogenates and supernatants) in rat buccal and intestinal tissues. There was about the same esterase activity in the buccal membrane and cytosolic fractions while there was greater activity in the intestinal membrane fraction than in the cytosolic fraction. These results confirm that whole homogenates provide a better indication of total esterase activity in the tissues than do homogenate supernatants. Similar results might be expected for other enzyme activities as well, although this was not addressed in this study.

Comparative levels of enzyme activities

Esterase, aminopeptidase, carboxypeptidase and endopeptidase activities in homogenates of rat and hamster buccal and intestinal tissues are graphically represented in Figs. 1 (esterase and aminopeptidase), 2 (carboxypeptidase) and 3 (endopeptidase). The esterase and aminopeptidase activities in intestinal homogenates were significantly ($P < 0.001$) higher than in buccal homogenates for both species. The results for aminopeptidase activity are in contrast to a previous report (Stratford and Lee, 1986) that showed similar levels of aminopeptidase activity in rabbit buccal and duodenal homogenates. This difference may be due to inter-species variation or the use of different substrates.

Buccal homogenates showed significantly ($P < 0.005$) higher carboxypeptidase activity than intestinal homogenates in both species. These results contrast with those found for esterase and aminopeptidase activities. A possible explanation for this observation is that carboxypeptidase is

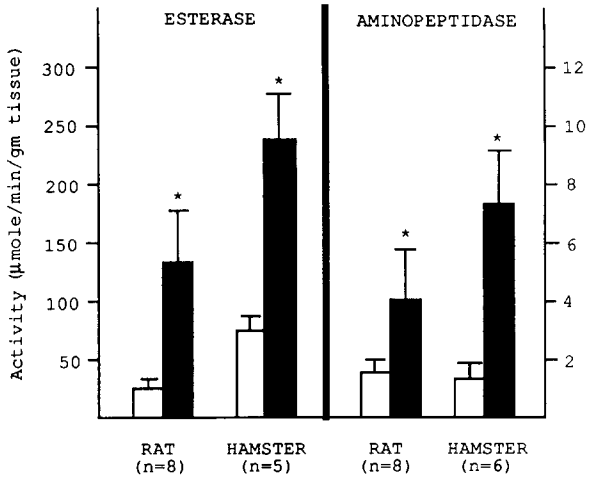

Fig. 1. Comparative levels of esterase and aminopeptidase activities at 37°C and pH = 7.40 in homogenates at rat and hamster buccal (□) and intestinal (■) mucosae. Assay methods described in text. Error bars represent one standard deviation. * = significantly higher ($P < 0.001$) as determined by a paired *t*-test.

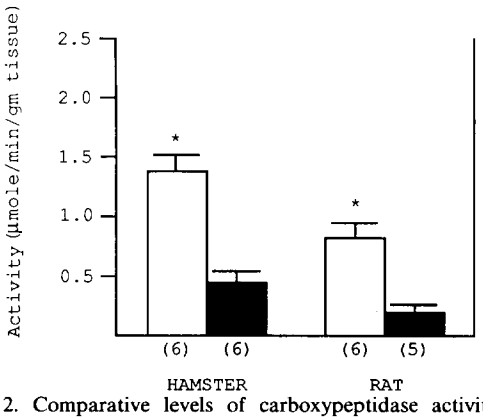

Fig. 2. Comparative levels of carboxypeptidase activity at 37 °C and pH = 7.40 in homogenates of rat and hamster buccal (□) and intestinal (■) mucosae. Assay method described in text. Error bars represent 1 S.D. for (n) determinations. * = significantly higher ($P < 0.005$) as determined by a paired t-test.

secreted by the pancreas into the GI tract, making it unnecessary for this activity to be duplicated in the intestinal mucosa.

No endopeptidase activity could be detected when hippuryl-L-phenyl-alanineamide (HPAA) was used as the substrate, possibly due to a lack of specificity of the enzyme for this dipeptide substrate which is derivatized at both the C- and N-termini. Endopeptidase activity toward the pentapeptide TAGPM in the rat was significantly ($P < 0.001$) higher in intestinal as compared to buccal homogenates, while in the hamster there

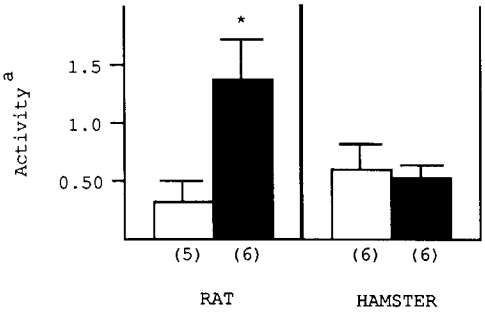

Fig. 3. Comparative levels of endopeptidase activities at 37 °C and pH = 7.40 in homogenates of rat and hamster buccal (□) and intestinal (■) mucosae. Assay method described in text. Error bars represent 1 S.D. for (n) determinations. * = significantly higher than buccal ($P < 0.001$). a = μmol TAGPM lost/min/g tissue.

was no significant ($P > 0.05$) difference between the activities in buccal and intestinal homogenates. It has been reported (Kashi and Lee, 1986) that the rates of hydrolysis of TAGPM in rabbit buccal and ileal homogenates are similar. This is in agreement with the results for hamster homogenates found in the present study. The difference in the relative levels of endopeptidase activity found for rat homogenates indicates inter-species variation.

Conclusions

The results of these studies demonstrate the wide variation in the types and distribution of enzyme activities among species. This makes it unlikely to be able to predict the degree to which an enzymatically-labile drug might be absorbed across the buccal mucosa of humans. However, the data do suggest that the human buccal mucosa is likely to contain enzymes that could limit the utility of this route for the delivery of some drugs. Alternatively, the presence of enzymatic activity in the oral mucosa may make the delivery of some compounds possible through a prodrug approach.

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