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Note

# Degradation of luteinizing hormone releasing hormone in buccal, liver, nasal and skin tissues

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**Abstract**

Degradation of luteinizing hormone releasing hormone (LHRH) in various pig tissues (i.e. buccal, liver, nasal and skin) and in their corresponding homogenates was determined. The degradation of LHRH followed apparent first-order kinetics. The degradation rates of LHRH in intact tissues were lower than in their corresponding homogenates. The lowest degradation rate [$(0.15 \pm 0.01) \times 10^3$ $min^{-1}$] was observed in intact skin tissue indicating that the skin has minimal proteolytic activity, thus decreasing the rate of LHRH degradation. © 1998 Elsevier Science B.V. All rights reserved.

*Keywords:* Degradation rate; LHRH; Enzymatic barrier; Intact tissue; Tissue homogenate; HPLC

The incomplete bioavailability of peptides and proteins from various routes of administration suggests that there is at least an enzymatic barrier limiting their absorption (Lee, 1988; Morimoto et al., 1992). The enzymatic barrier is composed of exopeptidases and endopeptidases that cleave peptides and proteins (Contijoch et al., 1990). Therefore, a major challenge in peptide and protein delivery is to overcome the enzymatic degradation that limits the amount of peptide and protein drugs that reach their targets.

LHRH is a highly potent decapeptide whose primary structure is PGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-$NH_2$. It has proven to be a useful compound both experimentally and clinically (Henzl, 1993). This study focuses on the susceptibility of LHRH to proteolytic breakdown in intact buccal, liver, nasal and skin tissues and in their corresponding homogenates in order to determine the suitable delivery systems and routes of LHRH administration.

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Fresh and viable buccal, liver, nasal and skin tissues from the pig were obtained from slaughter houses. Viable tissues of the same weight and from the same pig were used in this study. Three pieces of each type of tissues (i.e. buccal, liver, nasal and skin) of the same weight were used; one piece for each of the control, intact, and homogenate experiments. Particular care was taken to ensure the viability of the excised tissues. One of the three pieces was homogenized in ice-cold 0.02 M potassium phosphate buffer (pH 7.4) in a Silverson L4R Mixer (Silverson, East Longmeadow, MA). The homogenates were centrifuged for 5 min at $1000 \times g$ at 4°C in a Beckman RC-5 superspeed refrigerated centrifuge (Dupont De Nemours, Newtown, CT). The supernatant fraction was used as the source of peptidase activity and was stored at −80°C until assayed. The protein contents in various supernatants were determined by the modified Lowry method (Lowry et al., 1951). Intact tissue of the same weight was used, therefore the total protein content in the intact tissue was presumably the same as its supernatant. Thus, the protein contents in intact tissues and their homogenates were known. We varied LHRH concentration to get an LHRH to protein ratio of 1:10 with all tissues and their homogenates. Enzymatic degradation of LHRH due to peptidase was halted by heating the samples at 100°C for 6 min (Advis et al., 1982). A known concentration of LHRH incubated in a preboiled tissue (100°C for 6 min) was used as the control. Control and test samples containing a known concentration of LHRH in vials were maintained at $37 \pm 0.1$°C in a water bath. At specific time intervals, 0.2-ml samples were withdrawn from vials for LHRH analysis by a stability indicating and validated HPLC method (Bi et al., 1998). Briefly, a Hewlett Packard series 1050 liquid chromatograph (Hewlett Packard, Germany) was used for the analysis of LHRH. The mobile phase consisted of triethylammonium phosphate buffer (0.36 M, pH 2.5) and acetonitrile (86:14 v/v). A ZORBAX ODS column (4.6 mm × 15 cm, 5 μm, 100 Å), injection volume (100 μl), flow rate (1.5 ml/min), and detection wavelength (210 nm) were used in the HPLC analysis of LHRH in samples.

Semilogarithmic graphs of the residual amounts of LHRH versus time in intact tissues and in tissue homogenates were plotted. The degradation rate constant ($k$) was obtained from the slope of the semilog of concentration versus time plot by statistical regression analysis.

Figs. 1–4 illustrate the degradation of LHRH by buccal, liver, nasal, and skin tissues, respectively. LHRH was degraded in all of these tissues. There was no degradation in the control. The degradation of LHRH followed apparent first-order kinetics in all of the above tissues. Many enzymes are probably responsible for the degradation of LHRH present in the skin (Morimoto et al., 1992), nasal (Lang et al., 1996), and buccal (Yamamoto et al., 1988) tissues. The structure of LHRH reveals that this molecule with its unusual N-terminus (PGlu) is immune to attack by some proteases (e.g. aminopeptidases); its carboxy terminus with amide formation is also protective from carboxypeptidases. Therefore, LHRH should be very stable and can escape the proteolytic breakdown if a tissue has only exopeptidases. The liver is a chemical factory, which synthesizes and secretes most of the proteolytic enzymes, including proteases. Therefore, the liver is the primary organ of elimination for exogenously administered proteins and peptides (Ferraiolo et al., 1992).

Table 1 indicates the degradation rates of LHRH in different tissues and in their homogenates. The intact skin tissue demonstrated the lowest $[(0.145 \pm 0.01) \times 10^3 \text{ min}^{-1}]$ and liver the highest $[(2.12 \pm 0.08) \times 10^3 \text{ min}^{-1}]$ degradation rate among all of the four intact tissues. The enzymatic activity in the skin is less than 10% of the liver (Pannatier et al., 1978), and also its proteolytic activity is lower than other tissues (Zhou and Li wan po, 1990). These observations are consistent with our findings. Furthermore, in our study, nasal tissue has less proteolytic activity compared with buccal tissue as is evident from degradation rates in these tissues, and is consistent with earlier studies (Stratford and Lee, 1986; Lee et al., 1987).

Table 1 also shows that the intact tissues exhibited less degradation of LHRH than those of corresponding homogenates. Studies with super-

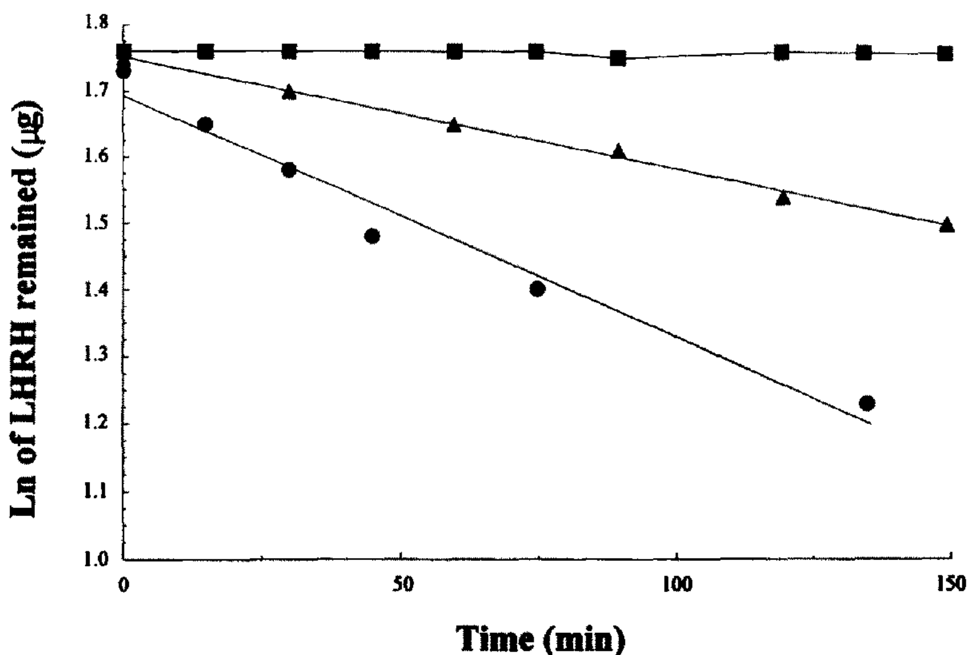


Fig. 1. First-order plot of LHRH degradation by buccal tissue. ■, Control; ▲, intact buccal tissue; ●, buccal tissue homogenate.

natants allowed us to determine the maximum enzymatic degradation in a particular tissue and to estimate the total protein content in a given amount of the tissue. It should be noted that the supernatant fraction as a source of peptidase activity is arbitrary. The conditions of centrifuga-

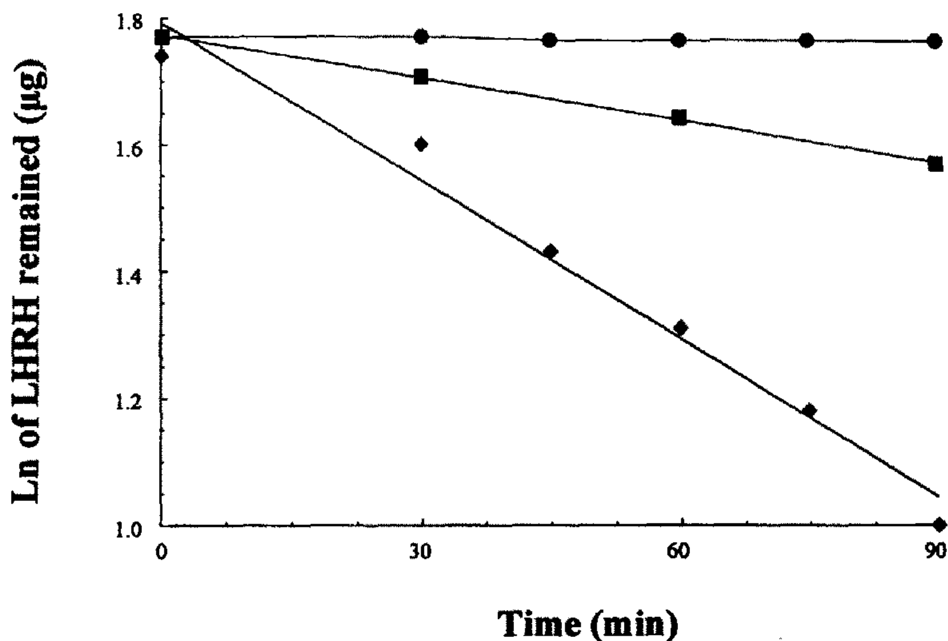


Fig. 2. First-order plot of LHRH degradation by liver tissue. ●, Control; ■, intact liver tissue; ◆, liver tissue homogenate.

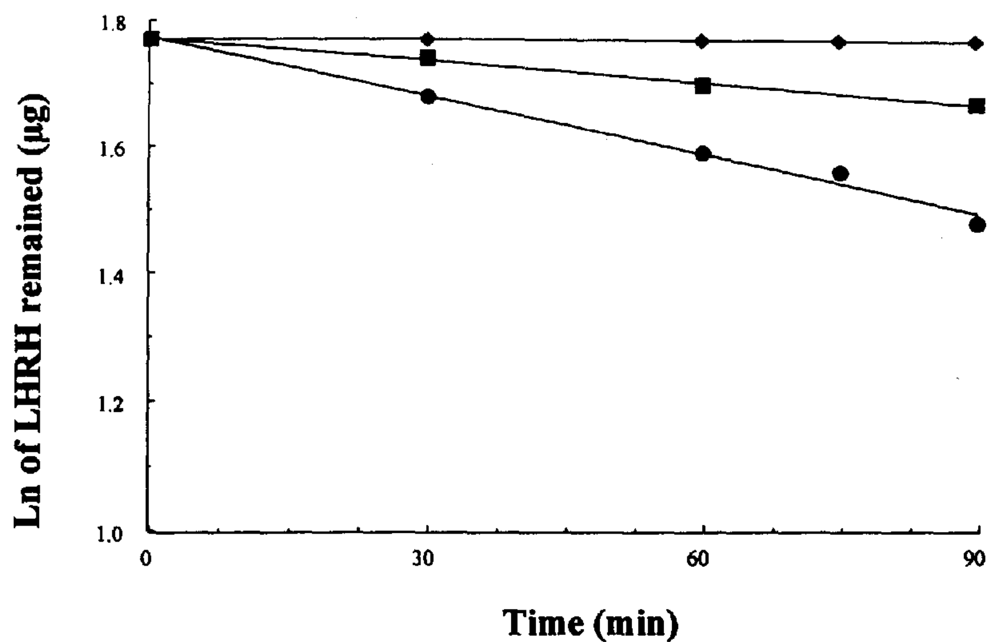


Fig. 3. First-order plot of LHRH degradation by nasal tissue. ◆, Control; ■, intact nasal tissue; ●, nasal tissue homogenate.

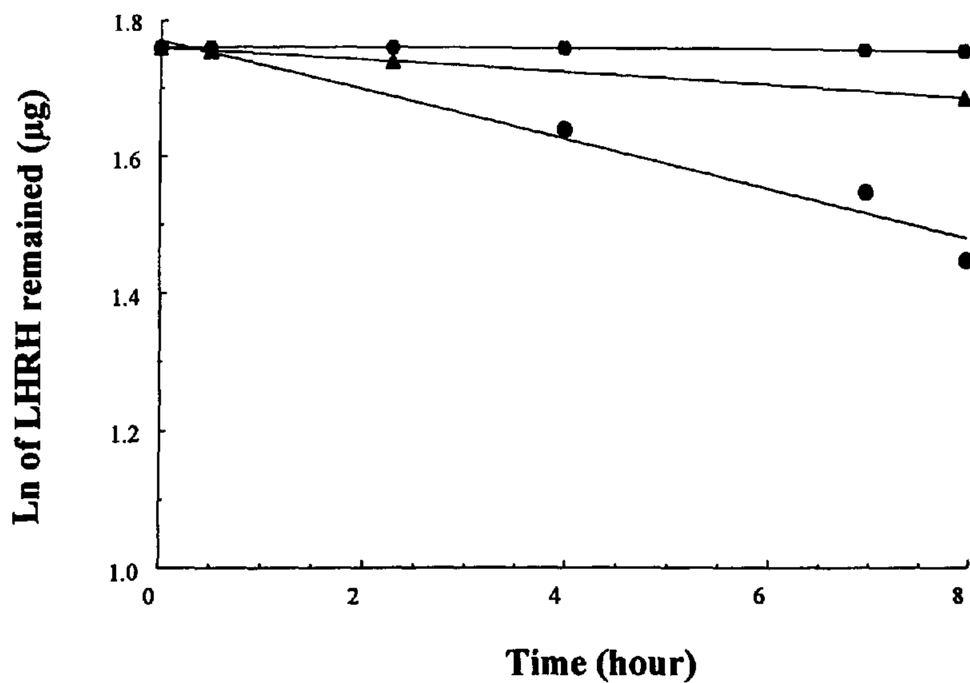


Fig. 4. First-order plot of LHRH degradation by pig skin tissue. Filled hexagon, control; ▲, intact skin tissue; ●, skin tissue homogenate.

Table 1
Degradation rates of LHRH in various pig tissues and their homogenates

| Tissue | Degradation rate constant $\times 10^3$ ($min^{-1}$) |
|---|---|
| Skin | |
| Homogenate | 0.60 |
| Intact tissue | 0.15 |
| Nasal | |
| Homogenate | 3.08 |
| Intact tissue | 1.13 |
| Liver | |
| Homogenate | 7.89 |
| Intact tissue | 2.12 |
| Buccal | |
| Homogenate | 3.64 |
| Intact tissue | 1.68 |

tion (10000 × g for 5 min) undoubtedly have allowed for much membrane-delimited material in the supernatant. Moreover, studies have demonstrated the presence of peptidase activity in the pellet resulting from homogenization and centrifugation procedures. The purpose of this study is to compare the degradation rates of LHRH in different tissues in order to help select proper delivery systems for this peptide. However, both permeability and metabolic characteristics of mucosal routes are important factors to consider in the selection of a given route for the administration of a therapeutic peptide or protein. In conclusion, LHRH was degraded in all of the four tissues. The degradation rate of LHRH was the least in the skin compared with other tissues. Therefore, transdermal delivery would provide a suitable route for administering LHRH for systemic therapeutic action considering enzymatic/ metabolic aspect of degradation.

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