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Differential expression of LHRH-receptor in bovine nasal tissue and its role in deslorelin delivery

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ABSTRACT

Deslorelin, a luteinizing hormone releasing hormone (LHRH) agonist, is transported via the LHRH-receptor (LHRH-R) and exhibits regional variation as follows: inferior turbinate posterior (ITP) > medium turbinate posterior (MTP) > medium turbinate anterior (MTA) of the bovine nasal mucosa. Differential LHRH-R expression in various regions of the nose is a potential explanation for regional variation in deslorelin transport. Thus, the objective was to determine whether LHRH-R expression exhibits regional variation in bovine nasal mucosa. LHRH-R density (B_{max}) and affinity constant (K_d) were determined by saturation experiments using 0.5 mg tissue in the presence of increasing amounts of I^{125}-deslorelin (100–100,000 cpm) at 4 °C for 4 h. The 50% inhibitory concentration (IC_{50}) was determined by competition experiments using various amounts of unlabelled deslorelin (0.01–3000 ng) at 4 °C for 4 h. LHRH-R mRNA and protein expressions were determined using real-time PCR and Western blot analysis, respectively. LHRH-R B_{max} and K_d varied between the regions of excised bovine nasal mucosa: ITP > MTP > MTA. The inhibition experiments yielded two IC_{50} concentrations which exhibited trends similar to B_{max} and K_d. Real-time PCR and Western blot analysis indicated that LHRH-R expression exhibits similar trends: ITP > MTP > MTA. We identified two deslorelin binding sites in the nasal tissues, with high affinity sites representing approximately 60–70% of the total sites available. In summary, regional differences in nasal deslorelin transport correlate with regional differences in LHRH-R expression, with LHRH-R expression, peptide binding, and transport being the highest in the inferior turbinate posterior region of the nose.

1. Introduction

The luteinizing hormone releasing hormone (LHRH) is a decapeptide of amino acid sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 [25,36]. There are at least two identified species of LHRH in several vertebrates including humans. These are designated LHRH-I and LHRH-II respectively [25]. It is synthesized and released from the hypothalamus and regulates mammalian reproduction [7] by regulation of the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) [34]. However, the native LHRH has a very short half-life leading to the use of peptide analogs for treatment of various diseases [28]. Currently newer generations of peptide and nonpeptide LHRH analogs are required [28].

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Deslorelin is a nine amino acid peptide of molecular weight 1285 Da with the amino acid sequence pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-ProNHEt [8,36]. Modifications at position 6 of deslorelin replacing Gly with D-Trp and at position 10 replacing Gly with ethylamide makes the synthesized deslorelin 144 times more potent than the native LHRH [8]. Deslorelin functions similar to LHRH by stimulating the release of LH and FSH following acute exposure and inhibits their release upon chronic administration. Hence, deslorelin is of therapeutic value in the treatment of endometriosis, uterine fibroids, precocious puberty, and breast and prostate cancers [31]. Both native LHRH and deslorelin exert their effects via the G protein – coupled receptor (GPCR) super family member – LHRH-receptor (LHRH-R) [16].

Similar to all the GPCRs the LHRH-R is characterized by the seven transmembrane (TM) domains connected by alternating intra- and extra-cellular loop domains [28]. Apart from the pituitary and reproductive tissues, LHRH-Rs have been shown to be expressed in non-reproductive tissues such as heart, kidney, placenta, and skeletal muscle [16]. Further, our previous reverse transcriptase-polymerase chain reaction (RT-PCR) studies indicated that normal respiratory tissues including the medium turbinate posterior (MTP) region of bovine nasal tissue, rat trachea and lungs express LHRH-R mRNA [19]. High LHRH-R expression is also present in cancers of prostate [12,35], breast [15], ovaries [15], and endometrium [11,14]. Although, several types of LHRHs and LHRH-Rs were identified in various chordate and vertebrate species [26], only one functional LHRH-R was found in humans [26]. The type II LHRH-R is silenced by stop codons and frame shift deletions [26,27], thereby suggesting that the LHRH-R I mediates the action of all LHRH ligands [23]. Further, the LHRH-R has higher affinity for the LHRH I and a 10-fold lower affinity for the LHRH II [24]. Further, the ligand binding sites in the LHRH-R are conserved in the LHRH-R among all the species implicating the residues in position 5, 7 and 8 of the LHRHs to confer binding and functional selectivity [26,34].

The nasal route offers the advantage of delivery of the peptide and protein drugs that suffer from low bioavailability to the systemic circulation. Currently available nasal sprays of LHRH agonists' including buserelin (Suprefact) and nafarelin (Synarel) allow LHRH agonist delivery to the systemic circulation via the nasal route [17]. The nasal sprays are predominantly deposited in the medium and inferior turbinates. The various turbinate regions of the bovine nose provide a useful in vitro model to assess the permeability and metabolism of therapeutic peptide drugs.

Nasal turbinates (inferior, medium, and superior) are highly vascularized, offer large absorptive surface, and they are responsible for warming and humidifying the inhaled air [5]. However, the superior turbinate region occupies the least surface area and is inaccessible to nasally administered drugs. Our previous permeability studies with medium and inferior turbinates indicated that deslorelin transport exhibits regional differences in the order: inferior turbinate posterior (ITP) > medium turbinate posterior (MTP) > me-medium turbinate anterior (MTA) [20]. However, the reasons for these regional differences are unclear. It is known since 1980's that the LHRH-R is involved in clathrin mediated internalization of the receptor ligand complexes [13]. In respiratory epithelial cells, we observed that a competing LHRH agonist as well as an antisense oligonucleotide against LHRH-R reduced deslorelin transport [19]. Further, our previous studies with the nasal mucosa indicated that deslorelin transport is reduced in the presence of cellular energy depletors and at low temperature, consistent with a cellular energy dependent transport process for deslorelin [20]. Thus, LHRH-R is involved in transcellular deslorelin transport. Thus, it is likely that differences in the expression of LHRH-R in various regions of the nose contribute at least in part to regional differences in deslorelin transport.

The hypothesis of the study was that regional differences in nasal expression of the LHRH-R underlie the previously observed regional differences in deslorelin transport. Thus, the objective of this study was to determine the LHRH-R expression in the various regions of the nose. In this study we employed a radioligand binding assay, quantitative real-time PCR, and Western blot analysis to investigate LHRH-R expression in various regions of bovine nasal mucosal tissue.

2. Methods

2.1. Chemicals

Deslorelin was a gift from Balance Pharmaceuticals, Inc. (Santa Monica, CA). The chemicals required for the preparation of buffers were obtained from Sigma–Aldrich (St. Louis, MO). I^{125} labeled deslorelin was obtained from Bachem California, Inc. (Torrance, CA).

2.2. Preparation of solutions

Receptor binding studies were conducted using an incubation buffer containing 40 mmol of Tris–HCl, 10 mmol of EDTA, and 0.5% BSA, adjusted to pH 7.4 [1,2].

2.3. Isolation of excised bovine nasal mucosa

Bovine tissues were obtained from a local slaughter house (J & J Meats, Elkhorn, NE). The nasal tissues were isolated and prepared for experiments as previously described [20]. The frontal part of the nasal conchae was dissected and the mucosal tissues were then separated from the cartilage using a pair of tweezers. Different regions of the bovine nasal tissue, including the medium turbinate anterior (MTA) – up to 2.5 in. from the frontal tip of the nares; the medium turbinate posterior (MTP) – beyond 2.5 in. past the tip of the nares; and the inferior turbinate posterior (ITP) – beyond 2.5 in. past the tip of the nares, below the MTP region and facing the floor of the nasal cavity, i.e., the inferior meatus, were isolated and used immediately for the experiments.

2.4. Receptor binding assay

2.4.1. Preparation of tissue for receptor binding assay

The isolated tissues (~200 mg) were homogenized using the Tissue Tearor® in 2 ml of Tris–HCl buffer. The tissue homogenate was then centrifuged at 15,000 × *g* for 15 min at 4 °C to