Immuno histochemical detection of Gonadotropin-Releasing Hormone (GnRH) in porcupine (Hystrix cristata) pancreas

B. G. TARAKÇI1*, M. YAMAN1, Ali BAYRAKDAR1 and O. ATALAR2

1 Department of Histology and Embryology, Faculty of Veterinary Medicine, Firat University, 23119, Elazığ-TURKEY
2 Department of Anatomy, Faculty of Veterinary Medicine, Firat University, 23119, Elazığ-TURKEY
* Corresponding authors: E-mail: btarakci@firat.edu.tr; myaman@hotmail; alibayrakdar@firat.edu.tr; oatalar@firat.edu.tr

SUMMARY

The expression of gonadotropin-releasing hormone (GnRH) was investigated in porcupine (Hystrix cristata, n = 5) pancreas by immunohistochemistry. The GnRH immunoreactive cells were exclusively distributed into glandular structures of the exocrine pancreas with a relative high frequency (mean ± S.D.: 63.8 ± 1.4 cells / microscopic field). By contrast, no GnRH positive cell was evidenced in pancreatic islets. These results show that GnRH is produced by exocrine pancreas of porcupine, suggesting that this hormone could be involved in the regulation of digestion.

Keywords: Gonadotropin-releasing hormone, porcupine, pancreas, immunohistochemistry.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a decapeptide widely known for its role in the control of reproduction cycle. Indeed the release of GnRH from the hypothalamus controls the synthesis and secretion of pituitary gonadotropins responsible for gonad development and growth in all vertebrates [1, 7, 11, 14]. The GnRH, also called luteinizing hormone releasing hormone (LHRH), is also synthesized by many non-hypothalamic tissues such as placenta [16, 23], gonads [3, 10] and mammary glands [2] of different kinds of mammals and GnRH analogs have been expressed in neoplastic breast and pancreatic cancer cells [15, 20]. However, GnRH is not only expressed in pancreatic cancer cells but also in normal rat and guinea pig pancreatic glands [24, 26] and it may express some physiological functions in normal tissue.

The porcupine belongs to the Hystricidae family which constitutes a small group of the order Rodentia [5, 9]. Although many studies were conducted on pancreas of various vertebrates including several species and strains of rodents, there are only a few studies concerning the porcupine pancreas. Recently, OZDEMIR [12] examined morphology of the porcupine pancreas using histochemical method and TIMURKAAN [25] studied endocrine reactive cells in the porcupine pancreas using immunohistochemical staining method. Several neuropeptides were found to be secreted by endocrine cells of porcupine pancreas [25]. However, no information knowledge concerning the occurrence of GnRH in porcupine pancreas is available. In rodents, little attention has been paid to the existence of GnRH in digestive system and a few existing reports have been carried out [24, 26]. Thus the present study investigated GnRH expression in porcupine pancreas by immunohistochemistry.

MATERIALS AND METHODS

1. ANIMALS AND TISSUE SAMPLES

Five adult female porcupines (Hystrix cristata) of different ages (2 to 4 years old) and hunted by villagers in Eastern Anatolia (Turkey) were used. Deep anaesthesia of animals was induced by initial injection of ketamine HCL (Ketanes 10-15mg/kg, I.M) followed by xylazine HCL (Rompun 0.10-0.15 mg/kg I.M). The tissue samples were taken from pancreas by biopsy puncture and fixed in 4% neutral-buffered formalin for 24 hr. They were then dehydrated through graded ethanol and embedded in paraffin. Seven µm-thick
sections were obtained and processed for immunohistochemical staining.

2. IMMUNOHISTOCHEMISTRY: PAP (PEROXIDASE-ANTI-PEROXIDASE) METHOD

Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H₂O₂) in methanol for 5 minutes [22]. In order to block unspecific binding, an incubation with normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 (Dilution 1:10) was performed. Sections were incubated for 16-20 hours at 4°C with rabbit IgG antibodies against luteinizing hormone releasing hormone (Chemicon, AB1567, Canada). Antibodies were diluted to 1:20 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark) followed by rabbit peroxidase antiperoxidase complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation step, and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate [18] for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and cover slips mounted with aqueous permanent mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by STERNBERGER [21] by using (including the replacement of) specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken. For semi-quantitative analysis, the average number of positive cells by microscopic field was determined throughout the identification and the counting of these cells onto 10 microscopic fields (magnification x 40).

Results

GnRH immunoreactive epithelial cells were observed in exocrine pancreas (Figures 1 and 2) and they were located into glandular structures where they constituted small groups (3 to 5 cells). These positive cells were big and round and they were encountered in the exocrine pancreas with a relative high frequency (mean ± S.D.; 63.8 ± 1.4 cells / microscopic field). The specific localization of GnRH positive cells in exocrine pancreas and their relative frequencies were found similar in the five animals (Table 1).

By contrast, no immunoreactive cell was detected in the endocrine pancreas of the 5 animals.

Discussion

GnRH has been reported to exist in many non-hypothalamic tissues such as the placenta, gonads and mammary glands [2, 3, 10, 16, 23]. The GnRH expression was also evidenced in rat mammary gland [6]. After detection of GnRH in the milk [4], the GnRH expression in breast was studied. It is now well established that GnRH is synthesized by the lactating mammary gland and that this peptide provides regulatory mechanisms for milk excretion. When suckling was prevented, serum LH concentrations of pups dropped to about 30%, and when suckling was allowed, the serum LH concentrations were restored within 1 h [4]. These
observations suggest that at least part of the milk GnRH is absorbed from the gastrointestinal tract of the suckling pup in a biologically active form. Indeed, the gastrointestinal tract of the neonatal mammals is largely permeable and allows the transport of peptides and proteins across the intestinal epithelium [8]. Similar results were also obtained in another study that demonstrated that milk GnRH may have a modulator role on the development of the infantile rat ovary [19]. In the mother, GnRH synthesized by the mammary gland may function as a paracrine agent within the mammary gland and/or on the anterior pituitary of the mother [13]. It seems that the mammary glands serve as complementary organs in a mechanism by which the mother exercises control over the infant development and metabolism. Milk provides the mechanism by which regulatory information is transferred from the mother to the progeny.

While details about the functional roles of GnRH in various extra-pituitary tissues are continuously being discovered, it was not known before WANG et al. [26] and our recent research [24] that GnRH is expressed in normal pancreatic tissue and especially in the exocrine part. The present study has clearly demonstrated that GnRH immunoreactive cells are localized in the exocrine portion of pancreas. This distribution is similar to those found in rat and guinea pig pancreas [24, 26]. If GnRH is expressed in pancreas of old species, it would be possible that this peripheral expression of this hormone exists also in more developed species. Because GnRH-immunoreactivity in pancreas was restricted to exocrine portion, we presume that GnRH may be secreted into the gut and would act locally as a regulatory peptide for digestion. On the other hand, this decapetide would be also directly involved in the autocrine and paracrine regulation of the exopancreas function.

Although GnRH-immunoreactive cells were not detected in endocrine pancreas in our study, SEEPLELA and WAHL-STROM reported positive immunoreactive cells in human pancreatic islets [17]. This may be due to differences in the antigen tested and/or the species investigated. Nevertheless, we could not exclude the possibility of GnRH existence in islets, albeit in situ hybridization has failed to evidence GnRH-mRNA positive cells in islets [26]. Consequently, if GnRH exists in islets, it should be synthesized by the pancreatic exocrine cells.

The present study is the first report of GnRH immunoreactive cells in the exocrine part of porcupine pancreas which may suggest that GnRH play a role in the regulation of digestion. Future studies on the effects of GnRH on the secretion of digestive hormones and/or enzymes are necessary to clarify the biological significance of GnRH in porcupine exopancreas.

References