

## Presence of Gonadotropin-Releasing Hormone (Gnrh) in Quine Pig Pancreas

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**Abstract:** In the present study, the existence of gonadotropin-releasing hormone (GnRH) was investigated in the quine pig pancreas and gastrointestinal tract by using immunohistochemical techniques. GnRH-immunoreactive cells were only detected in exocrine pancreas. No immunoreactivity was seen in pancreatic islets and also in any region of gastrointestinal tract. This is the first report suggesting the existence of GnRH in quine pig pancreas.

**Key words:** Gonadotropin-releasing hormone, quine pig, pancreas, immunohistochemistry

### INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a decapeptide and play a role in the regulation of reproduction. This peptide synthesized and secreted from hypothalamus<sup>[1-3]</sup> and when it reaches the pituitary gland, it induces the synthesis and secretion of follicle-stimulating hormone and luteinizing hormone (LH) which regulate gonadal functions<sup>[4]</sup>.

GnRH also called Luteinizing Hormone Releasing Hormone (LHRH) is also synthesized by many non-hypothalamic tissues such as placenta<sup>[5,6]</sup>, gonads<sup>[7,8]</sup> and mammary glands<sup>[9]</sup> of different kinds of mammals.

GnRH analogs have been expressed in neoplastic breast and pancreatic cancer cells<sup>[10,11]</sup>. As GnRH is not only expressed in pancreatic cancer cells but also in normal rat pancreatic gland<sup>[12]</sup>, it may have certain physiological functions in normal tissue. Except this study<sup>[12]</sup>, there still have been no reports concerning the existence and expression of GnRH in the mammalian digestive system. So we intended to carry out similar research to the quine pig pancreatic gland and gastrointestinal tract.

In order to detect and locate GnRH in the quine pig pancreas, immunohistochemical method was used.

### MATERIAL AND METHODS

Five female quine pig used in this study. The animals were anesthetized with ethyl ether. Then tissue samples were taken from pancreas, stomach, small and large intestine and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. 7 µm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the Peroxidase-antiperooxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 minutes<sup>[13]</sup>. In order the block unspecific binding, an incubation with (1:10) normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 was performed.

**PAP technique:** Sections were incubated for 16-20 hours at 4°C in rabbit anti-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 respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark), followed by rabbit peroxidase anti-peroxidase 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 and finally immersed in glucose oxidase-DAB-nickel ammonium sulphate substrate<sup>[14]</sup> for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger<sup>[15]</sup>.

### RESULTS

**Distribution of GnRH in quine pig pancreas:** In pancreas, GnRH immunoreactive epithelial cells were observed to be distributed in exocrine pancreas (Fig. 1 and 2). These cells big and round. No immunoreactive cells were detected in pancreatic islands (Fig. 3).

**Distribution of GnRH in quine pig gastrointestinal tract:** GnRH immunoreactive cells were not detected in any region of gastrointestinal tract (Fig. 4).

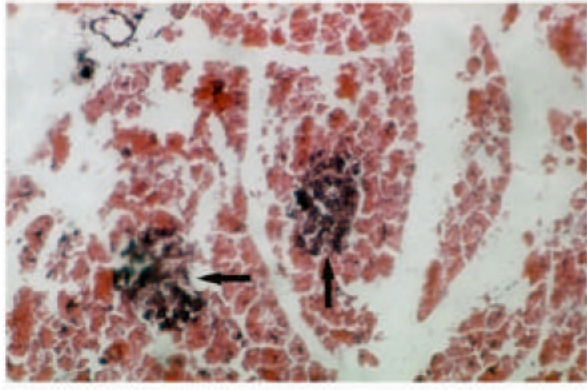


Fig. 1: Immunohistochemical demonstration of GnRH in quine pig exocrine pancreas (arrows). x 100

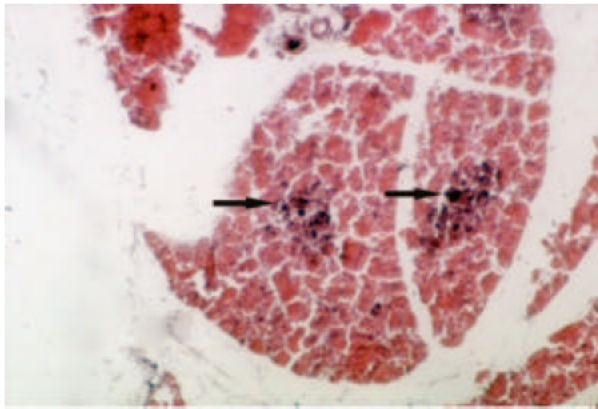


Fig. 2: GnRH immunoreactive cells in quine pig exocrine pancreas (arrows). x 100

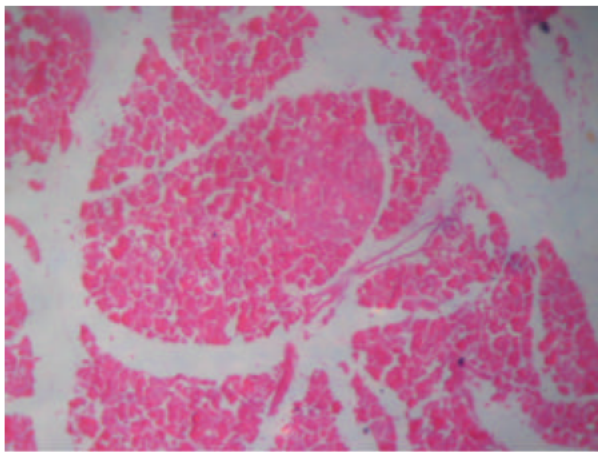


Fig. 3: The pancreatic islet is immunonegative. X50

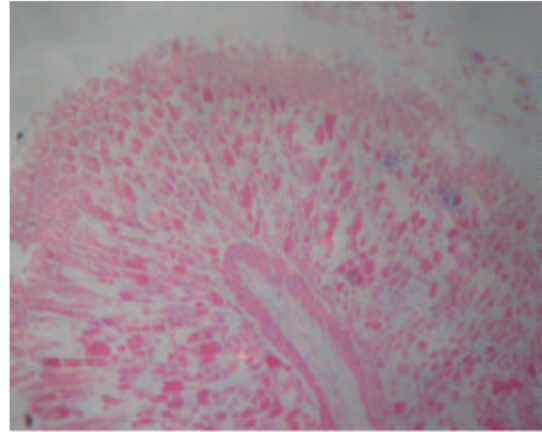


Fig. 4: No GnRH immunoreactivity was seen in any region of quine pig gastrointestinal tract examined. The stomach as shown in this photograph is immunonegative. x 50

#### DISCUSSION

While GnRH is synthesized from hypothalamus and it controls the production of pituitary gonadotropins, GnRH is also expressed extrahypothalamically and has extrapituitary activity.

Preliminary studies have revealed that GnRH regulation in placenta account for the unique time course of the hCG secretion during pregnancy<sup>[6]</sup> and indicated that GnRH analogs are able to alter steroidogenesis in the ovary<sup>[7]</sup>. While details about the functional roles of GnRH in various extrapituitary tissues are continuously being discovered, it was not known before recent studies by Wang *et al.*<sup>[12]</sup> that GnRH is actually expressed in normal pancreatic tissue nor has there been any previous indication of its distribution in the exocrine part.

Present study has clearly demonstrated that GnRH localized in the exocrine portion of pancreas. This result is the same as that in rat pancreas<sup>[2]</sup>. This finding was quite similar to the discovery of GnRH expression in rat mammary gland<sup>[8]</sup>. When the detection of GnRH was discovered in the milk, the study on GnRH in breast was started. GnRH has now been affirmed to be produced in mammary gland and provides a mechanism by which regulatory information is transferred from mother to progeny. In addition, it has been thought that GnRH synthesized by the mammary gland may affect the mother by a paracrine and / or an endocrine mechanism<sup>[9]</sup>. As GnRH-immunoreactivity in pancreas was restricted to exocrine portion, and no immunoreactivity was observed in any part of gastrointestinal tract, we presume that they may act by secretion into the gut and function there,

while, the possibility of paracrine or autocrine regulation can't be excluded.

Although, Present study, no GnRH-immunoreactive cells were detected in endocrine pancreas, Seppala reported positive immunoreactive cells in human pancreatic islets<sup>[20]</sup>. This may be due to the fact that different regions of the GnRH molecule were used as antigen determinant. In situ hybridization had shown GnRH-mRNA positive cells in exocrine part of rat pancreas but not in the islets<sup>[12]</sup>. So if GnRH is exist in islets, it should be synthesized by the exocrine cells in pancreas.

Present study is the first report of GnRH expression in the exocrine part of quine pig 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 are necessary to clarify a biological significance of GnRH in quine pig pancreas.

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