大韓獸醫學會誌(2001) 第41卷 第2號
Korea J Vet Res(2001) 41(2) : 133~138

An immunohistochemical study of the serotonin-immunoreactive cells in the developing pancreas of the chicken embryos

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(Accepted by May 15, 2001)

Abstract : The distributions and relative frequencies of the serotonin-immunoreactive cells were studied in dorsal, ventral, third and splenic lobes of developing chicken pancreas during embryonic periods (10 days of incubation to hatching) by immunohistochemical methods. The regions of pancreas were subdivided into three regions, exocrine, light and dark islets. Round and/or oval shaped serotonin-immunoreactive cells were detected in all four lobes. According to developmental stages, the types of lobes and the regions of pancreas, these immunoreactive cells were showed various distributions and relative frequencies. In exocrine portions, serotonin-immunoreactive cells were found in the splenic lobes at 13-14 days of incubation, in the third lobes from 10 days to 19 days of incubation, in the ventral lobes from 10 days of incubation to hatching and in the dorsal lobes from 11 days of incubation to hatching. In pancreatic islets, these cells were detected only in the dark islets of splenic lobes at 15 and 16 day of incubation with rare frequency. In conclusion, serotonin-immunoreactive cells decreased with developmental stages in all four lobes and their relative frequencies decreased with developmental stages.

Key words : Chicken embryo, developing pancreas, serotonin, immunohistochemistry

Introduction

It has been known that avian pancreas consisted of 2 lobes, dorsal and ventral. But Clara[1] asserted that the other parts that extended from the head of pancreas to the spleen regions existed and subdivided into 3 lobes, dorsal, ventral and splenic lobes. In addition, Mikami and Ono[2] reported the ventral lobe could be subdivided into 2 parts, ventral and third lobes. These reports[1,2] generally indicated that the pancreatic lobes of avian species consisted of 4 lobes, splenic, third, dorsal and ventral lobes[3]. A series of normal stages in development of chicken embryos were established by Hamburger and Hamilton[4]. The vascular supply of the chicken pancreas was reported[5] and the morphogenesis of chicken pancreas during embryonic period was also reported in Japanese chicken[6]. Especially, Ono[6] insisted that the differentiation of pancreatic lobes occurred after 10 days of incubation.

Differing from mammalian species, A cells producing glucagon and B cells producing insulin were distributionally segregated in the avian endocrine pancreas, and A cells were more than B cells. In addition, somatostatin-producing D cells in the avian pancreas were more than those of mammals[2,7]. Also differing from mammals, 2 types of the pancreatic islets, dark and light islets could be subdivided. Dark islet consisted of numerous A cells and a few D cells but light islet consisted of numerous B cells and a few or moderate frequency D cells. And the origin of these terms had different staining nature of A and B cell[2,3,7]. Iwanaga *et al*[7] insisted that these 2 types of islets showed different distribution with types of the pancreatic lobes.

Until now, many researches about avian endocrine pancreas were reported including electron microscopical studies and histochemical studies using silver techniques[8,9]. Especially, many researchers have showed great concern for the anatomical, histological and endocrinological structure of the splenic lobes. Recently, immuno-histochemistry using the specific antisera against hormone was settled[9]. Existence, distribution and relative frequency of various hormone producing cells including insulin-, glucagon-, somatostatin-, pancreatic polypeptide-, biotin-, serotonin- and chromogranin- immunoreactive cells were

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demonstrated in the pancreas of avian species including chicken[10-12], duck[14] and mallard[15,16]. In addition, some fragmental reports[12,17,18] about ontogeny of immunoreactive cells in the avian pancreas were reported and Ku *et al*[19] reported that the distribution and the relative frequency of insulin-, glucagon- and somatostatin-immunoreactive cells in each lobes of developing pancreas of the chicken embryos. However, no report shows changes in the distribution and the relative frequency of serotonin-immunoreactive cells in each lobes of developing pancreas of the chicken embryos.

In present study, according to Ono's report[1], the distribution and relative frequency of serotonin-immunoreactive cells were studied in the dorsal, ventral, third and splenic lobes of developing chicken pancreas after differentiation of the pancreatic lobes, from 10 days of incubation to hatching, by immunohistochemical methods using specific antiserum against serotonin.

Materials and Methods

One hundred and five chicken eggs (Harvard Co., USA) were used. The eggs were incubated by incubator (KE 300, Eunjo Incubator Co, Korea) under 70% humidity and at 37.8°C. The developmental stage of the embryos were divided by Hamburger and Hamilton's methods[4]. Each of 5 chicken embryos was used in this study without sexual distinction. The pancreas was sampled from chicken embryos after cracking under stereoscopy. At hatching, the pancreas was taken after being phlebotomized.

The sampled pancreas was fixed in the Bouin's fluid. Fixed pancreas was divided into each lobe under stereoscopy. After paraffin embedding, 3-4 μm serial sections were prepared by routine methods. Each representative section was deparaffinized, rehydrated and immunostained by the peroxidase antiperoxidase (PAP) methods[10]. Background blocking was performed with normal goat serum prior to incubation with the primary antiserum against serotonin (BioGenex Lab, Cat No BO68082C, 1:20). After rinsing in phosphate buffered saline (PBS, 0.01M, pH 7.4), the sections were incubated in the secondary antiserum. Then they were washed in PBS and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3, 3-diaminobenzidine tetrahydrochloride containing 0.01% of H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and immunoreactive cells were observed under light microscope.

Results

The lobes of pancreas of the chicken embryos were

Table 1. Regional distributions and relative frequencies of serotonin-immunoreactive cells in the splenic and third lobes of pancreas of the chicken embryos.

Incubation periods	Splenic lobe			Third lobe		
	Exocrine	Dark islet	Light islet	Exocrine	Dark islet	Light islet
10 days	*	*	*	+	**	−
11 days	*	*	*	+++	−	−
12 days	*	*	*	++	−	−
13 days	++	−	−	++	−	−
14 days	++	−	−	++	−	−
15 days	−	±	−	+	−	−
16 days	−	±	−	+	−	−
17 days	−	−	−	±	−	−
18 days	−	−	−	±	−	−
19 days	−	−	−	±	−	−
Hatching	−	−	−	−	−	−

*Splenic lobes were not detect in this ages; **These types of islets were not detected in this lobe; Remarks: − : not detected, ± : rare, + : a few, ++ : moderated, +++: numerous.

Table 2. Regional distributions and relative frequencies of serotonin-immunoreactive cells in the ventral and dorsal lobes of pancreas of the chicken embryos.

Incubation periods	Ventral lobe		dorsal lobe		
	Exocrine	Light islet	Exocrine	Dark islet	Light islet
10 days	+	−	−	*	*
11 days	+++	−	+++	*	*
12 days	++	−	+++	*	*
13 days	++	−	++	−	−
14 days	++	−	++	−	−
15 days	+	−	++	−	−
16 days	+	−	++	−	−
17 days	+	−	+	−	−
18 days	±	−	+	−	−
19 days	±	−	+	−	−
Hatching	±	−	+	−	−

*These types of islets were not detected in this lobe; Remarks: − : not detected, ± : rare, + : a few, ++ : moderated, +++ : numerous.

differentiated into 3 lobes, third, dorsal and ventral, from 10 days of incubation to 12 days of incubation. After 13 days of incubation, all of 4 types of lobes, splenic, third, dorsal and ventral, were observed. Dark and light islets were observed in all 4 types of lobes except for ventral lobes where dark islets were not observed (Table 1, 2).

Round and/or oval shaped serotonin-immunoreactive cells were observed in all 4 lobes (Fig 1). **In the splenic lobes**, they were detected in exocrine and dark

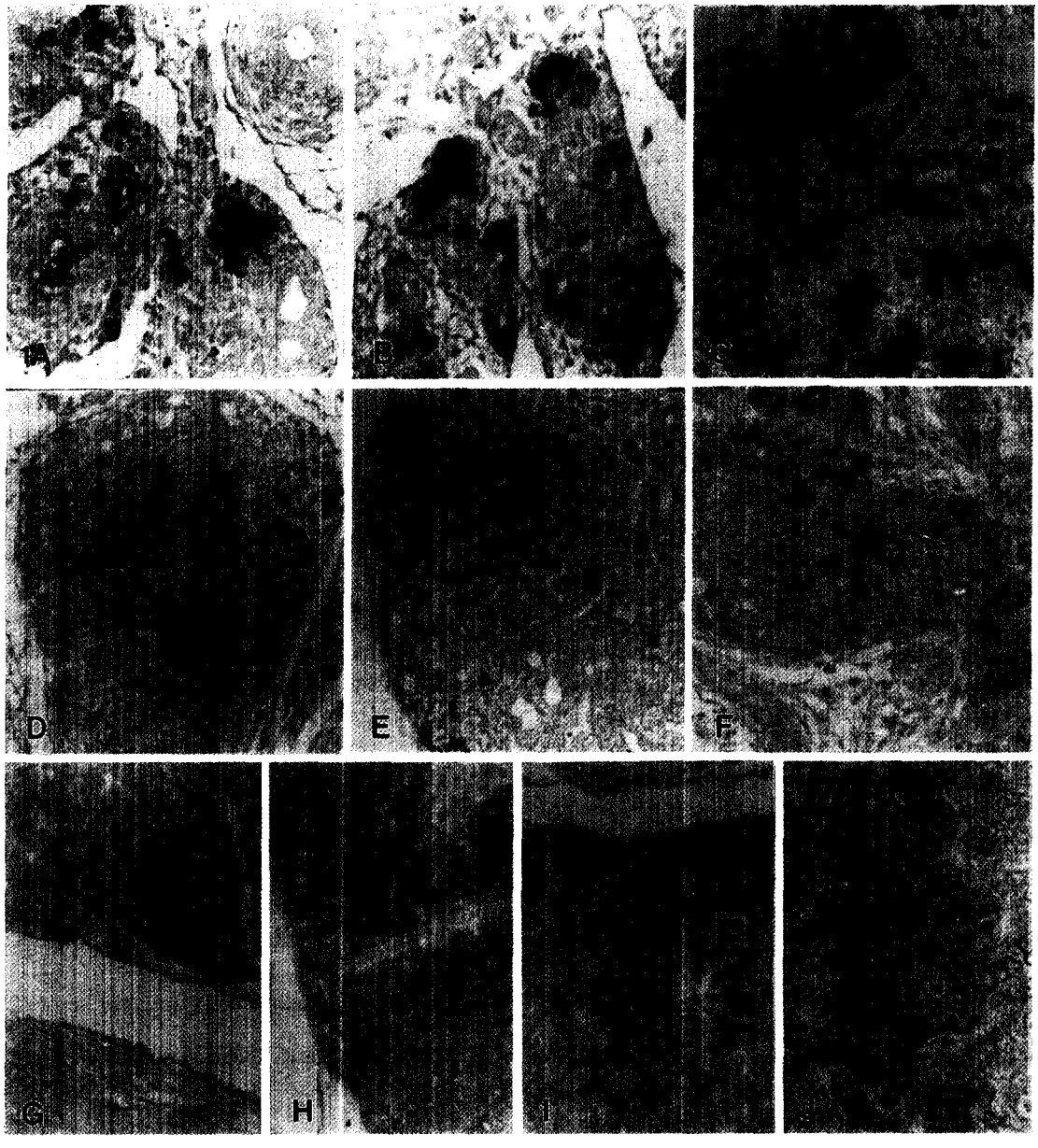

Fig 1. Serotonin-immunoreactive cells in the pancreas of the chicken embryos. Note that various distributions and relative frequencies of these immunoreactive cells were observed in third lobe of 11 days of incubation (A), ventral lobe of 11 days of incubation (B), dorsal lobe of 12 days of incubation (C), splenic lobe of 15 days of incubation (D), ventral lobe of 15 days of incubation (E), dorsal lobe of 15 days of incubation (F), dorsal lobe of 18 days of incubation (G), ventral lobe of 19 days of incubation (H), splenic lobe at hatching (I), dorsal lobe at hatching (J). A~I, X 150, J, X 400, PAP method.

islets. Only rare cells were detected at 15-16 days of incubation in dark islets. These cells were observed at 13-14 days of incubation in exocrine with moderate frequency. **In the third lobes**, these cells were restricted to exocrine from 10 days to 19 days of incubation. They increased to 11 days of incubation and reached the highest frequency but decreased with developmental stages. Finally, they were observed with rare frequency at 19 days of incubation. **In the ventral lobes**, these cells were detected in the exocrine only from 11 days of incubation to hatching. The changes of relative frequencies were similar to those of third lobes. **In dorsal lobes**, they were demonstrated in the exocrine from 11 days of incubation to hatching. They were decreased with developmental stages.

Discussion

Serotonin is secreted by enterochromaffin cells in the gastroeneteropancreatic system having various physiological regulating functions including contraction of smooth muscles[20]. About the appearance of serotonin-immunoreactive cells in the pancreas of animals, Ito *et al*[21] reported that serotonin-immunoreactive cells were detected in the pancreas of pig. In this report, two types of serotonin-immunoreactive cells were existed, namely, one was a stronger immunoreacted cell and the other was a weak immunoreacted cell. The former was detected in pancreatic islets and exocrine with a few frequency and was not colocalizated against insulin, glucagon, somatostatin, and PP antisera. Also Ito *et al*[21] suggested that the former was similar to the gastroenetroendocrine cells. The latter was detected in pancreatic islets and was colocalizated against insulin antisera. Owman *et al*[22] suggested that these immunoreactive cells were demonstrated in A and B cells of pigs, A cells of dog and B cells of guinea pig by immunofluorescence methods but Watanabe *et al*[23] reported that they were detected in A cells not in B cells of chicken. Lucini *et al*[14] demonstrated the appearance of these immunoreactive cells in the exocrine and pancreatic islets of duck and they also reported that some of these immunoreactive cells were colocalizated with glucagon (A)-immunoreactive cells. In present study, serotonin-immunoreactive cells were found in the splenic lobes at 13-14 days of incubation, in the third lobes from 10 days to 19 days of incubation, in the ventral lobes from 10 days of incubation to hatching and in the dorsal lobes from 11 days of incubation to hatching in exocrine. In pancreatic islets, these cells were detected only in the dark islets of splenic lobes at 15-16 day of incubation with rare frequency. Generally, these immunoreactive cells were decreased with developmental stages in all four lobes. These results were somewhat different from Lucini *et al*[14] but similar to Lee *et al*[15] who reported that no serotonin-immunoreactive cells were detected in pancreatic islets of mallard above 9 weeks old, and Ding *et al*[24] who suggested that these immunoreactive cells were restricted in exocrine of adult chicken. However, the changes according to developmental stages were difficult to compare with other works because there was no report that carried out the changes of serotonin-immunoreactive cells with developmental stages in pancreas of other avian species. In addition, Ku *et al*[25] reported that serotonin-immunoreactive cells were remarkably increased in chicken duodenum after 14 days of incubation, and consequently, it is considered that numerously detected serotonin-immunoreactive cells in early periods of this study were disappeared or decreased toward mid or late periods but it is also hardly excepted that these concentrated cells in early periods were dispersed into whole pancreas along with expansion of pancreas during developmental stages. So exact mechanisms of these phenomenons were unknown.

In conclusion, serotonin-immunoreactive cells showed various distributions and relative frequencies according to developmental stages, the types of lobes and the regions of pancreas. In future, the changes of the distribution and frequency of the other types of endocrine (immunoreactive) cells in the pancreas of the chicken or other species of birds with developmental stages will be clarified by using histochemical or immunohistochemical methods.

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大韓獸醫學會誌(2001) 第41卷 第2號
Korea J Vet Res(2001) 41(2) : 133~138

발생단계에 따른 닭 태자 췌장에서 serotonin 면역반응세포에 대한 면역조직화학적 연구

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(2001년 5월 15일 게재승인)

국문초록 : 부란 1일부터 부화직후까지의 닭 태자 췌장에서 serotonin 면역반응세포들의 부위별 분포 및 상대적 빈도를 면역조직화학적 방법으로 검색하였다. 췌장은 해부학적으로 배쪽, 등쪽, 제 3엽 및 비장엽의 4개엽으로 구분하였으며, 각 엽은 조직학적으로 외분비 부분, light 및 dark 췌장섬의 3부분으로 세분하였다. 이들의 각 발생단계에 따른 닭 췌장에서 serotonin 면역반응세포들의 분포 및 빈도는 췌장의 엽, 조직학적 부위 및 발생단계에 따라서 매우 다양하게 관찰되었으나, 대체로 원형 또는 난원형의 형태로 모든 엽에서 관찰되었다. 외분비 부분에서 serotonin 면역반응세포들은 비장엽의 경우 부란 13일과 14일에서만 국한되어 관찰되었고, 제 3엽에서는 부란 10일부터 부란 19일 동안 관찰되었다. 또한 배쪽엽에서는 부란 10일부터 부화 직후까지 관찰되었으며, 등쪽엽에서는 부란 11일부터 부화 직후까지 관찰되었다. 췌장섬에서 이들 면역반응세포는 비장엽의 dark 췌장섬에서만 부란 15일과 부란 16일에 국한되어 극소수 관찰되었고 다른 엽 또는 light 췌장섬에서는 관찰되지 않았다. 결론적으로 serotonin 면역반응세포들은 부란 발생 초기에 다수 관찰된 이후 발생단계에 따라 점차적으로 감소되며 이런 양상은 엽의 종류에 관계없이 나타나는 것으로 관찰되었다.

Key words : Chicken embryo, developing pancreas, serotonin, immunohistochemistry