Ontogeny of Somatostatin Cells in the Rat Fetal Pancreas

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ABSTRACT. The growth pattern of somatostatin cells was clarified immunohistochemically from day 12 to day 18 of gestation in the rat fetus. On day 12, somatostatin cells first appeared within the pancreatic anlage. The total number of somatostatin cells was gradually increased from day 12 to day 16 and rapidly increased thereafter. It may be concluded that such a sequence of events of development in somatostatin cells occurs in a fashion similar to that in B cells. — KEY WORDS: pancreatic islet, rat fetus, somatostatin cell.

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Somatostatin, consisting of 14 amino acids, was first isolated from the hypothalamus [4]. It has been confirmed that immunoreactivity for somatostatin is also found in D cells in the pancreatic islet and throughout the gastrointestinal tract [2, 3, 15]. Somatostatin inhibits the release of insulin and glucagon from B and A cells, respectively, in the pancreatic islet both *in vivo* and *in vitro* [1, 7, 8, 10, 11]. Early development of somatostatin cells has not been much studied in detail, although that of A and B cells has been widely investigated in the rat fetus [5, 6, 16–18]. The present study was performed to determine when somatostatin cells would appear and in what fashion they would grow in the rat fetus, by means of the immunocytochemical technique.

Wistar rats (CLEA, Tokyo) were given a commercial diet (Labo MR Breeder, Tokyo) and water both *ad libitum*. They were maintained on 12-hr on- and 12-hr off-light cycle at room temperature of 23 ± 2°C and humidity of 55 ± 10%. Females were placed with males overnight and the day on which sperm was detected in vaginal smear was designated as day 0 of gestation.

Maternal autopsy was made on each day from day 12 to day 18 of gestation. Fetuses were quickly removed from the uteri of mother rats. On days 12 and 13, each fetus was fixed in Bouin's fluid *in toto*. From day 14 to day 18, the fetal pancreas was removed together with the stomach and duodenum and was fixed in the same fluid. The fixed materials were then dehydrated in a graded series of ethanol, embedded in Paraplast (Sherwood Medical, St. Louis), and sectioned serially at 5 μm. All sections in each pancreas were immunostained with anti-somatostatin serum (ZYMED, Cat. No. 18–0078, San Francisco). The antiserum was diluted with phosphate buffer containing bovine serum albumin (1:500). The total number of somatostatin cells in each pancreas was counted. The counts were made on all of serial sections for each pancreas. Particular attention was paid for avoiding repeated counts of profiles of the same cell in adjoining sections.

The staining specificity of polyclonal antiserum for somatostatin was examined by pre-absorption test of the serum using serial sections of pancreas. The positive reaction by the antiserum was completely abolished after adding the corresponding peptide, at the concentration of 1.0 μg/m*l* synthetic somatostatin (Peptide Institute, Inc., Osaka).

On day 12 of gestation, several cells in a tubular pancreatic anlage reacted with anti-somatostatin serum (Fig. 1). These cells were small, but filled with immunoreactive materials. On day 13, the primitive islet became distinguishable from the exocrine part, and somatostatin cells were located evenly in the primitive islet and in the exocrine part. On days 14 and 15, somatostatin cells were mainly located and irregularly distributed in the primitive islet. On day 17, somatostatin cells were located in the peripheral portion of the primitive islet as those in adult. On day 18, somatostatin cells were located not only in the peripheral portion of islet but also in the exocrine duct system.

The total number of somatostatin cells was gradually increased day by day from day 12 to day 16 of gestation (Table 1), the daily increase rate being approximately 1.5 to 2.2. After day 17, the total number of somatostatin cells was increased rapidly (48, 2 samples on day 17, and 453, 2 samples on day 18).

As shown in the foregoing, somatostatin cells first appeared in the pancreatic anlage on day 12 of gestation, not in line with the previous reports that somatostatin was first detected on day 14 by radioimmunoassay (RIA) [14] and on day 15 by immunohistochemistry [18]. Such a discrepancy may be caused by the difference in the sensitivity between immunohistochemistry and RIA, and by the difference in the observation method between all serial sections and skipped sections at intervals.

In the developing pancreatic islet in the rat fetus, A cells first appear on fetal day 11 [6], followed by B cells on day 12 [18]. It is proposed that B cells of the fetal pancreas differentiate by 3 steps [9, 13]. In the first step up to day 15, B cells reveal no signs of cytological differentiation with constantly low insulin content and with undetectable insulin granules by ultrastructural analysis [16, 17]. In the second step around day 15, B cells reveal a cytodifferentiation characterized by the appearance of secretory granules and an exponential increase in the cell volume and the insulin content [12]. In the 3rd step on days 19 and 20, B cells attain their full differentiation. In the present study, total number of somatostatin cells was

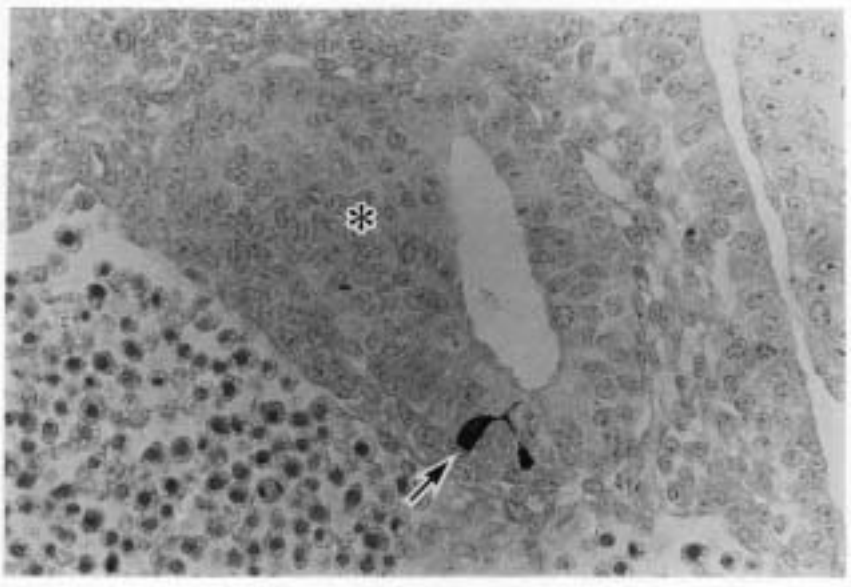

Fig. 1. Photomicrograph of a 12-day fetal pancreas. Somatostatin cells (arrow) are located within the tubular pancreatic anlage (asterisk). The anlage is obliquely sectioned. A section of the foregut is seen at the right upper corner. × 350.

Table 1. The total number of somatostatin cells (mean ± SEM)

Age in fetal days	No. of samples (litters)	Total number of somatostatin cells
12	7(3)	2.4 ± 0.3
13	8(3)	5.4 ± 0.6
14	9(3)	8.3 ± 0.9
15	8(3)	18.0 ± 1.7
16	8(3)	26.4 ± 2.2

very small from day 12 to day 16 (particularly on days 12, 13, and 14), followed by subsequent rapid increase. Therefore, it may be concluded that such a sequence of events of development in somatostatin cells occurs in a fashion similar to that in B cells.

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