Ontogeny of salivary peptide P-C like immunoreactivity in human pancreatic B-cells

Seiki Ito, Satoko Isemura¹, Eiichi Saitoh¹, Kazuo Sanada¹, Toshimitsu Suzuki² and Akira Shibata

First Department of Internal Medicine and Department of Pathology²,
Niigata University School of Medicine, Niigata 951 and
Department of Oral Biochemistry¹, Nippon Dental University, Niigata Faculty, Niigata 951, Japan

Abstract. An immunohistochemical study using antisera against proline rich salivary peptide P-C and insulin, glucagon, somatostatin and pancreatic polypeptide antisera was carried out on the foetal pancreas at different stages and on the newborn infant's, infant's, child's and adult pancreas to examine the time at which salivary peptide P-C like immunoreactivity appeared in the human pancreas. Salivary peptide P-C like immunoreactive cells first appeared as a few scattered cells in the foetal pancreas after 16 weeks of gestation and gradually increased in numbers during gestation. The cells corresponded only to insulin immunoreactive cells in the foetal, newborn infant's, infant's, child's and adult pancreas. Only some of the insulin immunoreactive cells in the foetal pancreas contained salivary peptide P-C like immunoreactivity while the majority of those in the infant's pancreas and all those in the child's and adult pancreas did so. The findings, together with the fact that the full sequence of salivary peptide P-C is identical to the COOH-terminal 44 amino acid residues of Salivary Protein C, led to the possibility that peptide P-C like immunoreactivity in the human pancreatic B-cells was not a moiety of the precursor of insulin and pro-insulin, but a moiety of Salivary Protein C. It has been suggested that, in saliva, Salivary Protein C aids in maintenance of the calcium concentration. Based on the hypothesis that peptide P-C like immunoreactivity in the human pancreatic B-cells may play some role in insulin release through the maintenance of the calcium concentration, the present finding seems to explain the fact that the mechanism for insulin release in the foetal pancreas is immature in spite of sufficient biosynthesis of insulin.

Recently, Isemura et al. (1979) isolated the salivary proline rich peptide P-C from human whole saliva and presented the primary structure of this peptide (Isemura et al. 1980). Although the pathophysiological role of this peptide remained to be elucidated, its tissue distribution was recently demonstrated by an indirect immunofluorescence technique using antisera against peptide P-C (Ito et al. 1983). Peptide P-C like immunoreactivity was present not only in the salivary glands but also in the pancreatic B-cells. As the antisera against peptide P-C did not have any cross-reactivity with other peptides, including insulin, human C-peptide, glucagon, somatostatin, pp and VIP, it was suggested that peptide P-C like immunoreactivity was present in the pancreatic B-cells independently of insulin and pro-insulin (Ito et al. 1983). Furthermore, peptide P-C like immunoreactivity was considered to play some role in the function of human pancreatic B-cells, since it was localized only in the B-cells among the four kinds of cells in the islets. The idea made us interested in the time at which salivary peptide P-C like immunoreactivity appeared in the human pancreatic B-cells, since it is well known that the mechanism for insulin release in the foetal pancreatic B-cells is immature in contrast to that in the adult pancreatic B-cells (Espinosa et al. 1970; Milner et al. 1972).

In order to elucidate the time at which salivary
peptide P-C like immunoreactivity appeared in the human foetal pancreas, an immunohistochemical study using antisera against peptide P-C was carried out on the foetal pancreas at different stages and on the newborn infant's, child's and adult pancreas.

**Materials and Methods**

**Antisera**

Antisera against peptide P-C were produced in rabbits by injections of peptide P-C-BSA conjugates which had been prepared from isolated salivary peptide P-C and BSA by using glutaraldehyde. Details of the conjugation procedure and immunohistological character of the antisera have been reported previously (Ito et al. 1983).

Glucagon, somatostatin and pp antisera were also produced in rabbits. Preparation methods and the immunological character of these antisera have been reported elsewhere (Ito et al. 1981, 1982, 1983).

Insulin antisera were produced in rabbits by injections of monocomponent insulin (Novo Institutes) emulsified with Freund's complete adjuvant.

**Tissues**

The foetuses studied were delivered by spontaneous abortion or hysterectomy, and varied between 16 and 26 weeks in gestational age. Foetal pancreas after 16 weeks (2 cases), 18 weeks (1 case), 20 weeks (1 case), 24 weeks (2 cases) and 26 weeks (2 cases) gestation were obtained from the above foetuses 4 to 24 h after death. Newborn infant's, infant's and child's pancrea were obtained from cadavers aged 2, 4, 28, 45, 90, 120, 210 days and 16 months, whose deaths were due to acute gastric ulcer (2 days) and congenital heart anomalies (4, 28, 45, 90, 120, 210 days and 16 months). They were fixed either in Bouin's solution or formalin at room temperature and embedded in paraffin. Adult pancreata were obtained from patients with gastric or ovarian cancer 6 to 12 h after death. Serial thin sections, 2 to 3.5 µm thick were examined by the PAP method described by Sternberger et al. (1970). Anti-rabbit IgG antisera and soluble peroxidase-anti-peroxidase complex were purchased from DAKO-immunoglobulins.

**Immunohistochemistry**

Deparaffinized sections were treated with 0.34% H₂O₂ in 0.1 M phosphate buffer saline (PBS) pH 7.4 at room temperature for 15 min. They were then incubated with normal porcine serum diluted to 1:10 at room temperature for 45 min to prevent non-specific reactions between unknown components in the antisera and unknown substances in the tissues. After washing out normal porcine serum with PBS, sections were allowed to react with the following primay antisera: 1) 1:3000 diluted insulin antisera pre-treated with 1 µg of peptide P-C, 2) 1:2000 diluted antisera against peptide P-C, 3) 1:1500 diluted glucagon antisera, 4) 1:5000 diluted somatostatin 1-14 antisera, 5) 1:15000 diluted pp antisera. After incubation with these primary antisera at room temperature for 90 min, sections were treated with anti-rabbit IgG diluted to 1:30 for 45 min. They were then incubated with soluble peroxidase-anti-peroxidase complex diluted to 1:30 for 45 min and stained by using diaminobenzidine and H₂O₂. After each procedure, the sections were washed with PBS for 30 min. As control studies, normal rabbit serum diluted to 1:500 and antisera against peptide P-C pre-incubated separately with porcine insulin, human C-peptide, glucagon, somatostatin, pp and isolated peptide P-C (1 µg of each antigen/1 ml of diluted antisera) were used to replace the primary antisera in the immunohistochemical staining process. Porcine insulin, glucagon and porcine pp were purchased from Novo Institutes and somatostatin1-14 was obtained from the Protein Research Foundation, Osaka, Japan.

**Results**

A moderate number of insulin, glucagon, somatostatin and pp immunoreactive cells was already present in the foetal pancreas at 16 weeks of gestation, as seen in Fig. 1a and 1c. In contrast, one or two peptide P-C immunoreactive cells were detected in these pancreata as shown in Fig. 1b. At 18 and 20 weeks gestation, insulin, glucagon, somatostatin and pp immunoreactive cells had increased in numbers in the pancreas. Peptide P-C immunoreactive cells which were distinctly larger in numbers than those in the pancreas at 16 weeks gestation were distributed only in the islets while insulin immunoreactive cells formed islets and were distributed as scattered cells as seen in Fig. 2a, 2b and 2c. At 24 to 26 weeks gestation, peptide P-C immunoreactive cells had increased in numbers in the pancreas compared with those at 18 to 20 weeks gestation. Thus, peptide P-C immunoreactive cells gradually increased in numbers during gestation. The cells were not identical to the glucagon, somatostatin, pp immunoreactive ones, but were to some of the insulin immunoreactive cells. From a comparison of the number of insulin immunoreactive cells with that of the peptide P-C immunoreactive ones, it was shown that one or 2% of insulin immunoreactive cells at 16 weeks, about 20% of those at 18 to 20 weeks and about 50% of those at 24 to 26 weeks gestation contained peptide P-C like immunoreactive cells. In the infant pancreas, about 95% of insulin immunoreactive cells contained peptide P-C like immunoreactivity. In the child's
and adult pancreas, all the insulin immunoreactive cells contained peptide P-C like immunoreactivity.

Detection of the peptide P-C immunoreactive cells was not inhibited in the sections in which antisera against peptide P-C pre-incubated with insulin, human C-peptide, glucagon, somatostatin and pp were used as the primary antisera in the immunohistochemical staining process, but was inhibited in the section in which antisera against peptide P-C pre-treated with peptide P-C were employed to replace the primary antisera.

Discussion

This study has shown that salivary peptide P-C immunoreactive cells first appeared as a few scattered cells in the foetal pancreas at 16 weeks gestation, started to form islets at 18 to 20 weeks gestation and gradually increased in numbers during gestation. Although peptide P-C immunoreactive cells in the foetal pancreas were not identical to glucagon, somatostatin, and pp immunoreactive cells, but to insulin immunoreactive ones, all insulin immunoreactive cells did not have peptide P-C like immunoreactivity in the foetal pancreas. In contrast, a large number of insulin immunoreactive cells in the infant's pancreas and all those in the child's and adult pancreas contained peptide P-C like immunoreactivity. In the previous study (Ito et al. 1983), peptide P-C like immunoreactivity was considered to be present in the human pancreatic B-cells independently of insulin and pro-insulin, since antisera against peptide P-C did not have any cross-reactivity with other peptides including insulin, human C-peptide and glucagon, etc. In addition, the present study showed that the peptide P-C like immunoreactivity in the pancreatic B-cells was not a moiety of precursor of insulin, since peptide P-C like immunoreactivity in the foetal pancreas was not present in all the insulin immunoreactive cells, though peptide P-C like immunoreactivity would be assumed to be present in all insulin immunoreactive cells if it was a moiety of precursor of insulin and pro-insulin. Thus, it was clear that a new substance unrelated to the biosynthesis of insulin was present in the human foetal, infant's, child's and adult pancreas.

Wong & Bennick (1980) recently presented the primary structure of a proline rich phosphoprotein named Salivary Protein C, which was isolated from human parotid saliva by Bennick & Connell (1971). The protein consists of a single polypeptide chain of 150 residues, and its COOH-terminal 44 amino acid residues are identical to the full sequence of the salivary peptide P-C isolated from human whole saliva by Isemura et al. (1979). The finding led us to question whether the peptide P-C like immunoreactivity detected in the human pancreatic B-cells is a salivary peptide P-C itself or a moiety of Salivary Protein C. In view of the present finding that peptide P-C like immunoreactivity does not appear to be a moiety of the precursor of insulin and pro-insulin, it seems unlikely that salivary peptide P-C itself is present in the human pancreatic B-cells, since two different peptides not produced from the same precursor are not thought to be present in the same endocrine cells. Thus, it seems reasonable to think that the peptide P-C like immunoreactivity in the human pancreatic B-cells may belong to a moiety of Salivary Protein C or to another unknown protein which has common antigenic determinants with salivary peptide P-C.

It has been reported that Salivary Protein C appears potentially capable of participating in the calcium exchange process in the oral cavity (Bennick & Cannon 1978). If the peptide P-C like immunoreactivity detected in the human pancreatic B-cells belongs to a moiety of Salivary Protein C, it seems probable that the peptide P-C like immunoreactivity in the human pancreatic B-cells may play some role in the calcium exchange process of pancreatic B-cells. This hypothesis together with the fact that calcium plays an important role in insulin release (Brisson et al. 1972; Valverde et al. 1979) leads to the possibility that the peptide P-C like immunoreactivity in the pancreatic B-cells may play some role in insulin release through the calcium exchange process of the pancreatic B-cells.

It is well known that the mechanism for insulin release in the foetal pancreatic B-cells is immature up to the end of gestation (Pronina & Sapronove 1976) or for a few months after birth (Stimmier et

---

**Fig. 1.**

Insulin (Figs. 1a and 1c) and peptide P-C (Fig. 1b) immunoreactive cells in the foetal pancreas at 16 week of gestation. One or two scattered insulin immunoreactive cells contained peptide P-C like immunoreactivity. (× 480).
Insulin (Figs. 2a and 2c) and peptide P-C (Fig. 2b) immunoreactive cells in the foetal pancreas at 18 week of gestation. About one fifth of insulin immunoreactive cells contained peptide P-C like immunoreactivity. (× 310).
al. 1964; Massi-Benedetti et al. 1980) in spite of sufficient biosynthesis of insulin and an increase in the total pancreatic insulin content throughout foetal life. The immaturity of the insulin release in the foetal pancreatic B-cells may be explained by the finding that only some of the insulin immunoreactive cells contained peptide P-C like immunoreactivity which may play some role in insulin release as calcium-binding protein. However, in the infant’s pancreas in which a large number of insulin immunoreactive cells contained peptide P-C like immunoreactivity, though it was unclear whether the content of peptide P-C like immunoreactivity in each one of the infant’s pancreatic B-cells was or was not the same as that in the adult pancreas, the mechanism for insulin release is known also to be immature compared with that in the adult ones. Thus, the development of another mechanisms for insulin release, in addition to the appearance of peptide P-C like immunoreactivity, seems to be necessary before the foetal and infant’s pancreas can achieve complete maturity regarding its function of insulin release.

Acknowledgment

The authors wish to thank Dr Iwanaga who works in the Department of Anatomy of Niigata University School of Medicine for giving us the paraffin-embedded tissues of foetal pancreas.

References


Received on January 25th, 1983.