Upsala J Med Sci 87: 81–85, 1982

Immunocytochemical Localization of Gastric Inhibitory Peptide (GIP) in the Human Foetal Pancreas

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ABSTRACT

The occurrence of gastrin and gastric inhibitory polypeptide (GIP) was investigated immunocytochemically in 17 foetal and neonatal human pancreata of gestational ages ranging from 12-41 weeks. GIP immunoreactive cells were observed in the pancreas of five foetuses with gestational ages of 18-20 weeks. These cells were located in islet-like cell clusters, at the base of tubular structures and among the exocrine-like acini. They were sometimes seen to emit a single long protrusion. The controls used, including preincubation of the antisera with anti-complement C1q, emphasized the specificity of the observed immunoreaction. No gastrin-immunoreactive cells were seen in any of the foetal or neonatal pancreata examined.

INTRODUCTION

In a previous report (3) it was postulated that in the absence of food ingestion - as in the case of hibernating animals and in foetal life - the gastrointestinal endocrine cells will lack the stimulus for releasing their secretory products, with the result of partial or complete blocking of their hormone release. Under such conditions it is hypothesized that the pancreas as a target tissue for gastrin and gastric inhibitory polypeptide (GIP) will replace the deficiency caused by this lack of hormone release by developing local gastrin and GIP cells, which will probably act through a paracrine mechanism. In order to test this hypothesis in the present study, the human foetal pancreas was investigated immunocytochemically for the occurrence of gastrin and GIP.

MATERIAL AND METHODS

Pieces from 17 foetal and neonatal human pancreata, gestational ages ranging from 12-41 weeks, were fixed in neutral buffered formalin for 24 h. The tissues were obtained from women undergoing legal abortion and at autopsy of newborn infants, who had died of non-endocrine disorders.

After dehydration and embedding in paraffin, 5 μm thick sections were cut and processed for demonstration of gastrin and GIP by the peroxidase-antiperoxidase

Table 1. Details of the absorption tests performed.

The antiserum	The antiserum dilution	The antigen and its amount per ml of diluted antiserum	Results†)
Anti-GIP (No. 378)	1:1600	50-125 µg of porcine GIP (purchased from J.C. Brown, Univ. Brit. Columbia, Canada)	+
Anti-GIP (No. 378)	1:1600	100-500 µg of porcine glucagon (Novo Industri A/S, Copenhagen, Reg. No. 8075)	-
Anti-GIP (No. 378)	1:1600	100-300 µg of secretin (Kabi Diagnostica, Studsvik, Sweden, Lot No. 162904)	-
Anti-GIP (No. 378)	1:1600	100 µg of porcine VIP (gift from Prof. V. Mutt, Karolinska Institute, Sweden)	-
Anti-GIP (No. 378)	1:1600	5-15 µg of glicentin (Novo Industri A/S, Copenhagen, Denmark)	-
Anti-GIP (No. 255)	1:400	50-125 µg of porcine GIP (purchased from J.C. Brown)	+
Anti-GIP (No. 255)	1:400	100-500 µg of porcine glucagon (Novo Industri A/S, Reg. No. 8075)	-
Anti-GIP (No. 255)	1:400	100-300 µg of secretin (Kabi, Lot No. 162904)	-
Anti-GIP (No. 255)	1:400	100 µg of porcine VIP (gift from Prof. V. Mutt, Karolinska Institute, Sweden)	-

†) + = complete inactivation of the antiserum; - = unaffected activity of the antiserum

(PAP) method (5). The antisera used were: rabbit antiserum against synthetic human gastrin-17 No. 4562, rabbit antiserum against synthetic mid-portion (6-13) of non-sulphated human gastrin-17 No. 4710 (both gifts from J.F. Rehfeld, Dept. of Medical Chemistry, Aarhus University, Denmark), rabbit antiserum against the mid-portion (9-20) of CCK No. 280, and rabbit antisera against porcine GIP Nos. 378 and 255 (gifts from J.M. Polak, Dept. of Histochemistry, Hammersmith Hospital,London, England). The antisera were tested at serial dilutions ranging from 1:50 to 1:15,000.

The controls used were: 1) Non-immunized rabbit or guinea pig serum as the first layer; 2) 100 μg rabbit γ-globulin or 50 μg rabbit IgG per ml tris-saline buffer as the first layer; 3) incubation of the antisera with rabbit anti-human Clq complement diluted 1:50 (Dako, Denmark, Lot No. 038B) and 4) preincubation of the first layer antiserum with the corresponding peptide or related peptide(s) for 24 hours at 4°C (see Table 1). In each series of staining experiments sections of adult human pancreas were included.

RESULTS AND DISCUSSION

In the pancreata of five foetuses with gestational ages of 18-20 weeks, GIP-immunoreactive cells were observed to intermingle with the islet-like cell clusters, in the basal part of tubular structures and among the exocrine-like acini (Fig. 1). They were round or polygonal and sometimes exhibited a single long

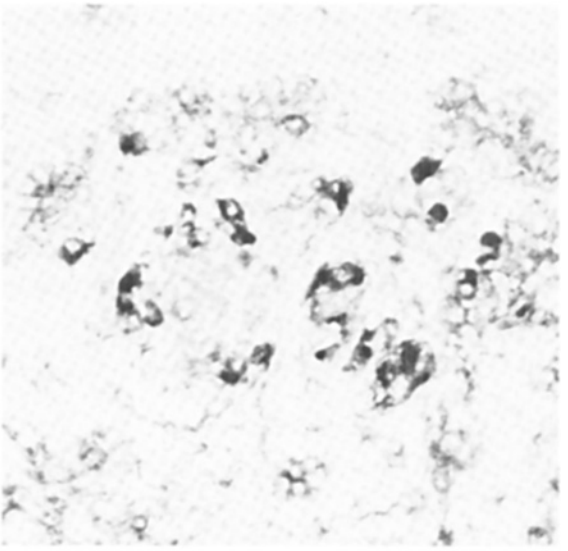

Fig. 1. GIP-immunoreactive cells in the human foetal pancreas. They are located at the islet-like cell clusters, at the basal part of tubular structures or in between the zymogen cells, and display a cytoplasmic protrusion. PAP technique. anti-GIP No. 378 (dilution 1:1,600). x 570.

protrusion. On the other hand, no gastrin or CCK immunoreactivity was detected in the pancreas in any of the foetuses examined. GIP immunoreactivity was found with both anti-GIP sera used in all the dilutions tested up to 1:1,600 of anti-GIP No. 378 and up to 1:400 of anti-GIP No. 255.

No staining was obtained when the antisera were replaced by rabbit serum, guinea-pig serum, rabbit γ-globulin, or rabbit IgG. Furthermore, incubation of the antisera with rabbit anti-human Clq complement had no effect on the positive immunostaining results (2). These findings together with the neutralization tests (Table 1), emphasize the specificity of the results obtained. It is noteworthy that the anti-GIP sera tested here did not react with any cellular component in the adult human pancreas.

The finding of GIP-immunoreactive cells in the human foetal pancreas is in line with earlier observations in the foetal porcine pancreas (1). These cells were, however, observed over only a limited gestational age, though the physiological state - the absence of food ingestion, which has been assumed to be the cause of their location in the pancreas - extends throughout the whole fetal life. This finding, together with the absence of gastrin-immunoreactive cells in all the foetal pancreata examined in the present study and previously (4) would seem to contradict our working assumption. However, in foetal life other physiological factors than the absence of the food ingestion - a condition shared with hibernating animals - must be taken into consideration, for example the continuous process of growing.

Whether the detected GIP-like immunoreactive substance is present in independent cell type or within other endocrine cell types, and the ultrastructural identification of these cells are problems which are now under study.

ACKNOWLEDGEMENTS

The authors wish to thank G. Hultquist for supplying us with some foetal material, and J.M. Polak and J.F. Rehfeld for providing us with the antisera.

This investigation was supported by a grant from the Swedish Medical Research Council (project No. 12X-102) and by a fellowship to M. El-Salhy from the Swedish Medical Research Council.

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