Immunohistochemical Demonstration of Somatostatin in the Pancreas of Fetal and Adult Sheep

S. Reddy,^A J. R. Oliver^B and R. B. Elliott^A

^A Department of Paediatrics, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand. (Address for reprints.)
^B Department of Medicine, Flinders Medical Centre, Flinders University of South Australia, Bedford Park, S.A. 5042.

Abstract
Pancreatic cells immunoreactive with rabbit anti-somatostatin serum were demonstrated in fetal and adult sheep by immunofluorescence histochemistry. Our observations provide evidence that in the sheep the pancreas is a major site of production of the hormone. It is suggested that the hormone may be important in modulating intra- and extra-islet function.

Introduction
Somatostatin, a tetradecapeptide, was initially isolated from sheep hypothalami (Brazeau et al. 1973) and was shown to be a potent inhibitor of growth hormone release. A peptide with identical structure to ovine somatostatin and with the same biological property was subsequently isolated from porcine hypothalami (Schally et al. 1976). Somatostatin is now recognized to be present in several mammalian species (for a review, see Luft et al. 1978; Schally et al. 1978).

Radioimmunoassay and immunocytochemical studies have revealed the presence of this peptide in specific cells outside the brain such as the D cells of the pancreas and duodenum (Hokfelt et al. 1975; Patel and Weir 1976). In the pancreas somatostatin is believed to inhibit the release of insulin and glucagon in a 'paracrine' manner (Mortimer et al. 1974; Unger and Orci 1977).

Ontogenic studies of pancreatic somatostatin in the human, rat and chicken and in the neonatal pancreas of the human have already been reported (Alumets et al. 1977; Rahier et al. 1980) but similar investigations in ruminant species have been lacking. As part of our study on the ontogeny of insulin, glucagon, somatostatin and pancreatic polypeptide in sheep, results are presented on the cellular localization of somatostatin in the pancreas of fetal and adult sheep by immunofluorescence histochemistry.

Materials and Methods

Pieces of pancreas from four adult and four fetal sheep (gestation: approximately 120–140 days) were removed immediately after decapitation of the animals and fixed in Bouin's fluid (acetic acid free) at room temperature for 24 h. Tissues were dehydrated through increasing concentrations of ethanol, cleared in xylol and embedded in paraffin at 56°C. Sections (5 μm) were cut, attached to glass slides and prepared for histological examination.

The somatostatin antigen was obtained from Bachem (California, U.S.A.). Rabbit anti-somatostatin serum was prepared by one of us (Dr J. R. Oliver) and its specificity has been reported previously (Buckerfield et al. 1981). The indirect immunofluorescence technique of Nairn (1976) was employed. Tissue sections were incubated with anti-somatostatin serum at a dilution of 1:40 (v/v) at 37°C for 1 h with phosphate-buffered saline (PBS) as the diluent. After washing the sections in excess PBS


0004-9417/84/010025$02.00
they were reacted with sheep anti-rabbit gammaglobulin labelled with fluorescein isothiocyanate (Wellcome Laboratories, U.K.) at a dilution of 1:10 (v/v) for 30 min at 37°C. After washing the sections in PBS they were mounted in glycerol–saline and their immunofluorescence observed with a Nikon fluorescence microscope. The specificity of the immunoreaction was confirmed by replacing the antiserum with either normal rabbit serum, PBS or anti-somatostatin serum after absorption with somatostatin (100 μg somatostatin per millilitre of undiluted anti-somatostatin serum). After observing the sections for immunofluorescence they were stained with haematoxylin and eosin.

Results

Immunofluorescent staining was observed in cells of pancreatic sections which were reacted with anti-somatostatin serum as the primary antiserum (Figs 1a, 2a). In the adult pancreas (Figs 2a, 2b) somatostatin immunoreactive cells were located within the islets, predominantly arranged in a ribbon-like manner, but occasionally occurring as clusters or just single cells. In the fetal pancreas, these cells were also mostly ribbon-like (Figs 1a, 1b). The number of immunoreactive cells in the adult and fetal specimens were similar.

Discussion

Using antibodies to somatostatin we have localized the hormone in cells of the pancreatic islets of adult and fetal sheep. The anti-somatostatin serum used in the present study showed less than 0·01% cross-reactivity by radioimmunoassay when tested against several peptides including β-endorphin, leu-enkephalin, insulin and glucagon (Buckerfield et al. 1981). Further confirmation of the specificity of the immunoreaction was obtained by parallel application of the antiserum to pancreatic sections after neutralization with somatostatin which resulted in complete abolition of the immunostaining.

Although somatostatin is known to be present in high concentrations in sheep hypothalamus and in the peripheral circulation, this study to our knowledge is the first report of the presence of the hormone in cells of fetal and adult islets of this species. The distribution of somatostatin immunoreactive cells in the pancreas of the various species examined is different; for example in the rat, the cells are located in the periphery of the islets, whereas in the human they appear interspersed among the A and B cells (Alumets et al. 1977; Watkins et al. 1980). In the present study we have demonstrated somatostatin immunoreactive cells appearing in a predominantly ribbon-like formation within the islets. Further studies would be necessary to determine the cell-type responsible for the synthesis of the peptide in the pancreas.

Our observation of somatostatin cells in the pancreas of fetal sheep at 120–140 days gestation requires further extension to determine its ontogeny. The maturation of somatostatin cells in the pancreas of various species examined occurs at different stages of gestation. In the rat and human (Alumets et al. 1977), somatostatin cells appear at 16–17 days and 15 weeks of pregnancy respectively. These observations are consistent with the known differential maturation of the neuroendocrine axes in these species. The presence of the hormone in the fetal pancreas of late pregnancy suggests a functional role for somatostatin, perhaps in modulating insulin and glucagon release from the islet cells, and it is likely that the hormone is present earlier in fetal life. Whether the maturation of somatostatin, insulin, glucagon, and pancreatic polypeptide cells in the sheep is simultaneous or occurs at different stages of fetal pancreatic development is currently under study.

Acknowledgments

This study was financed by the National Children’s Health Research Foundation of New Zealand. We thank the Auckland City Council Abattoirs for supplying the experimental tissue, and Ms K. Hyde for histological assistance.
Fig. 1. Immunofluorescence localization of somatostatin in pancreatic cells of fetal sheep. (a) Section stained by the immunofluorescence procedure showing somatostatin cells (arrow). (b) Same section subsequently stained by haematoxylin and eosin showing somatostatin cells (arrow).
Fig. 2. Immunofluorescence localization of somatostatin in pancreatic cells of adult sheep. (a) Section stained by the immunofluorescence procedure showing somatostatin cells in an islet (arrow). (b) Same section subsequently stained by haematoxylin and eosin showing somatostatin cells in same islet (arrow).
References


Manuscript received 4 November 1983, accepted 20 January 1984