AMYLASE AND INSULIN RELEASE FROM OVINE PANCREATIC SEGMENTS EVOKED BY ELECTRICAL FIELD STIMULATION

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Introduction

The functions of the exocrine and endocrine pancreas are controlled by the autonomic nervous systems as well as by gastrointestinal hormones and some nutrients. *In situ* preparations have been used to investigate the neural control. Recently, however, the electrical field stimulation (EFS) technique has been developed and adapted to the study of exocrine secretory mechanisms *in vitro*.

The aim of the present experiment was to extend the EFS technique to the endocrine pancreas and to investigate the role of autonomic nerves in the secretory functions, especially the release of amylase and insulin from ovine pancreas *in vitro*.

Materials and Methods

Pancreatic tissues isolated from sheep were dissected into small segments with scissors in an oxygenated Krebs-Henseleit solution kept at 37°C. The EFS and superfusing techniques were generally similar to those previously reported (Katoh and Tsuda, 1984; Katoh et al., 1986). The segments (150 mg) were transferred into a small column-shaped tissue chamber (6 mm, I.D. and 13 mm in length), and superfused with an oxygenated and warmed (37°C) solution using a peristaltic pump at a flow rate of 1 ml/min. A pair of platinum-electrodes at the bottom and the top of the chamber was connected to the output of a square pulse generator. The intrinsic nerves in the tissue segments were stimulated through the electrodes. The stimulation was repeated every 10 min and sustained for 6 min. The effluent samples from the chamber were collected every 2 min with a fraction collector. The amylase and insulin concentrations in the effluent samples were assayed by the blue-starch method and by radioimmunoassay, respectively.

Results

The optimal stimulus condition of EFS for amylase and insulin releases was 75 V in strength, 10-20 Hz in frequency and 1 msec in duration. The maximal release of amylase and insulin evoked by EFS was four- and two-fold larger than the basal levels in the control solution, respectively. A neurotoxin tetrodotoxin (TTX, 10^{-7} g/ml) completely suppressed, whereas a ganglion blocke hexamethonium (C6, 3×10^{-4} M) scarcely affected,

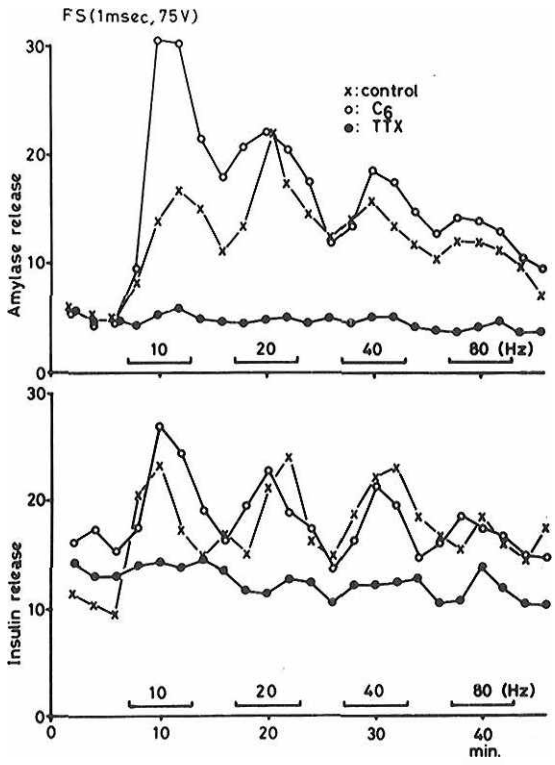

Figure 1. Effects of hexamethonium (C6) or tetrodotoxin (TTX) on amylase (u/g·min) and insulin (μu/g·min) release evoked by EFS. EFS (1 msec in duration and 75 V in strength) was repeated at 10, 20, 40 and 80 Hz in frequency for 6 min.

amylase and insulin release evoked by EFS.

The amylase release evoked by EFS at any frequency was completely abolished in a solution containing the muscarinic antagonist atropine (1.4×10^{-6} M), while it was not changed in a solution containing the alpha- and beta-adrenergic antagonists phentolamine (10^{-5} M) and propranolol (5×10^{-6} M).

The presence of phentolamine or phentolamine and propranolol in the solution enhanced, whereas atropine scarcely affected, the basal and EFS-evoked insulin release. The EFS-evoked insulin release could not be abolished even in a solution containing both muscarinic and adrenergic antagonists.

Discussion

The EFS-evoked responses were caused by the excitation of the post-ganglionic autonomic nerves in pancreatic tissue, since TTX completely abolished, but C6 scarcely affected, the responses.

The amylase release evoked by EFS was caused by the activation of the muscarinic receptors on the pancreatic acinar cells, since it was completely abolished by the presence of atropine.

The finding that phentolamine enhanced insulin release agrees with previous studies (Sasaki and Takahashi, 1980; Oda *et al.*, 1988) in that the stimulation of alpha-adrenergic receptors inhibits insulin release in sheep. Furthermore, the finding that insulin release evoked by EFS was detected even in solution containing muscarinic and adrenergic antagonists arises the possibility that non-cholinergic non-adrenergic transmitters are involved in insulin release.

From the present experiment employing the EFS technique in ovine pancreatic segments, it is concluded that 1) amylase and insulin release evoked by EFS are caused by the excitation of post-ganglionic autonomic nerves, 2) amylase release evoked by EFS is stimulated mainly by the activation of muscarinic receptors on the pancreatic acinar cells, 3) stimulation of the adrenergic receptors inhibits insulin release, and 4) non-cholinergic non-adrenergic transmitters might be involved in insulin release evoked by EFS.

(**Key Words**: Amylase Release, Insulin Release, Electrical Field Stimulation)

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