EFFECTS OF GLUCOSE AND ARGININE ON THE RELEASE OF IMMUNOREACTIVE SOMATOSTATIN FROM THE ISOLATED PERFUSED RAT PANCREAS

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1. Introduction

Somatostatin administered exogenously inhibits the release of insulin and glucagon [1–3]. This finding as well as the presence of the somatostatin in the D-cells of the islets of Langerhans [4–6] indicate that the peptide may play an important role in the regulation of functions of the endocrine pancreas. This question was further investigated by comparing the kinetics of insulin, glucagon and somatostatin release stimulated by glucose and arginine.

2. Materials and methods

Sprague-Dawley rats, weighing 200–250 g and fed ad libitum were used for the preparation of the perfused isolated pancreas. The animals were anesthetized by intraperitoneal injection of 50 mg/kg of pentobarbital, and the pancreas was isolated by a slight modification of the technique of Loubatières et al. [7]. The perfusate, Krebs-Ringer bicarbonate solution to which was added 0.8 g/l of glucose and 20 g/l of beef albumine, was administered into the coeliac artery, and run into the prepared pancreas by an open circuit 'non-recycling perfusion system'. The flow rate of the perfusate was approximately 2.5 ml/min.

Somatostatin antibodies were generated in white rabbits by subcutaneous injection of 1 mg cyclic somatostatin coupled to hemocyanin (KLH, Calbiochem) and emulsified with Freund's adjuvant. The coupling was accomplished with carbodiimide. This complex was then dialysed before the use. The antisera obtained after the third booster (rabbit 141 c) were used for the radioimmunoassay. Tyr^1-somatostatin was labelled with ^{125}I by the lactoperoxidase method [8], and separated on a carboxymethylcellulose column as described by Arimura et al. [9]. Phosphate buffer (0.04 M, pH = 7.4) containing 1% bovine serum albumin was used as the diluent for all components in the radioimmunoassay. Each reaction tube contained 0.4 ml of buffer, 0.1 ml of antiserum, 0.1 ml of standard, unknown or blank, and 0.1 ml of ^{125}I-Tyr^1-somatostatin (about 5000 counts per min). The final dilution of antibodies was 1:56 000. The incubations were conducted for 48 h at 4°C. Separation of bound from free tracer was accomplished with dextran coated charcoal as described by Arimura et al. [9]. Somatostatin concentrations ranging between 1.25–20 pg per tube could be easily determined by this method. Cross-reactivity of the antibodies was less than 0.01% with insulin, glucagon, substance P, LH–RH, vasopressin and oxytocin.

Insulin was determined by a double-antibody radioimmunoassay using insulin reagent Kits (Radiochemical Centre, Amersham), and a rat insulin standard. Glucagon was assayed by the charcoal separation technique [10], using an antibody specific for pancreatic glucagon (30K, kindly provided by Dr Roger Unger, Dallas, TX).

3. Results

Increase of glucose conc in the perfusate from 3.3 to 28 mM, as expected, induced marked and biphasic insulin release (fig.1). The somatostatin levels

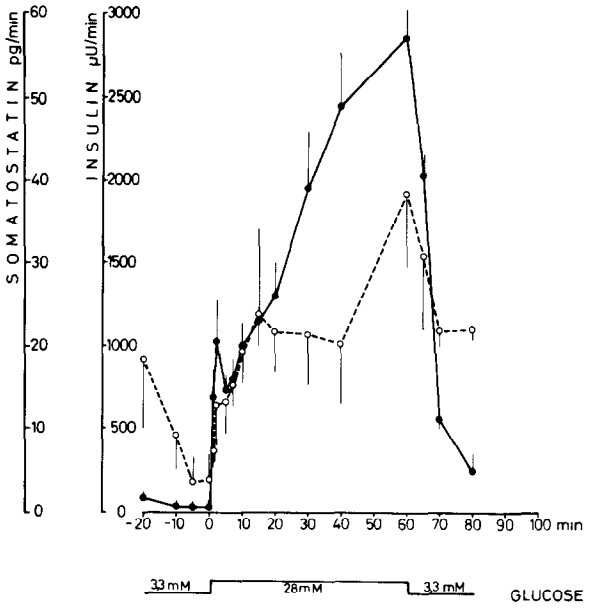

Fig.1. Effect of glucose on insulin (●——●) and somatostatin (○– – –○) release from the isolated perfused rat pancreas. Results are expressed as the mean ± SEM of 6 experiments.

decreased during the 20-min equilibration period, and increased considerably and continuously during the perfusion with 28 mM of glucose. Return to low glucose conc was accompanied by prompt decrease in the release of both hormones.

Arginine also induced significant and biphasic release of insulin and, in addition, of glucagon and somatostatin (fig.2).

4. Discussion

The present findings clearly demonstrate that the insulinogogues, glucose and arginine, also stimulate the release of somatostatin from the isolated perfused rat pancreas. These results are in accordance with findings on the perfused dog pancreas [11], on isolated perfused rat islets [12] and on rat islets incubated in vitro [13,14]. The instantaneous and parallel fall of somatostatin and insulin after withdrawal of the high glucose conc is in accordance with observations on the perfused dog pancreas [11], but does not agree with findings on perifused rat islets [12]. The latter

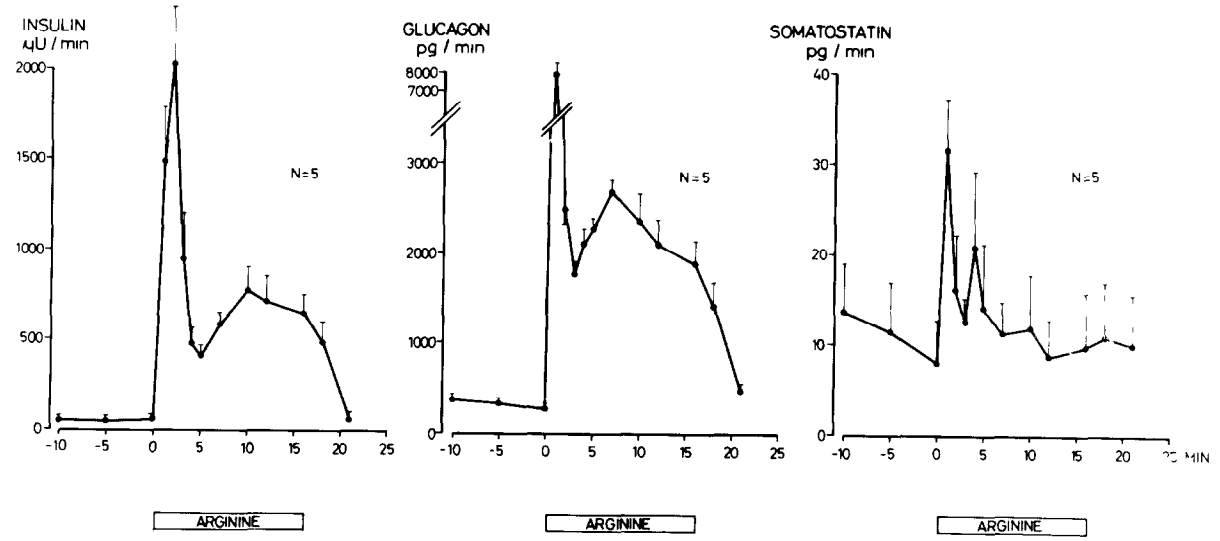

Fig.2. Effect of arginine (8 mM) on insulin, glucagon and somatostatin release from perfused rat pancreas. Following isolation pancreas was equilibrated for 10 min with 3.3 mM glucose in the perfusate. The arginine stimulus was applied between 0 and 17 min. Results are expressed as the mean ± SEM of 5 experiments.

authors described transient increase in somatostatin release when the glucose conc in the perfusion medium was lowered from 25 to 2 mM, implying that glucose, in spite of stimulating somatostatin release, also generates mechanisms responsible for inhibition of its release. Our data do not support such a hypothesis.

The response of the D-cells of the pancreas to glucose and arginine, resembling that of the B-cells to the same releasers and that of the A-cells to arginine, gives some insight into the mechanisms controlling insulin and glucagon release. The fact that the same stimulators enhance the release of insulin and glucagon, on the one hand, and of their inhibitor (somatostatin) on the other, suggests that the responsiveness of the D-cells may play an important role for the release of insulin and glucagon. A paracrine action may be the way by which somatostatin exerts this effect. We have previously presented data supporting such an action of somatostatin – released from the cat antrum – on gastrin release [15].

Acknowledgements

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