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Research Article

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Rapid Publication

Insulin Within Islets Is a Physiologic Glucagon Release Inhibitor

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Abstract. To determine if glucagon secretion is under physiological control of intra-islet insulin, pancreata from normal rats were perfused at a 100 mg/dl glucose concentration with either guinea pig antiinsulin serum or normal guinea pig serum in a nonrecirculating system. Perfusion of antiserum was followed within 3 min by a significant rise in glucagon that reached peak levels three times the base-line values and assumed a hectic pattern that returned rapidly to base-line levels upon termination of the antiserum perfusion. Nonimmune guinea pig serum had no effect.

To gain insight into the probable site of insulin neutralization, ^{125}I-labeled human γ-globulin was added to antiserum or nonimmune serum and perfused for 3 min. More than 83% of the radioactivity was recovered in the effluent within 3 min after termination of the infusion, and only 0.05±0.015% of the radioactivity injected was present in the pancreas 10 min after the perfusion. The maximal amount of insulin that could be completely bound to insulin antibody at a dilution and under conditions simulating those of the perfusion experiments was 20 mU/min.

It is concluded that insulin maintains an ongoing restraint upon alpha cell secretion and in its absence causes hectic hypersecretion of glucagon. This restraint probably occurs largely in the intravascular compartment. Loss of this release-inhibiting action of insulin may account for initiation of hyperglucagonemia in insulin-deficient states.

Introduction

The concept that insulin is a physiologic suppressor of glucagon secretion was first proposed by Samols et al. (1) in 1971, but unequivocal experimental support for this hypothesis has been difficult to come by. In man, low concentrations of intravenously infused insulin suppress glucagon, provided that glucose is co-infused to prevent hypoglycemia (2) and infused insulin paradoxically increases the glucagon response to arginine, presumably by inhibiting endogenous insulin secretion (3). However, it cannot be assumed from such in vivo results that this necessarily reflects a direct action of insulin on alpha cells. When insulin is perfused directly into the pancreatic artery of dogs (1) or isolated chicken pancreas in vitro (4), or added to isolated islets (5), extremely high concentrations are required to suppress glucagon release, and it is not certain that such effects reflect a physiologic function of insulin.

In the present studies we provide evidence that intrapancreatic insulin exerts an ongoing release-inhibiting action on alpha cells, and that this action occurs within the islets via an intravascular or endocrine route rather than an interstitial or paracrine pathway.

Methods

The pancreata of 300–400-g male Long-Evans rats were isolated and perfused by the method of Grodsky and Fanska (6). After a 24–30-h fast, the rats were anesthetized with 50 mg/kg of sodium pentobarbital injected intraperitoneally. 15 min later the pancreas and an adjacent 5-cm portion of the duodenum were isolated and within 30–40 min were transferred to a thermostatically controlled plexiglas perfusion chamber in which the celiac trunk and the portal vein were cannulated. With the temperature of the perfusate and of the external surface of

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the pancreas maintained between 37.0° and 37.5°C, nonrecirculating perfusion was begun at a constant flow rate of 3.6 ml/min. The perfusate was Krebs-Ringer bicarbonate buffer supplemented with 4.5% (wt/vol) Dextran T-70, 1% (wt/vol) bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 10 mmol arginine, 5 mmol sodium pyruvate, sodium fumarate, and sodium glutamate. Oxygenation was maintained by a bubble oxygenator using a gas mixture of 95% O_2 and 5% CO_2. The perfusate pH varied between 7.35 and 7.45, the PO_2 varied between 450 and 550 mm of Hg, and the pressure varied between 470 and 570 mm of water.

In one group of experiments, perfusate glucose concentration was maintained at 100 mg/dl throughout the 50-min experiment. 11 min after the start of the perfusion, 0.1 ml/min of guinea pig antiporcine insulin serum (lot GP29, Miles-Yeda Ltd., Israel) or of normal guinea pig serum (lot NGP28, Miles-Yeda Ltd.) was added via a side-arm. This was terminated at 40 min. (In other studies not shown, similar results have been obtained with GP26 and GP31 [Miles-Yeda Ltd.], and NGP28 and NGP27 [Miles-Yeda Ltd.].) The antiserum contained 139 pg/ml immunoreactive glucagon (IRG)[1], and the normal guinea pig serum contained 102 pg/ml IRG and 3 μU/ml immunoreactive insulin. IRG was assayed by a previously described method (7) using 30K antiserum. Insulin was measured by the Herbert modification (8) of the method of Yalow and Berson (9).

In another type of experiment designed to determine the intrapancreatic distribution of the perfused insulin antibodies, 20,000 cpm of ^{125}I-human gamma globulin (specific activity 2 mCi/mg) was mixed with the antiinsulin serum or with the normal guinea pig serum (0.1 ml/min) and was perfused for 3 or 10 min. This was followed by a 10-min perfusion with buffer free of serum and ^{125}I-human gamma globulin. Throughout these experiments the glucose concentration in the perfusate was 100 mg/dl. The IRG was determined in the effluent samples, and the radioactivity remaining in the pancreas at the end of the experiment was measured by counting the entire pancreas.

In a final group of experiments designed to estimate the maximal amount of insulin that can be completely bound to insulin antibody under the conditions of the perfusion, 54 pg of ^{125}I-insulin was mixed with unlabeled insulin in amounts from 10 to 200 mU in 3.6 ml of buffer, which is the volume perfused in 1 min. 0.1 ml of either guinea pig antiinsulin serum or nonimmune serum was added to this and incubated for 1 min at 37°C, pH 7.4, to simulate all of the conditions of the antiserum perfusion. At the end of the incubation, 0.5 ml of charcoal-Dextran was added and separation of free from bound ^{125}I-insulin was carried out in the usual fashion (8).

Statistical calculations were performed using the *t* test for two groups.

Results

Effect of antiinsulin serum on steady-state glucagon secretion. Glucagon levels rose significantly from a base-line average of 865±106 pg/ml to 2,365±520 pg/ml within 3 min of the start of perfusion of antiinsulin guinea pig antiserum, and continued in hectic fashion to a peak of 2,906±535 pg/ml at 26 min (Fig. 1). In all experiments, termination of the antiserum infusion was followed 1 min later by a paradoxical single-point rise in glucagon and then a return to base-line levels.

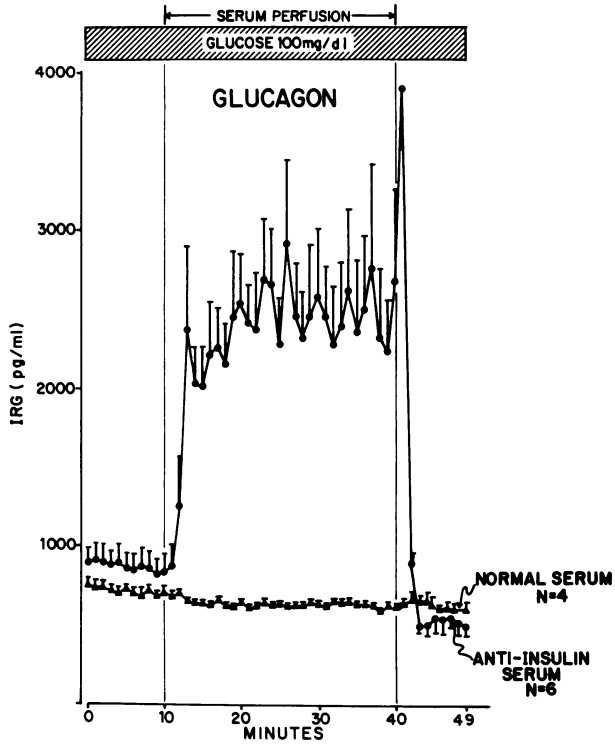

Figure 1. The effect of antiinsulin serum (closed circles) on glucagon secretion (mean±SEM) in the isolated perfused rat pancreas. Normal guinea pig serum (closed triangles) was used as a control.

Nonimmune guinea pig serum had no effect on glucagon levels (Fig. 1).

Appearance time and recovery of ^{125}I-labeled human IgG relative to antiserum effects. The foregoing experiments do not indicate if the insulin antibody was interacting with the hormone within the vasculature of the islets or within their interstitium. We therefore added ^{125}I-labeled human IgG to the antiserum and perfused the mixture into the pancreas for 3 min in six experiments. The appearance of radioactivity in the effluent resembled the configuration of the glucagon response to the antiserum, which began within 2 min of the start of the infusion and was rising within 3 min of the start. More than 83% of the radioactivity was recovered in the effluent within 3 min of termination of the 3-min perfusion period, at which point the effect of the antiserum upon glucagon was rapidly receding. At 10 min after termination, 0.05±0.015% was detected in the pancreas.

We also perfused the same mixture for 10 min in three experiments. 90% of the radioactivity injected was recovered within 3 min of termination of the infusion, and residual radioactivity in the pancreas 10 min after termination averaged 0.21% of the injected dose.

The maximal amount of insulin completely bound by the antibody-containing perfusate. The maximal amount of insulin

1. *Abbreviation used in this paper:* IRG, immunoreactive glucagon.

completely bound by the antiinsulin serum at the 1:37 final dilution at which it was perfused through the rat pancreas was 20 mU/min (Fig. 2).

Discussion

The results provide strong support for the concept first promulgated by Samols et al. (1) that insulin plays an ongoing physiologic role in the regulation of glucagon secretion. Previous direct evidence was limited to studies in which insulin was perfused directly into the pancreaticoduodenal artery of an intact dog at a rate of 0.8 mU/kg per min (1), infused at a 20 mU/ml concentration through the pancreas of the dog, perfused into isolated chicken pancreata (4), or incubated with rat islets (5). Others have failed to show an effect of perfused insulin upon arginine-stimulated glucagon secretion (10). In the present studies, the nonrecirculating perfusion of an antiinsulin serum at a concentration and under conditions that were estimated to bind up to 20 mU of insulin per minute elicited a striking increase in glucagon levels together with a chaotic pattern of fluctuation. The present studies indicate that insulin acts within the islet as a release-inhibiting hormone for glucagon. Loss of this insulin action probably accounts for the hyperglucagonemia that characterizes insulin deficiency states (11, 12).

Although the extraordinarily high antibody concentration prevented valid measurement of free insulin in the effluent, it seems reasonable to assume that most, if not all, of the within-islet insulin that normally reaches insulin receptors on alpha cells was neutralized within 3 min of the start of the infusion. This assumption of complete neutralization is based on the fact that a less than complete reduction of insulin levels does not seem to increase glucagon levels in vivo or in vitro (13; and Helderman, J. H., H. Maruyama, G. Bolli, and R. H. Unger, manuscript submitted for publication). If this assumption is correct, the maximum rate of insulin secretion by the rat pancreas during antiinsulin serum perfusion must be <20 mU/min, which is the maximal insulin-binding capacity of the antibody perfusion mixture under the conditions of these experiments. This figure is remarkably close to the insulin doses that suppressed glucagon in the previously cited experiments (1, 4, 5).

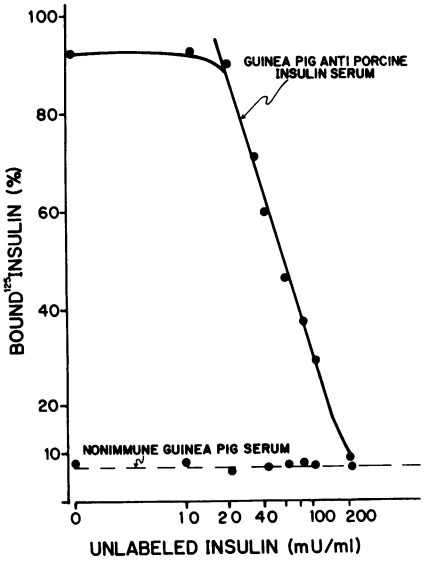

Figure 2. The 1-min insulin-binding capacity of the perfused antiinsulin antibody at 1:37 dilution at 37°C to simulate the dilution, temperature, and contact time of the antibody perfusion experiments. The broken line represents the nonimmune guinea pig serum.

The anatomical pathway by which insulin restrains glucagon secretion has not been clearly defined. Contiguity of alpha, beta, and delta cells has made it attractive to propose that paracrine interactions take place via the interstitium that separates these islet cells (14), although recent studies provide indirect evidence against a paracrine routing for somatostatin-mediated effects on the secretion of insulin and glucagon (15). The present data also tend to argue against a paracrine routing for the restraining action of insulin upon glucagon secretion, at least for the glucagon-restraining action of the insulin that is neutralizable by perfusion of antiserum. Barring an extremely rapid exchange of γ-globulin in the interstitium, the recovery pattern of radioactivity during perfusion of ^{125}I-labeled gamma globulin mixed with the antiinsulin serum, the postperfusion intrapancreatic radioactivity of <0.1%, and sudden onset and offset of the glucagon response to the antiinsulin serum suggest that most if not all of the insulin that restrains alpha cell function reaches its receptors via the circulation. Arteriolar blood of the rat islet flows first to the centrally located beta cells and then to the peripherally located alpha and delta cells (16), which are thus exposed to the highest insulin concentration that exists in the circulation.

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