The Order of Islet Microvascular Cellular Perfusion Is B → A → D in the Perfused Rat Pancreas

Ellis Samols, John I. Stagner, Robin B. L. Ewart, and Vincent Marks*
*Veterans Administration Medical Center and the Department of Medicine, University of Louisville, Louisville, Kentucky 40202; and the *University of Surrey, Surrey, GU1 4UG United Kingdom*

Abstract

In order to determine whether microvascular blood flow is important in the regulation of intra-islet cellular interactions, rat pancreata were isolated and perfused in vitro, both anterogradely or retrogradely, with and without anti-insulin or anti-somatostatin γ-globulin. Expressed as percent change, anterograde infusion of insulin antibody increased efflux concentrations of glucagon (110±20%, $P < 0.0005$) and somatostatin (2,112±73%, $P < 0.0005$) above their respective control. Retrograde infusion of insulin antibody did not affect efflux concentrations of glucagon ($P < 0.50$) or somatostatin ($P < 0.50$). The anterograde infusion of anti-somatostatin antibody had no effect upon insulin ($P < 0.50$) or glucagon ($P < 0.50$) efflux concentrations, whereas retrograde anti-somatostatin antibody infusion produced immediate increases in efflux concentrations of both insulin (115±33%, $P < 0.0005$) and glucagon (77±8%, $P < 0.0005$).

These results strongly suggest that (*a*) the vascular compartment is important in the regulation of intra-islet cellular interactions and further suggest that (*b*) the order of islet cellular perfusion and interaction is from the B cell core outward to the mantle, and (*c*) the mantle is further subordered with the majority of D cells downstream or distal to the majority of A cells. Thus, in the vascular compartment, B cells inhibit A-cell secretion and A cells stimulate D-cell secretion.

Introduction

The recognition that the islet is not a random or homogeneous mixture of cell types, but is an ordered structure containing distinct regions of cell types, has suggested that adjacent cells within the islet may influence one another (1–4). The discovery that both insulin and glucagon affected the secretion of the other in a negative-positive relationship (4) bolstered the concept that the net secretion of insular hormones may be the product of specific interactions (5, 6) within the islet. However, neither the sequence of cellular interaction nor the mechanism of intra-islet hormone delivery is clear. The latter may be by direct sequential vascular perfusion, paracrine interactions in the interstitial spaces, or a combination of both.

Address reprint requests to Dr. Samols, Research Service, VA Medical Center, 800 Zorn Avenue, Louisville, KY 40202.

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Recent histologic evidence, from serial reconstruction of the rat islet after ink injections, has suggested that the path of blood flow may be from the B cell core outward to the A and D cell mantle (7). Support for the physiological significance of this vascular flow model by Bonner-Weir and Orci (7) has been obtained from experiments in the rat (6) and dog (8) pancreas. These reports suggested that core-derived, microvascularly delivered insulin may tonically inhibit the A cell, but did not conclusively prove that the direction of blood flow, i.e., the order of cellular perfusion, is important in the regulation of islet hormone secretion. Indeed, it has been reported from a study of the canine pancreas (9) that directionality of blood flow does not exist, suggesting that intra-islet hormone interactions are not dependent upon the order of cellular perfusion as determined by blood flow. Moreover, despite the attractiveness of the report by Maruyama et al. (6) that the depletion of vascular insulin by infused anti-insulin serum increased glucagon secretion, it may be argued that the increase in glucagon efflux concentration may have resulted from a mechanism other than by an antibody-mediated decrease in microvascular insulin. The reported increase in glucagon (6) may have been the result of an insulin-mediated change in A cell glucose metabolism (10), leakage of the antibody into the interstitial space to affect paracrine interactions (11), or a paradoxical receptor-mediated A cell response (12) to anti-idiotypic antibodies reportedly produced during insulin and glucagon antibody production (12, 13).

We have previously suggested that the importance of the direction of intra-islet blood flow and the sequence of islet cellular perfusion may be tested by comparing the results of anterograde and retrograde (reversed flow) perfusion (8, 14). The use of this model system should definitively establish whether or not intravascular depletion of endogenous insulin was responsible for the reported increase in efflux glucagon concentrations (6, 8, 14). If the above hypothesis, that the path of islet blood flow and subsequent flow-dependent cellular interactions are from the B cell core outward to the A and D cell mantle is correct, then the A cell should be stimulated by an acute deprivation of vascularly delivered insulin which may be produced either by the anterograde infusion of insulin antibody (6, also Fig. 1) or by the reversal of the direction of islet cell perfusion (8, 14). If the reversal of islet cell perfusion prevents insulin from reaching the A cell (an acute state of insulin deprivation), then retrograde anti-insulin antibody should not further increase glucagon secretion above that obtained with retrograde perfusion alone. Alternatively, an increase in glucagon efflux concentrations during the retrograde infusion of anti-insulin antibody may be indicative of the presence of an idiotypic antibody stimulation as discussed above (12, 13). If

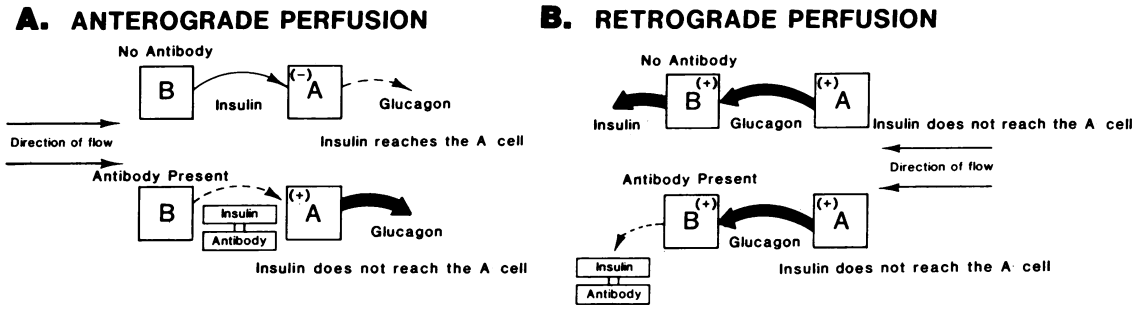

Figure 1. Expected results of anterograde vs. retrograde infusion of anti-insulin antibody. (*A*) If the A cell is perfused and tonically inhibited by intra-islet vascularly delivered insulin during normal (anterograde) perfusion, the addition of anti-insulin antibody may be expected to reduce the concentration of insulin bathing the A cell. The A cell may be stimulated by an acute deprivation of insulin despite a constant glucose concentration. (*B*) If the normal order of cellular perfusion is from the B cell to the A cell, then the reversal of the order of cellular perfusion (retrograde) would be expected to result in an increase in glucagon secretion inasmuch as the A cell would be in an insulin-deficient environment. Therefore the addition of anti-insulin antibody during retrograde perfusion has no effect upon the A cell despite the reduction of downstream vascular insulin concentrations.

the flow hypothesis is valid, the anterograde vs. retrograde perfusion technique provides a model system for the establishment of the order of cellular perfusion and interaction within the islet when used in concert with the anterograde and retrograde infusion of specific antibodies directed against islet hormones. Therefore in the present study we compared the response of the A cell to an antibody-induced lack of insulin or somatostatin during anterograde and retrograde perfusions in order to establish whether or not there is directionality of microvascular cellular perfusion within the islet.

Methods

Rat pancreata are isolated and perfused by the method of Grodsky and Fanska (15). Male Sprague-Dawley rats weighing 325–400 g were anesthetized with 50 mg/kg sodium pentobarbital injected intraperitoneally. Pancreata were maintained at 37°C and were perfused anterogradely and/or retrogradely at a rate of 2 ml/min with modified Krebs-Ringer buffer containing 1 mM mixed amino acids (11). The perfusate glucose concentration was maintained at 5.56 mM throughout the experiments except where noted. Samples of the efflux medium were obtained at 1-min intervals. Insulin, glucagon, and somatostatin concentrations in the effluent samples were analyzed by radioimmunoassay as described previously (16).

In one group of experiments, pancreata were perfused normally through the arterial system (anterograde perfusion) for 50 min with 4.89 mM glucose. The first 20 min were regarded as an equilibrium period during which samples were not obtained. 30 min after sample collection had begun, the direction of perfusion was reversed (retrograde perfusion) by the rapid switching of the influx and efflux tubing (9) so that the pancreata were perfused through the venous system. Pancreata were retrogradely perfused for an additional 30 min. In order to test for possible vascular damage and associated secretory artifacts, another group of pancreata were perfused anterogradely 15 min, retrogradely 25 min, and then anterogradely 20 min with 5.56 mM glucose.

In another group of experiments, pancreata were perfused either anterogradely or retrogradely throughout the experiment. After 40 min of perfusion, guinea pig anti-porcine insulin or ovine anti-somatostatin γ-globulin was infused for 10 min at a rate of 0.2 ml/min. As a test for complete perfusion of the pancreas during anterograde and retrograde perfusion, trypan blue dye was infused for 5 min at the conclusion of the experiments.

Guinea pig anti-porcine insulin serum was purchased from ICN Immunobiologicals, Cleveland, OH (lot GP 30 and GP 537). Ovine anti-somatostatin serum was produced by Guildhay (Surrey, U.K.). The γ-globulin fraction was isolated from the antiserum by ammonium sulfate precipitation (17) in order to remove the possible influences of specific and nonspecific contaminants. After dialysis with saline, the globulin fraction was reconstituted to half the original volume with perfusate buffer. The maximal amount of insulin or somatostatin bound to the respective antibodies under conditions which simulated perfusion parameters during a 1-min incubation was determined as described by Maruyama et al. (6). As a serum control, a portion of each antiserum was preabsorbed with the respective hormone prior to infusion. Excess unbound hormone was removed by reprecipitation and dialysis at pH 7.3, 4°C for 60 h. A subgroup of pancreata were infused anterogradely or retrogradely with the latter antibody preparations. Statistical calculations were performed by the use of analysis of variance and Student's *t* test. Results are reported as means±standard error.

Results

Maximal hormone binding by the antibody-containing perfusate. The maximal amount of insulin bound during a 1-min incubation at a dilution of 1:21 (0.1 ml per 2.0 ml of perfusate) was 100 mU/min for the anti-insulin globulin fraction. Maximal somatostatin binding was in excess of 2 ng/ml for anti-somatostatin globulin. Neither antibody preparation, anti-somatostatin or anti-insulin, demonstrated glucagon binding nor cross-reactivity with its antipode, insulin or somatostatin, respectively.

Effects of anterograde and retrograde perfusion upon insulin and glucagon secretion. During retrograde perfusion (4.89 mM glucose) both insulin and glucagon secretion increased significantly above the respective anterograde mean values. The integrated mean anterograde concentration of insulin and glucagon were 20±1 μU/ml and 296±24 pg/ml respectively, while the retrograde integrated 30-min mean insulin and glucagon concentrations were 41±1 μU/ml and 623±161 pg/ml, respectively ($n = 6$, $P < 0.0005$). In the alternating perfusion experiments (5.56 mM glucose, $n = 5$), efflux glucagon was 71±2 pg/ml anterogradely, 83±2 pg/ml retrogradely ($P < 0.0005$), and 56±2 pg/ml anterogradely ($P < 0.0005$). There was no difference in efflux volume between anterograde and retrograde perfusions.

Effects of anterograde and retrograde anti-insulin antibody infusion. The anterograde infusion of anti-insulin γ-globulin increased perfusate glucagon concentrations from a mean control level of 74±2 pg/ml to a mean level of 148±16 pg/ml, ($n = 6$, $P < 0.0005$). The peak glucagon level of 237±65 pg/ml, occurred late in the infusion, although a statistically significant rise in efflux glucagon was observed within 3 min of antibody infusion. By contrast, the retrograde infusion of anti-insulin γ-globulin did not further change efflux glucagon concentrations from the respective baseline (111±23 vs. 120±8 pg/ml, $n = 6$). Efflux concentrations of somatostatin (24±2 pg/ml) were promptly increased by the anterograde infusion of insulin antibody (434±156 pg/ml, $P < 0.0005$, $n = 6$). During the retrograde infusion of anti-insulin antibody, efflux somatostatin concentrations did not change from control levels (1±5% change) compared with the immediate augmentation of somatostatin secretion obtained during the anterograde infusion of anti-insulin antibody (2,112±73% change). Nonimmune guinea pig serum was without effect upon glucagon secretion. The infusion of preabsorbed antiserum did not change glucagon efflux concentrations above their respective anterograde or retrograde mean values ($n = 7$, $P < 0.45$).

Effects of anterograde and retrograde anti-somatostatin antibody infusions. Efflux concentrations of glucagon (57±5 pg/ml) and insulin (49±20 μU/ml) were not changed by the anterograde infusion of somatostatin antibody (glucagon 60±8 pg/ml, $P < 0.5$ and insulin 50±23 μU/ml, $P < 0.5$, $n = 5$). Conversely retrograde efflux concentrations of glucagon (57±5 pg/ml) and insulin (64±12 μU/ml) were increased by the retrograde infusion of somatostatin antibody (glucagon 76±8 pg/ml, $P < 0.001$ and insulin 135±25 μU/ml, $P < 0.0025$).

Trypan blue infusion. During both anterograde and retrograde infusions, the entire pancreas was colored within 2 min and was cleared of the dye within 2–3 min after the cessation of dye infusion. There was no noticeable difference in the distribution of the dye between anterograde and retrograde perfusions.

Discussion

The revision of the earlier anatomical model of the microvasculature of the rat islet (18) by Bonner-Weir and Orci (7) has become very important in considering attempts to define intra-islet interactions and strongly suggests that intra-islet blood flow may be from the B cell core outward to the A and D cell–containing mantle. Because it seems likely that the vascular compartment may influence cellular interactions, the determination of flow-dependent intra-islet interactions is fundamental to the understanding of the physiology of the intact, in situ perfused islet. Moreover, the physiological role of the microvasculature must be established before the existence of paracrine interactions can be properly studied (5). Recent reports from experiments using both the perfused rat (6) and dog (8) pancreas suggest that the A cell is tonically inhibited by vascularly delivered intra-islet insulin, but because of the lack of serum controls in the former (6) and the unknown anatomy of the dog islet in the latter (8), these studies do not conclusively prove that directionality of intra-islet blood flow is important in the regulation of islet hormone secretion. The present study, bolstered by the above reports (6, 8) suggests that the vascular compartment and the direction of capillary blood flow is very important, if not central, to the regulation of intra-islet hormone interactions. To date, clear evidence for a paracrine interstitial regulatory pathway separate from blood borne hormone interactions not only is lacking (5), but is extraordinarily difficult to elucidate without knowledge of the possible role or contribution of the islet microvasculature in islet cellular interactions.

In the present study, the anterograde infusion of insulin antibody produced an acute rise in somatostatin and glucagon secretion in agreement with a report by Maruyama et al. (6) for glucagon. In contrast, the retrograde infusion of anti-insulin antibody did not increase glucagon or somatostatin secretion above the respective retrograde control. These results suggest that during retrograde perfusion, the A and D cells are in an insulin-free environment (Fig. 1), further supporting the hypothesis that intra-islet blood flow is from core to mantle and that intra-islet cellular interactions are flow dependent. The failure of retrogradely infused anti-insulin γ-globulin to increase glucagon concentrations in the efflux is strong evidence that the increase obtained with anterograde anti-insulin γ-globulin was the result of vascular insulin deprivation and was not a nonspecific effect of the antibody nor the result of leakage of antibody into the interstitial space, where it may have induced paracrine interactions or may have stimulated the A cell by blocking insulin receptors (12, 13). Because it has been demonstrated that antibody proteins do not readily cross the capillary endothelium (19), the failure of retrogradely infused insulin antibody and of anterogradely and retrogradely perfused preabsorbed serum to influence A cell secretion is strong evidence against a possible nonspecific or idiotypic (10, 11) antibody effect and also argues against the rapid leakage of antibody into the interstitial spaces where it may influence possible paracrine interactions (12). The failure of the retrograde infusion of anti-insulin antibody to increase glucagon or somatostatin efflux concentrations and the immediate and large increase in insulin efflux concentrations obtained during the retrograde infusion of antisomatostatin antibody is strong evidence in support of the core to mantle perfusion hypothesis (6–8, 14).

The sequential perfusion (anterograde, retrograde, anterograde) of the same pancreata demonstrates that the increase in glucagon levels during retrograde perfusion was not caused by vascular damage, because the increase in retrograde efflux glucagon levels was reversed by anterograde perfusion. These results also support our previous suggestion, based on nonantibody studies in the dog (14), that the mantle is further subordered (14) with the majority of D cells located downstream or distal to the majority of A cells. The absence of an increase in glucagon secretion during the anterograde infusion of anti-somatostatin antibody suggests that the A cells vascularly precede the D cells. Consistent with this order of interaction (from B to A to D) was the major and prompt increase in somatostatin obtained during the anterograde infusion of insulin antibody which was associated with a significant increase and presumed influx of endogenous glucagon to the D cells. Further confirmation of the B → A → D order of vascular cellular perfusion was provided by the prompt increase in efflux glucagon and insulin concentrations obtained during the retrograde infusion of antisomatostatin gamma globulin.

In summary these results suggest that, in the vascular compartment, B cells inhibit A cell secretion and A cells stimulate D cell secretion. A cells appear not to significantly stimulate B

cells, nor do D cells appear to inhibit A cells in terms of microvascular communication. The pathophysiological significance of the A cell in general preceding the D cell is uncertain, but the reported dual elevation of glucagon and somatostatin, in insulin deficiency diabetes (4, 5) is consistent with the observed vascular influence of the A cell upon the D cell.

The present report is the first conclusive demonstration of intra-islet intercellular interactions by exclusively intravascular communication. We conclude that the intra-islet vascular compartment is very important in islet regulatory events and that intra-islet interactions are dependent upon the direction of flow and the cellular order of perfusion (B → A → D) through the islet microvasculature. With knowledge of the microvascular contribution to intra-islet interactions, it becomes possible, for the first time, to discover potential paracrine interactions in the regulation of islet hormone secretion.

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