β → α → δ Pancreatic Islet Cellular Perfusion in Dogs

JOHN I. STAGNER, ELLIS SAMOLS, AND SUSAN BONNER-WEIR

Intraislet communication between α-, β-, and δ-cells and their secretory products may theoretically occur via the paracrine (interstitial) and/or vascular routes. Recently, we have shown that there is a directed microvascular circulation in the rat islet with a cellular order of perfusion of β → α → δ. The direction of microvascular perfusion of cells within the dog islet has been controversial. Anterograde (arterial) perfusion and retrograde (reversed or venous) perfusion of a segment of isolated dog pancreas with potent insulin antibodies yielded results similar to those found in the rat pancreas (anterograde, 158 ± 44% increase in glucagon and 65 ± 20% increase in somatostatin; retrograde, no change in glucagon or somatostatin). Anterograde infusion of glucagon antibody (no change in insulin, −33.5 ± 3% decrease in somatostatin) or somatostatin antibody (no change in insulin or glucagon) also yielded the same results as in the rat pancreas. Anterograde infusion of 500 pg/ml glucagon caused a larger increase in insulin secretion (245 ± 10%) than retrograde infusion (45 ± 4%), whereas somatostatin was stimulated more retrogradely (339 ± 17%) than anterogradely (121 ± 9%). Anterograde infusion of somatostatin produced a larger decrease in insulin and glucagon than did retrograde perfusion ($P < .0001$ for both comparisons). The retrograde infusion of 0.3 mU/ml insulin caused a decrease in glucagon but was without effect anterogradely. The results from the infusion of exogenous hormones suggest that the sensitivity of the α-, β-, and δ-cells to insulin, glucagon, and somatostatin is determined by the β → α → δ order of perfusion. The antibody studies indicate that directed microvascular perfusion is central to intraislet regulation of insular secretions in dogs. *Diabetes* 37:1715–21, 1988

From the Veterans Administration Medical Center and the Department of Medicine, School of Medicine, University of Louisville, Louisville, Kentucky; and the Joslin Diabetes Center, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. John Stagner, Research Service (151), VA Medical Center, 800 Zorn Avenue, Louisville, KY 40202.

Received for publication 2 November 1987 and accepted in revised form 7 June 1988.

The regulation of islet hormone secretion may occur by one or both of two major routes of intraislet communication, the paracrine route, utilizing hormone diffusion through interstitial spaces, and a vascular route, which may deliver endogenous hormones to islet cells located downstream. The paracrine route is thought to operate only over short distances, requiring cells to be contiguous or close to one another (1). The intraislet vascular route allows cellular interactions to occur at greater distances, i.e., within the confines of the islet. Because the mammalian islet is organized into distinct regions, with the β-cells separate from the α- and δ-cells, we propose that the direction of perfusion is important in regulating islet hormone secretion. We recently demonstrated that there is a specific order of islet cellular perfusion in the rat islet, β → α → δ, which is important in the regulation of intraislet cellular interactions (2,3). These studies were performed by the anterograde (normal arterial perfusion) and retrograde (reversed or venous perfusion) infusion of potent antibodies directed against insulin, glucagon, or somatostatin. Retrograde perfusion results in a reversal of the cellular order of vascular perfusion and offers a mechanism in which to test the importance of direction of intraislet capillary blood flow and the cellular order of perfusion and interaction within the in situ pancreatic islet. Controversy exists concerning the importance of the cellular order of perfusion, i.e., direction of cellular interaction in the dog islet. Kawai et al. (4) have suggested that in the canine pancreas the direction of cellular perfusion in the islet is either not important in the regulation of hormone secretion or that it does not exist. Their report is in striking opposition to our previous report that the direction of intraislet blood flow in the canine islet is important in the regulation of islet hormone secretion and that intraislet blood flow is normally from the β-cell core outward to the α- and δ-cell mantle (5). We have attempted to resolve this controversy using techniques that defined the vascular order of cellular perfusion in the rat islet as the paradigm for the determination of the vascular order of cellular perfusion in

TABLE 1
Effect of anti-insulin γ-globulin on glucagon and somatostatin secretion

	Anterograde infusion of γ-globulin				Retrograde infusion of γ-globulin			
	n	Control	γ-Globulin	Change (%)	*n*	Control	γ-Globulin	Change (%)
Glucagon (pg/ml)	6	38 ± 12	99 ± 37*	158 ± 44	5	112 ± 41	107 ± 46†	−18 ± 12‡
Somatostatin (pg/ml)	5	70 ± 28	102 ± 37*	65 ± 20	6	51 ± 23	46 ± 20†	−5 ± 5‡

Values are means ± SE; *n* = number of experiments.
*$P < .025–.005$ and †NS vs. control, by Student's *t* test for paired data.
‡$P < .00025$ vs. percent change, by Student's *t* test for unpaired data.

the dog islet (2,3). Specific islet hormone antibodies were infused anterogradely or retrogradely into the isolated perfused canine pancreas. The order of vascular islet cellular perfusion, as determined by antibody infusion, was further corroborated by the anterograde and retrograde infusion of exogenous insulin, glucagon, and somatostatin.

MATERIALS AND METHODS

Fasting male mixed-breed German shepherd dogs weighing 20–25 kg were used as pancreas donors. Pancreatectomy was performed as described elsewhere under surgical levels of anesthesia induced by intravenous pentobarbital sodium (5–7). Isolated pancreases were perfused anterogradely or retrogradely as previously described with modified Krebs-Ringer buffer containing 1 mM mixed amino acids and 4.89 mM glucose in a nonrecirculating system at a flow rate of 10 ml/min (5). Pancreases were perfused anterogradely (normal arterial perfusions) for 60–90 min. Thereafter, the direction of perfusion was reversed (retrograde perfusion) by rapidly switching the influx and efflux tubing. Retrograde perfusion was continued for an additional 60–100 min. Antibodies are reported not to readily cross the endothelium of pancreatic capillaries (8) into the interstitial-fluid spaces and are not removed by pancreatic tissues (9). Therefore, specific hormone antibodies were infused anterogradely or retrogradely to neutralize vascular concentrations of endogenous hormone so that the direction and sequence of intraislet cellular perfusion could be determined. Hybridoma-produced insulin antibody was obtained by contract (Berkeley Antibody, Berkeley, CA) as ascites fluid. Glucagon antibody was generously provided by Guildhay (Surrey, UK). The γ-globulin fraction from the ascites fluid (anti-insulin) and serum (antiglucagon) were isolated and purified by precipitation with ammonium sulfate (10). After precipitation, the γ-globulin was dialyzed with saline and reconstituted to the original volume with perfusate buffer. To minimize the volume of costly antibody consumed by each perfusion, the appropriate γ-globulin was infused at a rate of 0.4 ml/min anterogradely or retrogradely into an isolated portion of the dorsal pancreatic lobe, which was ~3.75–6.0 cm^2. The perfusion flow rate was maintained at 4.0 ml/min in this series of experiments only. The remainder of the pancreas was clamped on each side and was excluded from the perfusion. The maximum amount of insulin or glucagon bound by the respective antibody during a 1-min incubation was determined as described by Maruyama et al. (9). Anti-insulin γ-globulin was infused anterogradely for 10 min after a 50-min equilibration period. Thirty minutes after anterograde antibody infusion, the direction of perfusion was reversed. Anti-insulin γ-globulin was infused for 10 min after a retrograde equilibration period of 60 min. In a similar experiment, antiglucagon γ-globulin was infused anterogradely for 10 min after a 50-min equilibration period. As a separate series of perfusion experiments, involving the whole pancreas, 0.3 mU/ml exogenous insulin (a gift from Lilly, Indianapolis, IN), 500 pg/ml glucagon, or 4 and 80 ng/ml somatostatin was infused anterogradely or retrogradely for 30 min after an equilibration period of 60 min. These experiments were performed to test the effects of exogenous hormones on the secretion of endogenous hormones as a function of the direction and the cellular order of perfusion. Flow rate, perfusion pressure, and efflux oxygen content were monitored throughout the experiment. Samples were obtained at 1-min intervals with a fraction collector. At the end of all experiments, a solution of trypan blue was infused for 5 min to assess the degree of pancreatic perfusion. Insulin, glucagon, and somatostatin were measured by radioimmunoassay with a charcoal separation method (7). Canine C-peptide was measured as previously described (11). Statistical calculations were performed by analysis of variance (ANOVA) and Student's *t* test. Results were reported as means ± SE. A level of $P < .01$ was considered significant.

TABLE 2
Effect of anterograde and retrograde infusion of glucagon on insulin and somatostatin secretion

	Anterograde infusion of glucagon (*n* = 5)		Retrograde infusion of glucagon (*n* = 7)	
	0 pg/ml	500 pg/ml	0 pg/ml	500 pg/ml
Insulin (μU/ml)	118 ± 11	378 ± 39*	126 ± 5	171 ± 9*
Somatostatin (pg/ml)	110 ± 0.1	243 ± 10*	36 ± 5	158 ± 22*

Values are means ± SE; *n* = number of experiments.
*$P < .0001$ vs. baseline, by Student's *t* test for paired data.

RESULTS

Effects of anterograde and retrograde perfusion. There were minimal changes in the parameters of perfusion pressure and oxygen uptake when the direction of perfusion was reversed. Perfusion pressure increased an average of 1.3 ± 0.36 mmHg (*n* = 43) after reversal from an arterial baseline of 37.7 ± 0.63 mmHg. The flow rate of 10 ml/min did not change after reversal of flow. The oxygen content of the efflux did not change after perfusion reversal (anterograde efflux 5.3 ± 0.57 ppm oxygen, retrograde efflux 5.35 ± 0.48 ppm oxygen; *n* = 12). Influx oxygen content

TABLE 3
Effect of anterograde and retrograde infusions of somatostatin on insulin and glucagon secretion

		Anterograde infusion of somatostatin			Retrograde infusion of somatostatin		
	n	0 ng/ml	4 ng/ml	80 ng/ml	0 ng/ml	4 ng/ml	80 ng/ml
Insulin (μU/ml)	11	228 ± 20	158 ± 9*	105 ± 4*†	201 ± 8	178 ± 7‡	139 ± 5*§
Glucagon (pg/ml)	9	105 ± 8	65 ± 4*	66 ± 5*‖	115 ± 7	121 ± 6‖	78 ± 5*†

Values are means ± SE; comparisons were by Student's *t* test for paired data.
*$P < .0005–.0001$ vs. baseline (0 ng/ml).
†$P < .0005–.0001$ vs. 4 ng/ml somatostatin.
‡NS vs. baseline.
§$P < .001$ vs. 4 ng/ml somatostatin.
‖NS vs. 4 ng/ml somatostatin.

was 11.5 ± 0.96 ppm at an infusion rate of 10 ml/min. In anterograde and retrograde perfusions, the pancreases were uniformly colored within 3 min from the infusion of trypan blue. At the cessation of dye infusion, the pancreases were cleared within 3–4 min. No differences in dye distribution between anterograde and retrograde perfusions were observed.

Effects of insulin antibody on glucagon and somatostatin secretion. The maximum insulin-binding capacity of the insulin antibody during a 1-min incubation at 0.1 ml of antibody/ml perfusion buffer (total vol 1.1 ml) was 100–200 mU/ml. The γ-globulin fraction did not bind glucagon or somatostatin. During anterograde infusion of insulin antibody, both glucagon and somatostatin efflux concentrations increased above the respective preinfusion concentration (Table 1). In contrast, during retrograde perfusion, the antibody had no effect on either glucagon or somatostatin secretion.

Effects of glucagon antibody on insulin and somatostatin secretion. The maximum glucagon-binding capacity of the glucagon antibody during a 1-min incubation at 0.1 ml antibody/ml perfusion buffer was >2000 pg/ml. Glucagon antibody did not bind insulin or somatostatin. Insulin concentrations during antibody infusion (165 ± 77 μU/ml) were not different from preinfusion insulin concentrations (142 ± 69 μU/ml, $n = 5$, NS). Thus, the anterograde infusion of glucagon antibody had no effect on insulin secretion (1 ± 5.6% change from control). In contrast, somatostatin efflux concentrations during the infusion of glucagon antibody (128 ± 10 pg/ml) were significantly reduced from preinfusion concentrations (195 ± 12 pg/ml, $n = 5$, $P < .0025$; −33.5 ± 3% change from control). Normal guinea pig and ovine sera or the respective γ-globulin fractions did not bind insulin, glucagon, or somatostatin, and infusion of these preparations did not affect islet hormone secretion.

Effects of anterograde and retrograde glucagon infusion. During anterograde perfusion, the infusion of 500 pg/ml exogenous glucagon significantly increased efflux concentrations of both insulin and somatostatin above preinfusion concentrations (Table 2). The retrograde infusion of glucagon induced a mild increase in efflux insulin concentrations compared with anterograde results. Retrogradely, somatostatin concentrations were markedly elevated by the infusion of exogenous glucagon. However, the mean somatostatin concentrations 20 min before the glucagon infusion (36 ± 5 pg/ml) were lower than the concentrations observed during the first 30 min after the reversal of perfusion (108 ± 22 pg/ml, $P < .0001$). ANOVA testing of the effect of direction on hormone secretion during the control periods suggested that the direction of perfusion had a significant effect on the secretion of insulin ($F = 63$, $P < .0001$) and somotostatin ($F = 92$, $P < .0001$). ANOVA comparison of the effects of glucagon infusion on insulin secretion within anterograde and retrograde perfusions indicated that exogenous glucagon affected insulin secretion ($F = 79$, $P < .0001$) and somatostatin secretion ($F = 247$, $P < .0001$).

Effects of anterograde and retrograde somatostatin infusion. Exogenous somatostatin inhibited insulin secretion in a dose-related manner in both modes of infusion (Table 3). Insulin secretion was inhibited by the anterograde infusion of 4 ng/ml somatostatin and was further inhibited by 80 ng/ml somatostatin. However, in retrograde perfusions only 80 ng/ml somatostatin significantly inhibited insulin secretion ($P < .0005$). There did not appear to be a dose-related inhibition of glucagon secretion by the anterograde infusion of somatostatin in that maximal α-cell inhibition was apparently achieved by the infusion of 4 ng/ml somatostatin. During retrograde infusion, 4 ng/ml somatostatin had no effect on glucagon secretion, whereas maximal glucagon inhibition was achieved by 80 ng/ml somatostatin. ANOVA by direction did not suggest a difference in insulin secretion between anterograde and retrograde controls ($F = 0.64$, NS), although glucagon secretion during the respective control period was significantly different ($F = 13$, $P < .0001$) and

TABLE 4
Effect of anterograde and retrograde infusions of insulin on C-peptide and somatostatin secretion

		Anterograde infusion of insulin			Retrograde infusion of insulin	
	n	0 mU/ml	0.3 mU/ml	*n*	0 mU/ml	0.3 mU/ml
Somatostatin (pg/ml)	8	140 ± 10	154 ± 12	6	98 ± 9	104 ± 6
C-peptide (pM/ml)	5	0.171 ± 0.03	0.167 ± 0.02	5	0.232 ± 0.16	0.274 ± 0.11

Values are means ± SE; *n* = number of experiments. Comparisons by Student's *t* test for paired data were not significant.

TABLE 5
Effect of hormone antibody infusion in rat and dog pancreases

	Insulin antibody		Glucagon antibody		Somatostatin antibody	
	Glucagon secretion	Somatostatin secretion	Insulin secretion	Somatostatin secretion	Insulin secretion	Glucagon secretion
Rat						
Anterograde	Increase	Increase*	No change	Decrease†	No change	No change*
Retrograde	No change	No change	Decrease	No change	Increase	Increase
Dog						
Anterograde	Increase	Increase	No change	Decrease	No change	No change‡
Retrograde	No change	No change				

*Ref. 2.
†Ref. 3.
‡Ref. 12.

dependent on the direction of perfusion. ANOVA comparison of experimental (i.e., exogenous somatostatin infusions) and control perfusions suggested that exogenous somatostatin had a significant effect on the secretion of insulin ($F = 102$, $P < .0001$) and glucagon ($F = 38$, $P < .0001$). In addition, ANOVA testing of the effects of both direction and exogenous somatostatin infusions indicated an interaction between the direction of perfusion and the presence of exogenous somatostatin on both insulin ($F = 28$, $P < .0001$) and glucagon ($F = 18$, $P < .001$) secretion.

Effects of anterograde and retrograde insulin infusion. The results of the infusion of 0.3 mU/ml exogenous insulin on glucagon have been reported elsewhere (5). Anterograde insulin infusion had no effect on glucagon or C-peptide concentrations ($P < .25$ and $< .35$, respectively). Retrogradely, the infusions of 0.3 mU/ml insulin significantly inhibited glucagon secretion ($P < .0005$). In contrast, neither somatostatin nor C-peptide secretion was affected by exogenous insulin, regardless of the direction of infusion, as indicated by ANOVA ($F = 0.2$, NS; Table 4).

DISCUSSION

Researchers have demonstrated that exogenous hormones affect the secretion of endogenous islet hormones (1,12). The observation that insulin and glucagon influenced the secretion of one another in a negative-positive interaction (13) bolstered the concept that islet hormone secretion may be regulated by specific intraislet cellular interactions. However, physiological studies of infusions of glucose or exogenous islet hormones into the pancreas have not been able to definitively establish whether there is a standard sequence of cellular perfusion and interaction within mammalian islets. It has been argued that α- and δ-cells regulate the β-cell (from mantle to core) or that the β-cell regulates the α-cell and possibly the δ-cell (from core to mantle), depending on the assumed order of vascular cellular perfusion and potential paracrine interactions within the islet (1,14,15). This controversy has been further clouded by the report that the regulation of hormone secretion from the dog islet does not depend on intraislet interactions (4). A correct interpretaton of the effects of exogenous and endogenous hormones on the regulation of islet hormone secretion is impossible without knowledge of the direction and order of cellular vascular perfusion within the islet.

We have demonstrated that the order of islet cell vascular perfusion in the rat islet is β → α → δ (2,3). In the rat islet, the β-cell regulates the α-cell as predicted by the microvascular perfusion model proposed by Bonner-Weir and Orci (16). In terms of the vascular order of cellular perfusion in the rat islet, the δ-cell is the farthest downstream. The δ-cell is thus vascularly neutral and does not vascularly affect the α- or β-cells during anterograde perfusion (2,3). Therefore the α-cell and possibly the β-cell regulate δ-cell secretion in the rat islet. These conclusions do not prohibit paracrine regulation of islet hormone secretion through the diffusion of islet hormones through interstitial fluid spaces (1,12). α-Cells and δ-cells, as well as the outer rim of β-cells, may be affected by endogenous hormones entering the interstitial fluid spaces. Indeed, we have suggested that intraislet endogenous somatostatin may affect the α- and β-cells through a mechanism that is countercurrent to the direction of vascular flow (17). However, true paracrine regulation has not been unequivocally demonstrated (1,12), and it cannot be proved or disproved without knowledge of the functional contribution of intraislet cellular vascular perfusion. The rat islet was used as the model for testing flow-dependent cellular interactions in the dog islet because the path of the microvasculature in the dog islet has not been anatomically determined. We have reported that, physiologically, the direction of blood flow within the dog islet is from the β-cell core outward to the α- and δ-cell–containing mantle (5). This conclusion was based on the differential responses of the α-cell to glucose or insulin during anterograde and retrograde perfusion. In contrast, a similar study found no relationship between the order of islet cellular perfusion and net hormone secretion (4), questioning the role of intraislet interactions in the regulation of insular secretion.

The order of islet cellular perfusion and interactions cannot be conclusively determined from the infusion of exogenous hormones. However, because γ-globulins are reported not to cross the endothelium of pancreatic capillaries (8) or to be adsorbed by pancreatic tissues (9), the infusion of hormone antibodies can be used to neutralize vascular concentrations of specific hormones, effectively reducing or abolishing their effect on the cell types located downstream. If the dog islet were similar to the rat islet (2,3,16) in terms of the vascular sequence of islet cellular perfusion (β → α → δ), the anterograde infusion of anti-insulin antibody should result in an increase in efflux glucagon and somatostatin. The rat experiments have shown that the increase

in somatostatin can be attributed to the increase in glucagon secretion, which itself was the result of insulin antibody–induced insulin deficiency (Table 5). Our results are consistent with this hypothesis and confirm our previous report that intraislet blood flow in the dog islet is from the β-cell core outward to the mantle (5; Table 1). Because the retrograde infusion of insulin antibody had no effect on glucagon or somatostatin secretion, we conclude that the β-cell is definitely perfused before the α-cell during normal anterograde perfusion and that the direction of islet vascular perfusion is probably β → α → δ.

The vascular relationship between the α- and δ-cells in the mantle was determined by the anterograde infusion of glucagon antibody. If the α-cell preceded the δ-cell, as in the rat islet (2,3), a decrease in vascular glucagon bathing the δ-cell should have resulted in a decrease in somatostatin secretion. If the δ-cell preceded the α-cell, a decrease in vascular glucagon should have had no effect on somatostatin secretion, whereas a fully random α- and δ-cell arrangement should have resulted in no significant change, or at most a minor decrease in somatostatin secretion. As noted, the anterograde infusion of glucagon antibody had no effect on insulin secretion but strongly decreased somatostatin secretion. Thus, in the dog islet mantle, most of the α-cells precede most δ-cells in terms of vascular perfusion. The increase in somatostatin secretion during the anterograde infusion of insulin antibody is indeed the result of an increase in endogenous glucagon bathing the δ-cell. These results demonstrate a β → α → δ cellular sequence of vascular perfusion in the dog islet and are further evidence of the importance of the direction of vascular perfusion in the regulation of islet hormone secretion. Rat and dog islets are therefore similar in terms of islet cellular vascular perfusion as determined by their response to the infusion of specific antibodies (Table 5).

Based on the knowledge of the order of vascular cellular perfusion within the islet, it is now possible to predict and interpret responses to the anterograde and retrograde infusion of exogenous hormones. The anterograde and retrograde infusion of exogenous glucagon resulted in an increase in insulin and somatostatin secretion (Table 2). The anterograde increase in insulin secretion was threefold larger (245 ± 10% change) than the glucagon-stimulated increase in insulin obtained during retrograde perfusion (45 ± 4% change, $P < .0001$). The difference between anterograde and retrograde glucagon-stimulated insulin secretion is the result of the order of islet cellular perfusion, because glucagon is reported not to be adsorbed by pancreatic tissues (18). Anterogradely, the β-cell is in a glucagon-free environment and is sensitive to low concentrations of exogenous glucagon. During retrograde perfusion, the sequence of cellular perfusion is reversed, with the α-cell preceding the β-cell. Under these circumstances, endogenous glucagon may occupy glucagon receptors on the β-cell membrane (19), rendering it less sensitive to exogenous glucagon. This conclusion is supported by the rapid increase in insulin and glucagon efflux concentrations obtained immediately after retrograde perfusion (5). The 60-min retrograde equilibration period before the infusion of exogenous glucagon is more than adequate time for receptor occupancy to occur. Although exogenous glucagon stimulated somatostatin secretion regardless of the direction of perfusion (Table 2), the magnitude of the δ-cell response according to the direction of perfusion is of interest. Anterogradely, somatostatin secretion was increased from a stable preinfusion somatostatin concentration. During retrograde perfusion, somatostatin efflux concentrations initially increased and then decreased after 30 min during continued perfusion (5,20). After 60 min of retrograde perfusion, somatostatin efflux concentrations stabilized at the reduced concentration of 36 ± 5 pg/ml. The reversal of the β → α → δ sequence of vascular perfusion during retrograde perfusion significantly reduced the amount of endogenous glucagon reaching the δ-cell, thereby decreasing tonic glucagon-stimulated somatostatin secretion and increasing δ-cell sensitivity to exogenous glucagon.

We suggest that an increase in δ-cell sensitivity to exogenous glucagon during retrograde perfusion accounts for the difference between anterograde and retrograde glucagon-stimulated somatostatin secretion, expressed as the percentage of change from control, 121 ± 9 and 339 ± 17%, respectively. These observations and conclusions are independent of possible differences in somatostatin adsorption in the exocrine tissues during anterograde and retrograde perfusion (21,22), because each was calculated as the percent change relative to the respective control somatostatin concentration. If the effect of glucagon on the δ-cell were equal anterogradely and retrogradely, then the percent change in somatostatin should be greater anterogradely than retrogradely if adsorption of somatostatin by the exocrine pancreas were a major factor. Therefore, we conclude that δ-cell secretion depends on the direction of intraislet blood flow and that the δ-cell depends on glucagon for the maintenance and modulation of somatostatin secretion.

The possible importance of the direction of intraislet blood flow on intraislet cellular interactions was further tested by the infusion of exogenous somatostatin. There was a concentration-dependent inhibition of insulin secretion during anterograde somatostatin infusion (Table 3). Glucagon secretion was not further inhibited by an increased somatostatin concentration, suggesting that α-cell somatostatin receptors were saturated at the lower somatostatin concentration, resulting in a maximal inhibition of secretion (19). In contrast, during retrograde perfusion, a significant ($P < .0005$) inhibition of the α- and β-cells was obtained only during the infusion of 80 ng/ml somatostatin. We postulate that the differences between anterograde and retrograde infusion may be the result of receptor occupation by endogenous somatostatin during retrograde perfusion, because the δ-cell is normally distal to the α-cell as well as to the β-cell. The uptake or extraction of exogenous somatostatin during retrograde infusion was considered as a possible explanation for the observed anterograde and retrograde differences; there are reports that ~54% of exogenous somatostatin may be extracted by the pancreas, presumably by exocrine tissue (21,22). Thus, during retrograde perfusion, the islets may have received 2 or 40 ng/ml of exogenous somatostatin, in contrast with 4 or 80 ng/ml during anterograde infusions. Maximal retrograde inhibition of the α- and β-cells was obtained only with the high dose of somatostatin, which is ~10-fold higher than the low dose given anterogradely that produced a similar degree of inhibition

(Table 3). Therefore, the possible retrograde extraction of exogenous somatostatin does not explain the anterograde and retrograde differences in α- and β-cell inhibition. These results support the possibility of receptor occupancy, or desensitization, during retrograde perfusion. This conclusion is strengthened by the increase in insulin and glucagon secretion reported from the retrograde infusion of somatostatin antibody into the rat pancreas (Table 5). Thus, the higher concentration of exogenous somatostatin may have been required to overcome receptor occupancy during retrograde perfusions, especially because a prolonged retrograde equilibration period, 60 min, was used.

The presence and importance of directed intraislet blood flow was further tested by the infusion of exogenous insulin. As previously reported, the anterograde infusion of 0.3 mU/ml exogenous porcine insulin had no effect on efflux concentrations of C-peptide or glucagon (5,6). There was no change in endogenous somatostatin secretion (Table 4). We have suggested that the lack of change in anterograde islet hormone release during the infusion of low concentrations of exogenous insulin is the result of the tonic exposure of α- and δ-cell insulin receptors to endogenous insulin in a core-to-mantle sequence of cellular vascular perfusion (2,3,5,8,19). In contrast, retrograde infusion of 0.3 mU/ml insulin had no effect on somatostatin or C-peptide efflux concentrations (Table 4) but significantly inhibited glucagon secretion (5). These results further confirm that vascular flow within the islet is from the β-cell core to the α- and δ-cell mantle and that the direction of flow is important in regulating intraislet cellular interactions.

We considered that retrograde perfusion may have affected net hormone secretion from the islet as a result of increased venous perfusion pressure or changes in hemodynamics. We have noted that increased pressure during anterograde perfusion results in increased insulin efflux concentrations (23); however, there was a concomitant decrease in glucagon and somatostatin concentrations consistent with β → α → δ cellular sequence of vascular perfusion. Because retrograde perfusion pressure remained constant and a 60-min equilibration period was used before the infusion of exogenous hormones or antibodies, and because of the marked differences in response depending on the specific γ-globulin or peptide infused anterogradely or retrogradely, our results cannot be explained on the basis of perfusion artifacts induced by pressure or collateral circulation within the pancreas. We have reported elsewhere that in experiments perfusing the same pancreas anterogradely, retrogradely, and then anterogradely, normal anterograde levels of insulin and glucagon were obtained after anterograde perfusion was reestablished, confirming that no vascular damage had occurred that may have affected hormone secretion (24). The results presented herein confirm our previous hypothesis that intraislet cellular interactions are flow dependent (2,3,5) and further suggest that, analogous to the kidney glomerulus, a single or continuous capillary system is present within the individual islets. The concept of receptor compartmentalization by an intercellular barrier system (25) is not supported by these results or by recent evidence that tight junctions are not present in islets fixed in situ (26,27).

Compartmentalization is a concept proposed to explain the sensitivity of the α- and β-cells to the infusion of exogenous somatostatin (25). We propose that the sensitivity of the α- and β-cells to exogenous somatostatin or somatostatin entering the pancreas from the general circulation is readily explained by the direction or sequence of cellular vascular perfusion, with the α- and β-cells preceding the δ-cell. Therefore, the α- and β-cells are not exposed to intraislet endogenous somatostatin. We propose that the normal separation of glucagon receptors on the β-cell from highly concentrated intraislet glucagon, i.e., compartmentalization, is achieved by the direction of blood flow within a continuous intraislet capillary system.

In summary, this study confirms that the direction of intraislet blood flow is important in the regulation of intraislet cellular interactions in the dog as well as in the rat islet (2,3,5). These observations also indicate that there is an order or hierarchy of cellular perfusion within the islet, β → α → δ. This direction of islet blood flow is central to the regulation of islet hormone secretion.

ACKNOWLEDGMENTS

We thank J. Faircloth, M. Lewis, M.J. Mauch, and V. Weis for skillful technical assistance and S. James for preparing the manuscript.

This research was supported through the Veterans Administration and the Norton Research Fund.

REFERENCES

1. Samols E: Glucagon and insulin secretion. In *Handbook of Experimental Pharmacology*. Vol. 66/I. Lefebvre PJ, Ed. Berlin, Springer-Verlag, 1983, p. 485–518
2. Samols E, Stagner JI, Ewart RBL, Marks V: The order of islet microvascular cellular perfusion is B-A-D in the perfused rat pancreas. *J Clin Invest*. In press
3. Samols E, Stagner J: Intra-islet regulation. *Am J Med*. In press
4. Kawai K, Chiba Y, Okuda Y, Yamashita K: Hormone release from pancreatic islets perfused from venous side. *Diabetes* 36:256–60, 1987
5. Stagner JI, Samols E: Retrograde perfusion as a model for testing the relative effects of glucose versus insulin on the A-cell. *J Clin Invest* 77:1034–37, 1986
6. Stagner J, Samols E, Polonsky K, Pugh W: Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. *J Clin Invest* 78:1193–98, 1986
7. Weir GC, Samols E, Loo S, Patel YC, Gabbay KH: Somatostatin and pancreatic polypeptide secretion: effects of glucagon, insulin, and arginine. *Diabetes* 28:35–40, 1979
8. Kvietys PR, Percy MA, Grange DN: Permeability of pancreatic capillaries to small molecules. *Am J Physiol* 245:G519–24, 1983
9. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296–99, 1984
10. Campbell DH, Garvey JS, Cremer NE, Sussdorf DH: *Methods in Immunology*. New York, Benjamin, 1964, p. 118–20
11. Polonsky K, Jaspan J, Pugh W, Cohen D, Schneider M, Schwartz T, Mossa AR, Jage H, Rubenstein AH: Metabolism of C-peptide in the dog: in vivo demonstration of the absence of hepatic extraction. *J Clin Invest* 72:1114–23, 1983
12. Samols E, Weir GC, Bonner-Weir S: Intra islet insulin-glucagon-somatostatin relationships. In *Handbook of Experimental Pharmacology*. Vol. 66, part II. Lefebvre PJ, Ed. Berlin, Springer-Verlag, 1983, p. 133–73.
13. Samols E, Tyler J, Marks V: Glucagon-insulin interrelationships. In *Glucagon. Molecular Physiology, Clinical and Therapeutic Implications*. Lefebvre PJ, Ed. Oxford, UK, Pergamon, 1972, p. 151–73
14. Fujita T: Morphology of islet blood supply. In *Methods in Diabetes Research*. Vol. I. Larner J, Pohl SL, Eds. New York, Wiley, 1985, p. 325–32
15. Pipeleers D: Islet cell interactions with pancreatic B-cells. *Experientia* 40:1114–26, 1984
16. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883–89, 1982
17. Stagner J, Samols E, Taborsky J: Mediation of B-cell secretion by intraislet somatostatin (Abstract). *Diabetes* 36 (Suppl 1):7A, 1987
18. Kawai K, Unger RH: Inhibition of glucagon secretion by exogenous glu-

cagon in the isolated, perfused dog pancreas. *Diabetes* 31:512–15, 1982
19. Patel YC, Anherdt M, Orci L: Quantitative electron microscopic autoradiography of insulin: glucagon and somatostatin binding sites on islets. *Science* 217:1155–56, 1982
20. Samols E, Stagner J: Flow versus compartmentalization in the determination of intra-islet hormone interactions (Abstract). *Clin Res* 34:687A, 1986
21. Kawai K, Orci L, Unger RH: High somatostatin uptake by the isolated perfused dog pancreas consistent with an insulo-acinar axis. *Endocrinology* 110:660–62, 1982
22. Taborsky GJ, Ensinck JW: Extraction of somatostatin by the pancreas. *Endocrinology* 112:303–307, 1983
23. Stagner J, Samols E: In vitro insulin cycles from the isolated canine pancreas—possible control by an intrinsic nonadrenergic nervous system (Abstract). *Diabetes* 29 (Suppl. 2):26A, 1980
24. Stagner J, Samols E: Comparison of insulin and glucagon pulsatile secretion between the rat and dog pancreas in vitro. *Life Sci* 43:929–34, 1988
25. Kawai K, Ipps E, Orci L, Perrelet A, Unger RH: Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin-poor compartment. *Science* 218:477–78, 1982
26. in't Veld PA, Pipeleers DG, Gepts W: Evidence against the presence of tight junctions in normal endocrine pancreas. *Diabetes* 33:101–104, 1984
27. Yamamoto M, Kataoka K: A comparative study on the intercellular canalicular system and intercellular junctions in the pancreatic islets of some rodents. *Arch Histol Jpn* 47:485–93, 1984