Exogenous Insulin Dose-Dependently Suppresses Glucopenia-Induced Glucagon Secretion From Perfused Rat Pancreas

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To clarify the role of insulin in modulating the glucagon response to glucose concentration changes, we investigated the effects of exogenous insulin (10 mU/mL, 100 mU/mL, and 3.3 U/mL) on responses to high glucose (5.6 → 16.7 mmol/L), low glucose (5.6 → 1.4 mmol/L), and arginine (10 mmol/L) stimulation using the perfused rat pancreas. Although glucagon levels were slightly suppressed by all of the exogenous insulin concentrations tested for the initial few minutes at 5.6 mmol/L glucose, baseline levels were maintained thereafter. Glucagon responses to high or normal glucose concentrations were not altered, but glucopenia-induced glucagon secretion was significantly suppressed as compared with that of controls (0.77 ± 0.14 ng/min [10 mU/mL, n = 5], 0.55 ± 0.14 ng/min [100 mU/mL, n = 5], 0.27 ± 0.13 ng/min [3.3 U/mL, n = 5] *v* 1.38 ± 0.20 ng/min [controls, n = 9], $P < .05$, respectively). The first phase of the glucagon response to arginine was potentiated (2.03 ± 0.24 *v* 1.17 ± 0.22 ng/min, $P < .05$) by 10 mU/mL exogenous insulin. The second phase of the glucagon response to arginine was significantly suppressed in the presence of higher concentrations of exogenous insulin (1.16 ± 0.23 ng/min [100 mU/mL], 0.96 ± 0.08 ng/min [3.3 U/mL] *v* 1.57 ± 0.17 ng/min, $P < .05$, respectively). These results suggest that glucagon secretion is modified by the combined suppressive effects of glucose and insulin, although it is mainly glucose that mediates glucagon secretion in the physiological glucose range. Glucopenia- or arginine-induced glucagon secretion is suppressed by insulin.

LOCAL INSULIN SECRETION is thought to exert a major influence on glucagon secretion.[1-4] Studies of the microcirculation of the rat islet indicate that blood flows from the B-cell–rich islet medulla to its A-cell–rich cortex before leaving the islet.[5] A cells are thus exposed to the highest insulin concentration in the body. It seems that insulin within the islet microcirculation acts as a modulator of glucagon secretion.[6,7] However, the regulatory role of insulin in pancreatic A-cell function remains controversial. Inhibition of glucagon secretion could be mediated by insulin alone[3,8-12] or by glucose in conjunction with insulin,[13,14] probably via enhanced glucose transport into pancreatic A cells.[15,16]

Glucagon secretion decreases in response to high, glucose stimulation and increases in response to low glucose stimulation in the perfused rat pancreas, just as it does in vivo.[17,18] In the present experiment, we used the perfused rat pancreas to investigate the effects of exogenous insulin on glucagon responses to high glucose, low glucose, and arginine stimulation to elucidate the role of insulin in the modulation of glucagon secretion.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 300 to 400 g were used.

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Experimental Procedure

Pancreata were isolated and perfused using a modification of the Grodsky and Fanska method,[19] as described previously.[20,21] Our institution's guidelines for the care and use of laboratory animals were followed. The perfusate was Krebs-Ringer bicarbonate buffer supplemented with 4.5% (wt/vol) dextran T-70 (Pharmacia LKB Biotechnology, Uppsala, Sweden), 1% (wt/vol) bovine serum albumin (Miles, Kankakee, IL), and 5 mmol/L sodium pyruvate, sodium fumarate, and sodium glutamate (Sigma, St Louis, MO), and the flow rate was set at a constant 3.0 mL/min. The partial pressure of oxygen was maintained between 450 and 550 mm Hg by a bubble oxygenator using a 95% O_2/5% CO_2 gas mixture. The perfusate pH was maintained between 7.35 and 7.45. A preperfusion period of approximately 15 minutes sufficient to stabilize glucagon levels was used in all protocols. Over the course of a 75-minute experimental period, the perfused pancreata were successively exposed to glucose concentrations of 5.6, 16.7, 5.6, 1.4, and 5.6 mmol/L for 20, 10, 10, 15, and finally 20 minutes, respectively. L-Arginine hydrochloride (Sigma) was added at 65 minutes for 10 minutes to achieve an arginine concentration of 10 mmol/L in the perfusate. This protocol was used for the control and all the following experiments. The glucagon concentration of the perfusate was determined at 1-minute intervals for the entire experimental period in the control study, and glucagon concentrations were measured every 2 minutes in the other studies.

In the first experiment, to clarify the effect of exogenous insulin on glucagon secretion, insulin (insulin human [synthesis], Actrapid Human; Novo Nordics, Copenhagen, Denmark) was added to the perfusion buffer via an infusion pump (flow rate, 0.1 mL/min) at 5 minutes, to achieve an insulin concentration of 10 mU/mL in the perfusate. In the second and third experiments, the effects of 100 mU/mL or 3.3 U/mL insulin were examined.

Measurement of Hormone Levels

The immunoreactive glucagon (IRG) level was measured by a previously described method using antiserum to synthetic glucagon(19-29).[22]

Statistical Analyses

All data are expressed as the mean ± SEM, and statistical comparisons of means within a group were made using Student's

paired *t* tests. Comparisons between the means of different groups were made by one-way ANOVA, followed by Dunnett's multiple comparison test if the null hypothesis was rejected by the former. Differences were considered significant when P was less than .05.

RESULTS

Glucose and Arginine Control Over Glucagon Secretion (control study)

As shown in Fig 1, the control preparations were perfused with a 5.6-mmol/L glucose perfusate, and when glucose was increased to 16.7 mmol/L glucagon secretion was immediately suppressed. When the perfusate glucose was changed back to 5.6 mmol/L, the glucagon concentration returned approximately to baseline. Glucose was subsequently decreased to 1.4 mmol/L, producing a monophasic glucagon-release pattern. When the perfusate glucose was restored to 5.6 mmol/L, the glucagon concentration again returned to baseline. The addition of 10 mmol/L arginine to the perfusate produced a biphasic glucagon-release pattern.

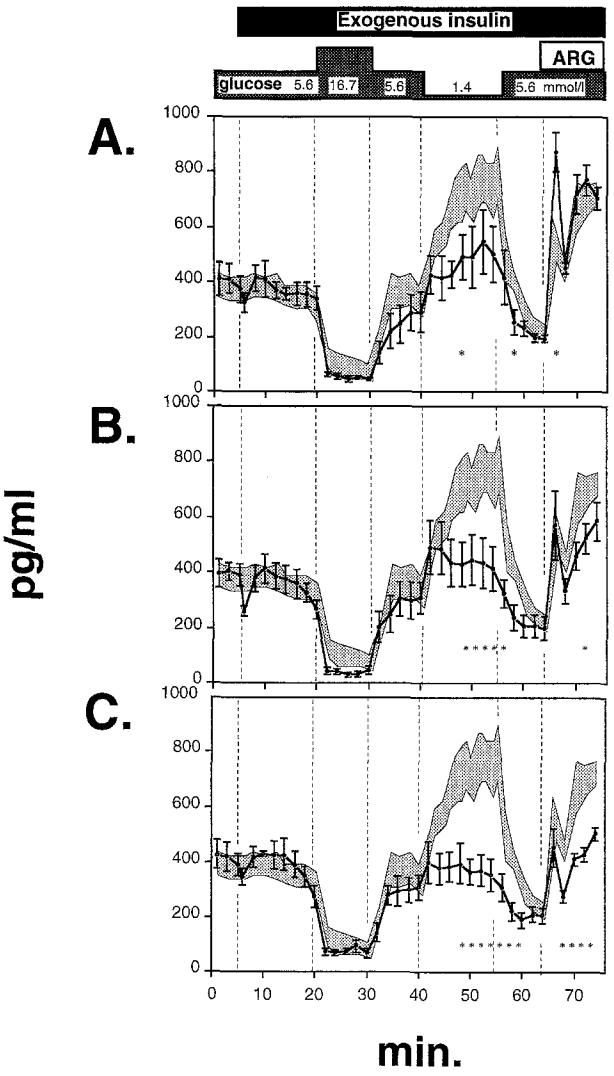

Fig 1. Effects of exogenous insulin on glucagon (IRG) secretion in response to changes in the glucose concentration and arginine (ARG). Means ± SE (pg/mL) of IRG responses of controls are indicated by shaded areas (n = 9). (A) With 10 mU/mL exogenous insulin (n = 5); (B) with 100 mU/mL exogenous insulin (n = 5); (C) with 3.3 U/mL exogenous insulin (n = 5). *P < .05 compared with controls. ARG, 10 mmol/L.

Exogenous Insulin Control Over Glucagon Secretion

In the first experiment, 10 mU/mL insulin was added to the perfusate, and in the second and third experiments 100 mU/mL or 3.3 U/mL insulin were added at 5 minutes.

Glucagon secretion was slightly suppressed by all tested concentrations of exogenous insulin for the initial few minutes at 5.6 mmol/L glucose (not significant), but returned to the baseline level immediately thereafter.

Glucagon suppression in response to high glucose was not changed by any of the exogenous insulin concentrations tested.

On the other hand, glucopenia-induced glucagon secretion was significantly suppressed (P < .05) as compared with that of the control in a dose-dependent manner (Fig 1, Table 1).

The first phase of the glucagon response to arginine was potentiated (P < .05) by 10 mU/mL exogenous insulin. The second phase of the glucagon response to arginine was significantly suppressed (P < .05) in the presence of higher concentrations (100 mU/mL, 3.3 U/mL) of exogenous insulin (Fig 1, Table 1).

DISCUSSION

Although we observed no suppression of glucagon secretion at either 5.6 or 16.7 mmol/L glucose at any exogenous insulin concentration, insulin did significantly suppress glucopenia-induced glucagon secretion as compared with that of controls in a dose-dependent manner.

In the normal pancreas, endogenous insulin may persistently suppress glucagon secretion at physiological glucose levels.[3] Thus, it might be impossible to suppress glucagon secretion any further in response to exogenous insulin at a normal or higher glucose level. At 1.4 mmol/L glucose, endogenous insulin is almost completely inhibited and the insulin concentration around A cells is very low. Exogenous insulin may be capable of suppressing glucagon secretion at this glucose level.[1]

Our data suggest that insulin can suppress glucopenia-induced glucagon secretion. However, the suppression of glucopenia-induced glucagon secretion by insulin is subject to limitations, possibly mediated by the ambient glucose concentration.[23,24]

From another standpoint, high doses of exogenous insulin suppress endogenous insulin secretion.[2] With the application of exogenous insulin, the insulin concentration around A cells could be maintained at almost the same level as the exogenous insulin concentration. Our results indicate that a change in the glucose concentration rather than the insulin level is essential for glucagon modulation at normal or high glucose concentrations, ie, glucose directly suppresses glucagon secretion, whereas insulin has only a permissive role. If there is sufficient insulin, which is normally the case in proximity to pancreatic A cells at

Table 1. Effects of Exogenous Insulin on Glucagon (IRG) Secretion in Response to Changes in the Glucose Concentration and to Arginine Stimulation

	Basal Secretion Rate (ng/min)		Maximal Secretion Rate (ng/min)	Change (ng/min)
Glucose 5.6 → 1.4 mmol/L				
Control (n = 9)	1.03 ± 0.17		2.41 ± 0.26	1.38 ± 0.17
10 mU insulin (n = 5)	0.88 ± 0.22		1.64 ± 0.35	0.77 ± 0.12*
100 mU insulin (n = 5)	0.93 ± 0.17		1.48 ± 0.28	0.55 ± 0.12*
3.3 U insulin (n = 5)	0.93 ± 0.14		1.20 ± 0.23	0.27 ± 0.10†
+Arginine 10 mmol/L				
Control (n = 9)	0.69 ± 0.08	1st phase	1.71 ± 0.23	1.17 ± 0.20
		2nd phase	2.21 ± 0.13	1.57 ± 0.15
10 mU insulin (n = 5)	0.59 ± 0.04	1st phase	2.61 ± 0.21	2.03 ± 0.22*
		2nd phase	2.12 ± 0.12	1.73 ± 0.16
100 mU insulin (n = 5)	0.60 ± 0.13	1st phase	1.72 ± 0.38	1.12 ± 0.24
		2nd phase	1.76 ± 0.20	1.16 ± 0.20*
3.3 U insulin (n = 5)	0.62 ± 0.04	1st phase	1.37 ± 0.20	0.75 ± 0.16
		2nd phase	1.52 ± 0.06	0.90 ± 0.08†

NOTE. Values are the mean ± SEM.
*$P < .05$, †$P < .01$: compared with controls.

physiological glucose levels, increased insulin secretion is not necessary for the suppression of glucagon secretion by a high glucose concentration.[13,14] However, with persistently elevated insulin around A cells, glucopenia-induced glucagon secretion would be strongly suppressed. Thus, a decreased intraislet insulin concentration is important for increased glucagon secretion in response to glucopenia, but may not be essential.

Asplin et al[25] reported that hyperinsulinemia within the physiological range achieved by insulin infusion inhibited B-cell secretion and, via a paracrine mechanism, potentiated arginine-induced glucagon secretion in a nondiabetic human glucose-clamp study. Furthermore, it has been reported that in an insulin-dependent diabetic human glucose-clamp study[25] and in a diabetic perfused rat pancreas,[1] endogenous insulin is minimal and exogenous insulin suppresses arginine-induced[1,25] or epinephrine-induced[1] glucagon secretion. In our study, the relatively low concentration of exogenous insulin (10 mU/mL) promoted the first phase of arginine-induced glucagon secretion. However, higher concentrations (100 mU/mL or 3.3 U/mL) of insulin significantly suppressed the second phase but not the first phase of arginine-induced glucagon secretion. Ten milliunits per milliliter of insulin appears to be insufficient to suppress arginine-induced glucagon secretion. Weir et al[1] reported that 20 mU/mL exogenous insulin did not suppress arginine-induced glucagon secretion from the perfused normal rat pancreas.

We speculate that exogenous insulin suppresses endogenous insulin surrounding A cells and that the concentration of insulin around A cells is almost the same as the exogenous insulin concentration. If this is the case, the insulin concentration surrounding A cells would be expected to decrease when exogenous insulin is lower than endogenous insulin. This situation would be seen with arginine stimulation in vivo hyperinsulinemic clamp studies. Furthermore, if the concentration of exogenous insulin is higher than that of endogenous insulin, glucagon secretion is suppressed because the insulin concentration surrounding A cells increases.

According to the intraislet portal system concept[12,26] (Fig 2), endogenous insulin continuously influences glucagon secretion via the microcirculation rather than via gap junctions between islet cells. Our data could be explained by this mechanism, operating without a paracrine contribution.

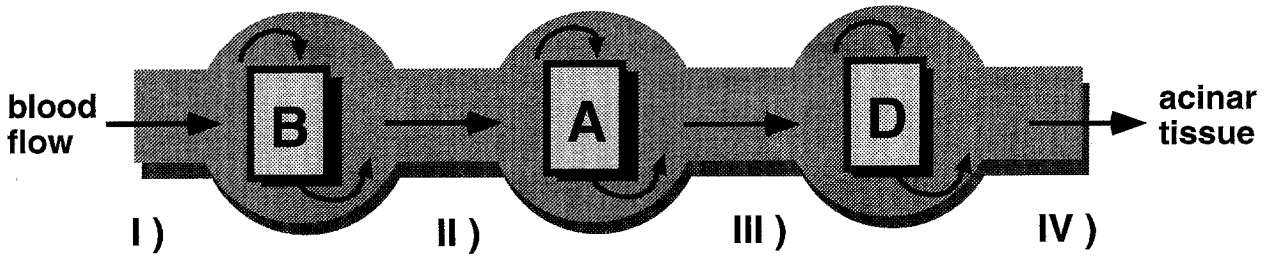

Fig 2. Intraislet portal microcirculation system.[26] According to this hypothesis, A cells and D cells are exposed to the highest insulin concentration in the body. B cells are influenced by several hormones such as epinephrine and incretin hormones. B cells may also be influenced by glucagon or somatostatin via the systemic circulation. In the rat, B cells have been found to have two capillaries to cell surface contacts. Such cellular surface specialization usually reflects either a sensory or secretory function.[30] (I) Incretin hormones (glucagon-like peptide-1, gastric inhibitory peptide, etc) + epinephrine (from adrenal gland); (II) I + insulin + gamma-aminobutyric acid?; (III) II + glucagon; (IV) III + somatostatin.

Filipponi et al[13] reported that exogenous insulin at a concentration of 20 mU/mL did not suppress glucagon release (glucose concentration, 3.9 mmol/L) in the alloxan-treated, endogenous insulin-depleted, perfused rat pancreas. Thus, in the present study, it is unlikely that exogenous insulin suppressed glucagon secretion at a low glucose concentration because endogenous insulin was very low.

The glucagon-promoting factors operating during hypoglycemic stress should be distinguished from factors maintaining glucagon secretion within a physiological glucose range. Endogenous norepinephrine is released in response to glucopenia from the perfused canine pancreas.[27] In the perfused rat pancreas, an α-adrenoceptor inhibitor was shown to suppress glucopenia-induced glucagon secretion.[28] Some neurotransmitters appear to enhance the glucagon secretion induced by glucopenia in the perfused rat pancreas. Thus, a difference in promoting factors could underlie the exogenous insulin suppression of glucagon secretion at a low glucose concentration, but have no effect at normal or high glucose concentrations in the perfused rat pancreas.

Furthermore, we demonstrated in preliminary studies using a single sequence of glucopenic stimulation that exogenous insulin did suppress this glucopenia-induced glucagon secretion (n = 4, data not shown).

In conclusion, glucagon secretion is modified by the combined suppressive effects of glucose and insulin. It is mainly glucose that mediates glucagon secretion in response to physiological changes in the glucose concentration, but this modulatory effect might be imprecise without an adequate quantity of ambient insulin. Glucagon secretion, whether induced by glucopenia or arginine, is suppressed by insulin. Glucopenia-induced glucagon secretion may be suppressed by insulin, since it is mediated mainly by endogenous neurotransmitters, such as norepinephrine, in the perfused rat pancreas, as well as in vivo.[29]

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