The alpha cell response to glucose change during perfusion of anti-insulin serum in pancreas isolated from normal rats

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Summary. To determine the effect of neutralization of endogenous insulin upon the glucagon response to a rise and fall of glucose concentration, pancreata isolated from normal rats were perfused with either a potent anti-pork insulin guinea pig serum or a nonimmune guinea pig serum for 30 min. During this period glucose concentration was changed from 100 mg/dl to either 130, 180 or 80 mg/dl for 10 min. Antiserum perfusion at 100 mg/dl caused an approximately two-fold increase in glucagon which was not suppressed by an increase in glucose concentration to either 130 or 180 mg/dl, although glucagon secretion was significantly suppressed in the control experiments in which nonimmune serum was perfused. However, the 0.38 ± 0.21 ng/min rise in glucagon secretion in response

to a reduction in glucose concentration to 80 mg/dl in the control experiments was not abolished by antiserum perfusion but, instead, was enhanced (2.66 ± 0.60 ng/min). These findings suggest that insulin may be required for glucose-mediated suppression of glucagon in the isolated pancreas of normal rats but not for stimulation of glucagon secretion by mild glucopenia. Alternatively, neutralization of insulin-mediated release-inhibition of glucagon secretion may simply have altered alpha cell responsiveness in a direction that desensitized it nonspecifically to suppression and sensitized it to stimulation.

Key words: Alpha cells, glucagon, anti-insulin serum.

Recent studies in isolated pancreata of normal rats suggest that endogenous insulin passing through the islet microcirculation exerts a constant tonic restraining influence upon basal glucagon secretion during euglycaemia [1]. However, it is not known if, in addition, changes in insulin secretion mediate alpha cell responses to acute changes in glucose concentration, as might be inferred from the fact that the glucagon response to hyper- and hypoglycaemia disappears following spontaneous or experimental destruction of the B cells of the islets of Langerhans [2–5]. However, such functional deficits in diabetic alpha cells could just as well be the consequence of other factors such as post-inflammatory changes in the islet tissue or of the antecedent hyperglycaemia [6–8] as of the loss of endogenous insulin. Therefore, the present study was designed to assess the role of insulin in the glucagon response of normal alpha cells to glycaemic change during acute in vitro insulin deficiency induced with neutralizing anti-insulin serum in normal rat islets, thereby eliminating antecedent hyperglycaemia as a possible variable.

Materials and methods

Pancreata of 300–400 g male Long-Evans rats were isolated and perfused using the methods of Fanska and Grodsky [9] with modifications as previously described [10]. The perfusate contained 10 mmol arginine HCl, a standard practice designed to maintain high baseline glucagon secretion. Glucose concentration was maintained at 100 mg/dl for the initial 21 min, changed to 130, 180 or 80 mg/dl for 10 min and returned to 100 mg/dl for the final 19 min. The reduction in glucose (from 100 to 80 mg/dl) was kept within the physiologic range of glycaemia because of previous evidence that the glucagon response to more profound glucopenia is mediated largely by release of catecholamines [10]. From 11 to 40 min after the start of the perfusion, 0.1 ml/min of undiluted guinea pig anti-porcine insulin serum (Miles-Yeda lot GP-29) or of a normal guinea pig serum (Miles lot NGP-28) was added via a sidearm.

IRG was assayed by a previously described method using 30 K antiserum [11]. Insulin was measured by the Herbert modification [12] of the method of Yalow and Berson [13]. Free insulin determinations could not be made in perfusate specimens collected during antiserum perfusion because of an extraordinarily high concentration of antibodies which could not be eliminated despite repeated polyethylene glycol treatment [1]. Antiserum GP-29 contained 139 pg/ml immunoreactive glucagon (IRG) and NGP-28 contained 102 pg/ml IRG and 3 μ U/ml IRI. All data were expressed as the mean $\pm$ SEM. Statistical significance was calculated using the paired Student's t-test. Glucagon secretion rate was calculated by averaging the mean of glucagon determinations for a given period and multiplying by the flow rate for the period.

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Fig. 1. A The effect of an increase in perfusate glucose concentration from 100 mg/dl to 130 mg/dl during the perfusion of either anti-pork insulin guinea pig antiserum ($n=5$) or nonimmune guinea pig serum ($n=7$) in pancreata isolated from normal rats. Perfusate contained 10 mmol arginine. B The effect of an increase in perfusate glucose concentration from 100 mg/dl to 180 mg/dl during the perfusion of either anti-pork insulin guinea pig antiserum ($n=3$) or nonimmune guinea pig serum ($n=5$). Insulin measurements were not performed in perfusate specimens containing antiserum. Asterisks indicate significant change in glucagon compared with the baseline value ($p<0.05$)

Results

The effect of an increase in glucose concentration upon glucagon secretion by the normal rat pancreas in the presence and absence of anti-insulin serum

In control experiments in which nonimmune guinea pig serum was perfused, an increase in glucose concentration from 100 mg/dl to 130 or to 180 mg/dl for 10 min elicited a significant reduction of glucagon secretion at almost all time points ($p<0.05$) (Fig. 1). The glucagon secretion rate during the 10 min was significantly below the rate observed in both the initial and final 10-min periods of perfusion at 100 mg/dl ($p<0.01$) (Tables 1 B, C). Upon restoring the perfusate glucose concentration of the 100 mg/dl level, a modest and transient increase in glucagon above its original value was observed (Fig. 1). Insulin, which for unexplained reasons was significantly suppressed during nonperfusion of nonimmune serum, rose in the familiar biphasic pattern when glucose concentration was increased.

In experiments in which guinea pig anti-pork insulin antiserum was perfused, a prompt two-fold rise in glucagon occurred as previously reported [1]. In this setting an increase in perfusate glucose concentration from 100 mg/dl to 130 or to 180 mg/dl (Fig. 1) failed to reduce the glucagon secretion rate significantly below either the preceding or subsequent 100 mg/dl perfusion periods (Table 1 B and C).

The effects of a reduction of perfusate glucose concentration on the glucagon response of the normal rat pancreas in the presence and absence of anti-insulin serum

To determine the role of endogenous insulin within the islets in the stimulation of glucagon secretion by glucopenia, the concentration in the perfusate was reduced from 100 mg/dl to 80 mg/dl for 10 min during a 30-min perfusion of either anti-insulin or nonimmune guinea pig serum.

During perfusion with nonimmune serum, the slight glucopenia elicited a small rise in glucagon that was sig-

Table 1. Effect of a change in perfusate glucose concentration on the rate of glucagon secretion by isolated pancreata from normal rats during perfusion of non-immune or anti-insulin guinea pig serum

Time period (min)	Glucagon secretion rate (ng/min) ^a			
	19–20		21–30	31–40
A. Glucose concentration (mg/dl)	100		100	100
Non-immune serum (<i>n</i> =4)	1.27 ± 0.07	NS	1.29 ± 0.05	1.29 ± 0.06
Anti-insulin serum (<i>n</i> =6)	9.97 ± 1.35	NS	10.06 ± 1.28	9.80 ± 1.70
B. Glucose concentration (mg/dl)	100		130	100
Non-immune serum (<i>n</i> =7)	3.61 ± 0.26	<i>p</i> < 0.01	2.85 ± 0.55	3.67 ± 0.85
Anti-insulin serum (<i>n</i> =5)	6.90 ± 1.49	NS	7.78 ± 1.82	7.01 ± 1.78
C. Glucose concentration (mg/dl)	100		180	100
Non-immune serum (<i>n</i> =5)	3.00 ± 0.45	<i>p</i> < 0.01	1.93 ± 0.31	2.60 ± 0.36
Anti-insulin serum (<i>n</i> =3)	5.06 ± 0.12	NS	5.76 ± 0.71	5.96 ± 1.14
D. Glucose concentration (mg/dl)	100		80	100
Non-immune serum (<i>n</i> =6)	2.26 ± 0.28	NS	2.63 ± 0.47	2.02 ± 0.29
Anti-insulin serum (<i>n</i> =6)	9.63 ± 0.66	<i>p</i> < 0.01	12.30 ± 0.94	10.63 ± 0.94

^a Calculation based on mean of glucagon levels at all time points within the specified time period multiplied by the flow rate. Mean ± SEM

Fig. 2. The effect of a small reduction in perfusate glucose concentration upon glucagon secretion during the perfusion of either anti-pork insulin guinea pig antiserum (*n*=6) or nonimmune guinea pig serum in pancreata isolated from normal rats (*n*=6). Perfusate contained 10 mmol arginine. Insulin measurements were not performed in perfusate specimens containing antiserum. Asterisks indicate significant change in glucagon compared with the baseline value (*p* < 0.05)

nificant at only one time point, and a reciprocal decline in insulin levels (Fig. 2). Glucagon secretion rate rose from 2.26 ± 0.28 ng/min to 2.63 ± 0.47 ng/min (NS), and declined to 2.02 ± 0.29 ng/min (*p* < 0.05) upon restoration of the glucose level to 100 mg/dl (Table 1 D). Insulin declined significantly during the glucopenic period (Fig. 2). During perfusion of antiinsulin serum the glucagon secretion rate rose significantly from 9.63 ± 0.66 ng/min to 12.3 ± 0.94 ng/min (*p* < 0.01). The mean increase in secretion rate was 2.66 ± 0.60 ng/min compared to an increase of 0.38 ± 0.21 ng/min in the control experiments with nonimmune serum (Table 1 D). When glucose concentration was maintained at 100 mg/dl throughout the experiment (Table 1 A), there was no increase in glucagon at the comparable time interval during antiserum perfusion.

Discussion

Samols and coworkers in 1965 first identified the glucagon suppressing effect of insulin [14], and postulated a regulatory role for insulin upon the alpha cell response to glycaemic change [15, 16]. According to the hypothesis, the suppression of glucagon by hyperglycaemia and its stimulation by hypoglycaemia might at least in part be mediated by glucose induced changes in insulin secretion, a construct that would account for the loss of these alpha cell responses following destruction of B cells [2–5].

Against this hypothesis are two pieces of evidence suggesting that alpha cells can respond to a change in glucose concentration independently of insulin and B cells. In insulin-deprived dogs with alloxan-induced deficiency of endogenous insulin, glucagon can be sup-

pressed normally by glucose provided that hyperglycaemia is first eliminated [6]; in Type I diabetic patients the glucagon response to insulin-induced hypoglycaemia remains normal for the first 15 months following destruction of the B cells [17]. However, since the foregoing evidence was obtained in diabetic islets in which B cells were destroyed by cytotoxic or immune-mediated events and which had been chronically exposed to hyperglycaemia, they may not be relevant to the physiologic role of insulin in normal islets that have never been damaged or exposed to a diabetic metabolic milieu. Prolonged hyperglycaemia itself may impair islet cell function [6–8]. To dissociate the effects of insulin deficiency from the effects of concomitant hyperglycaemia, the influence of an acute deficiency of endogenous insulin upon the alpha cell response to glycaemic change was examined in islets from normal rats without antecedent hyperglycaemic experience. The prompt rise in glucagon induced by antiserum perfusion and its fall soon after its termination was construed as evidence that the antiserum had lowered the insulin concentration within the islet to below the level required to restrain glucagon secretion [1]. Measurements of free insulin in antiserum-containing pancreatic effluent could not be made because of the persistence of antibodies despite repeated extractions. However, aliquots of the antiserum-containing perfusate employed in these studies (Miles-Yeda GP-29) are known to neutralize 100% of 20 mU/ml of insulin [1], the estimated concentration of insulin within the islet circulation [18], during a 1-min incubation at 37° in simulation of the conditions of antiserum contact with endogenous insulin during its transit through the pancreas [1]. Moreover, since electronmicroscopic examination of pancreas obtained after perfusion with antiserum at a constant 100 mg/dl glucose concentration (not shown) revealed no B cell degranulation, it is likely that in this constant metabolic milieu antiserum does not cause a compensatory increase in insulin secretion that might exceed the neutralizing capacity of the perfusate. Therefore, its neutralizing effect was probably constant throughout the experiment.

Perfusion of antiserum blocked glucagon suppression by a 30 mg/dl and 80 mg/dl increase in glucose but failed to prevent the stimulation of glucagon secretion by a 20 mg/dl decrease in glucose. Instead, the glucagon response seemed greater, making it unlikely that insulin played an obligatory role in the alpha cell response to glucopenia. This conclusion is consistent with other *in vivo* studies [17]. It is also unlikely that norepinephrine, which mediates at least 70% of the response of the alpha cell to more profound glucopenia [10], was the mediator, since in the present experiments glucopenia was well within the physiologic range in which the role of norepinephrine is thought to be minimal [10]. The marked exaggeration of the glucagon response to glucopenia during antiserum perfusion may therefore

point to potentiation of a mediator other than norepinephrine or insulin.

A second interpretation of these data is that by eliminating a constant restraining influence upon alpha cells the antiserum simply shifted alpha cell responsiveness in a direction that sensitized them to stimulation and desensitized them to suppression. This is consistent with studies in Type I diabetic patients who demonstrate an exaggerated glucagon response to stimuli such as arginine [2] and its correction by insulin [19, 20]. However, it is not consistent with the demonstration that glucagon is suppressed by glucose *in vivo* in the complete absence of insulin in insulin-deprived alloxan-diabetic dogs rendered chronically hypoglycaemic by phloridzin pretreatment [6]. The latter finding suggests that alpha cells can be suppressed by glucose independently of insulin and cells. It is not known if the apparent conflict in findings is due to species difference, the higher baseline glucose concentration of the *in vitro* studies (100 mg/dl vs. ~60 mg/dl), or the many other experimental and anatomical differences between the two preparations, including the lack of cells in the alloxan-diabetic dog islets and/or the severance of neural connections in the isolated rat pancreas.

In the experiments depicted in Figures 1 B and 2, a paradoxical single point rise in glucagon was observed immediately upon termination of the perfusion of anti-insulin serum. This phenomenon had been observed earlier [1], and is probably due to norepinephrine release, which has been reported to be caused by insulin [21, 22]. Coperfusion of phentolamine and propranolol throughout perfusion experiments using anti-insulin antiserum abolished the post-antiserum rise in glucagon (Maruyama, unpublished observation).

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