Divergent effect of glucagon antibodies on arginine and glucose-stimulated insulin secretion in the rat

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Summary. The effects of glucose and arginine on insulin secretion in the presence of glucagon antibodies were investigated in rats *in vivo*. In contrast to controls, animals given glucagon antibodies showed an inhibition of arginine-stimulated ($p < 0.001$), but not glucose-stimulated, insulin secretion. That these effects were not due to incomplete neutralisation of endogenous glucagon is evidenced by the presence of large antibody excess throughout the duration of the experiments. Both the glucagonotropic effect of arginine ($319 \pm 60 \text{ ng/l}$, $p < 0.01$) and the insulinotropic effect of exogenous glucagon

($8.3 \pm 0.8 \text{ } \mu\text{g/l}$, $p < 0.001$) were demonstrable under our experimental conditions in the absence of exogenous glucagon antibodies. These observations suggest that different mechanisms are involved in the stimulation of insulin release by arginine and by glucose, and that glucagon may play an important physiological role in the mediation and regulation of insulin secretion by secretagogues, such as arginine.

Key words: Paracrine, insulin secretion, glucagon secretion, glucagon antibodies, arginine, glucose, rat.

It is now established that the pancreatic islet of Langerhans contain A, B, D and PP cells which secrete, respectively, glucagon, insulin, somatostatin and pancreatic polypeptide [1]. By virtue of their anatomical juxtaposition, the sharing of a common extra-cellular space, and the existence of both tight and gap junctions, it seemed reasonable to assume that some mechanism exists whereby the pancreatic hormones suppress or stimulate each other's secretion [2–5]. Our understanding of these paracrine interrelationships has derived largely from pharmacological experiments and there has been some controversy regarding their physiological significance.

Understanding of the A- and B-cell interactions has remained confused. Physiological doses of glucagon are known to be hyperglycaemic and insulinotropic. Although this insulinotropic effect of glucagon was demonstrated as long ago as 1965 [6], there is still no proof that it is physiologically important. Conversely, it has not been established whether stimulation of the B cell can occur in an A-cell free environment, since no chemical agents have been found that are capable of selectively destroying A cells. Hence it has been virtually impossible to study B-cell function in isolation from A cells.

We have approached this problem using antibodies specific for pancreatic glucagon in an attempt to neutralise A-cell function and/or block its secretion. In an earlier study, we showed that glucagon antibodies administered intraperitoneally did not affect plasma glucose, insulin or somatostatin concentrations despite extensive endogenous glucagon neutralisation [7]. Using this model, insulin secretion in response to arginine and glucose were investigated under conditions of glucagon deficiency.

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Materials and methods

Glucagon antibodies

A high titre (1:180,000 final) C-terminal reactive rabbit anti-glucagon serum was obtained from Guildhay Antisera, Guildford, UK. The binding characteristics and capacity of the antibody and its ability *in vivo* to cause extensive neutralisation of endogenous glucagon have been described earlier [7]. Further studies on the specificity of the antiserum, and in particular, the absence of insulin antibodies were performed by incubating the glucagon antiserum in the presence of ^{125}I -insulin. An insulin antiserum (Guildhay Antisera, Guildford, UK) acted as control. The recovery of insulin in the presence of glucagon antibodies was studied by the addition of exogenous insulin to plasma samples containing antiglucagon immunoglobulin at 1:50 and 1:100 dilutions and assaying for insulin.

An immunoglobulin fraction of the pooled glucagon antiserum was prepared by ammonium sulphate precipitation and the immunoglobulin fraction reconstituted with saline (0.154M). An immunoglobulin fraction of normal rabbit serum was similarly prepared.

Fig. 1. Effects of glucagon (1.5mg/kg bodyweight, ●) and saline (○) on plasma insulin and glucose concentrations in rats. Both groups ($n=6$) received normal rabbit immunoglobulins (0.5ml) before the administration of glucagon. Results are mean $\pm$ SEM ($n=6$) (**= $p<0.001$ and *= $p<0.005$ compared with saline control group)

Fig. 2. Plasma insulin and glucose concentrations following administration of D-glucose (2g/kg bodyweight) in rats ($n=6$) given normal rabbit immunoglobulins (0.5ml, ○) or anti-glucagon immunoglobulins (0.5ml, ●). Results are mean $\pm$ SEM ($n=6$) (*= $p<0.01$; **= $p<0.005$; ***= $p<0.001$ compared with value at zero time within the same group). No significant differences were observed between the two groups

Experimental protocols

Glucagon (Eli-Lilly, Indianapolis, Indiana, USA, 1.5mg/kg bodyweight in saline) was administered intraperitoneally to six conscious male Wistar rats (150g) given normal rabbit immunoglobulins (0.5ml) by intraperitoneal injection 45 min earlier. Six control rats received normal rabbit immunoglobulins (0.5ml) and saline intraperitoneally. The plasma glucose and insulin responses were observed at different time intervals. In a second series of experiments antiglucagon immunoglobulins (0.5ml) were injected intraperitoneally into conscious fed rats. Control rats received 0.5ml of the normal rabbit serum immunoglobulin fraction. After 45 min, D-glucose (2g/kg body weight) or L-arginine (1.5 g/kg body weight) was administered intraperitoneally. Blood samples were obtained from the tail vein and assayed for plasma insulin, glucagon, glucose and excess antibody binding.

Chemical analyses

Immunoreactive insulin and pancreatic glucagon were determined by radioimmunoassay as previously described [7]. Plasma glucose was measured using a glucose oxidase method in a Beckman glucose analyser (Beckman Instruments, Fullerton, CA, USA).

Statistical analysis

Statistical analysis was performed using a two-tailed Student's *t*-test for unpaired data. Results are compared with values at zero time of

the same group using the paired *t*-test, unless otherwise indicated. Results are expressed as mean $\pm$ SEM.

Results

Glucagon (1.5mg/kg bodyweight) stimulation

The insulinotropic and hyperglycaemic effects of glucagon were confirmed under the experimental conditions described (Fig. 1). At 15 min, plasma insulin ($8.3 \pm 0.8 \mu\text{g/l}$, $p<0.001$) and plasma glucose ($8.7 \pm 0.3 \text{ mmol/l}$, $p<0.001$) were significantly raised over basal levels.

D-Glucose (2g/kg bodyweight) stimulation

D-glucose administration resulted in significant increases in plasma insulin concentrations in both the control animals given normal rabbit immunoglobulins ($p<0.001$) and the test animals given glucagon antibodies ($p<0.01$) (Fig. 2). There were no significant differences between the two groups. Plasma glucose levels were significantly raised above basal levels in both groups (Fig. 2), whilst a fall in plasma glucagon concentration was observed at 15 and 30 min after glucose injection in the control animals ($p<0.02$; Fig. 4).

L-Arginine (1.5g/kg bodyweight) stimulation

In control rats treated with normal rabbit immunoglobulins, arginine stimulated insulin secretion ($p<0.005$; Fig. 3). In contrast, the insulinotropic effect of arginine was inhibited in animals given glucagon antibodies ($p<0.001$ compared with controls). Plasma glucose levels rose significantly in the control animals ($p<0.01$) and a small but insignificant rise was also observed in the test animals followed by a rapid return to basal levels by 30 min (Fig. 3). No significant differences in plasma glucose concentrations were found between the two groups at any time point. Plasma glucagon levels were significantly raised 15 min after arginine injection in the control animals ($p<0.01$; Fig. 4).

Plasma antiglucagon concentrations after glucagon antiserum and glucose or arginine stimulation

Plasma samples collected from test animals after glucose or arginine administration were serially diluted with freshly prepared ^{125}I -glucagon and assessed for titre and percentage binding of unbound antibodies. The extent of antibody binding of glucagon by undiluted rat plasma was made by extrapolating the percent binding of the antibody to zero dilution [8]. Excess antibodies were detectable in both experimental, ie glucose and arginine, groups throughout the duration of the study (range 75–78% Bound/Total ^{125}I -glucagon) in concentrations equivalent to approximately 40 nmol/l glucagon binding capacity (Fig. 5).

Fig. 3. Effects of L-arginine (1.5g/kg bodyweight) on plasma insulin and glucose concentrations in rats ($n=6$) given normal rabbit immunoglobulins (0.5ml, ○) or anti-glucagon immunoglobulins (0.5ml, ●). Plasma insulin concentrations in the test animals were significantly different from control group at 15 (**= $p<0.001$) and 30 min (*= $p<0.005$). Plasma glucose concentration was significantly raised in the control group at 15 min (**= $p<0.02$ compared with value at zero time) but not in the test group. No significant differences were observed for plasma glucose concentrations between the two groups. Results are mean $\pm$ SEM ($n=6$)

Fig. 4. Effects of D-glucose (2g/kg, ●) and L-arginine (1.5g/kg, ○) on plasma glucagon concentration. Both groups received normal rabbit immunoglobulins. Results are mean $\pm$ SEM ($n=6$) (*= $p<0.02$; **= $p<0.01$)

Fig. 5. Plasma antiglucagon levels as determined by percentage binding of ^{125}I -glucagon following D-glucose (2g/kg, ●) and L-arginine (1.5g/kg, ○) stimulation. Results are mean $\pm$ SEM ($n=6$)

Discussion

In this study, a pool of antiglucagon antiserum raised in rabbits was used. Its binding capacity, avidity and titre were more potent than antisera used in previous studies [9–12]. Evidence for the specificity of the glucagon antibodies are described.

A major obstacle to elucidating the physiological role of glucagon has been the absence of any method

that can selectively block A-cell function and/or secretion. In recent years, however, glucagon specific antibodies have been used for this purpose and have proved to be a powerful tool in unravelling glucagon's physiology by neutralising the effects of endogenous glucagon [12]. Using this approach, we have been able to demonstrate the inhibition of arginine-stimulated insulin release by glucagon antibodies. Administration of glucagon antibodies in vivo abolished the insulin response after arginine stimulation in rats. In addition, the glucagonotropic effect of arginine and the insulinotropic action of glucagon in the rat were also confirmed. These observations are consistent with the hypothesis that arginine and other amino acids that stimulate both glucagon and insulin secretion do so in a step-wise manner with glucagon serving as a mediator or potentiator of the insulinotropic effect [5]. Antibodies entering the islets may neutralise the A-cell glucagon and prevent its stimulation of the B cell.

In contrast to arginine, the glucose-stimulated insulin secretion was not impaired by glucagon antiserum. This divergence suggests that different mechanisms are involved in insulin release by parenteral glucose and arginine. Failure of the antibody to modify glucose-stimulated insulin secretion cannot, however, be attributed to incomplete neutralisation of endogenous glucagon as evidenced by the presence of excess antibodies at all time points in concentrations equivalent to approximately 40nmol/l glucagon binding capacity. This is excess of 1000 times the concentration of basal plasma glucagon levels of around 30pmol/l [7].

There is general agreement that glucose has a direct effect on the B cell [13] either through being metabolised and forming specific metabolites or co-factors which initiate insulin release [14] or by activating glucoreceptors in the B-cell membrane [15]. Glucagon antiserum would not be expected to have any effect on such a direct B-cell action. In contrast, evidence from the present study suggests that the insulinotropic effect of arginine is glucagon dependent.

In an earlier study using a single pass perfusion system in the isolated rat pancreas, glucagon antibodies failed to affect somatostatin and insulin secretion following arginine stimulation [16]. Although the insulin response to arginine with glucagon antiserum tended to be lower than with non-immune serum, it was not found to be statistically significant. However, as conceded by the authors, since their experimental system is a single pass perfusion, access of the antibodies to the paracrine space may be difficult leading to incomplete neutralisation of glucagon. Our results are however consistent with those obtained by Tsiolakis [17] and Nishino et al. [18] who demonstrated inhibition of arginine-stimulated insulin secretion following intravenous glucagon antibody administration.

In their elegant studies using purified single A, B and D cells from rat islets, Pipeleers et al. [19, 20] reported that single B cells responded 2–4 fold more effective-

ly to glucose when they were perfused together with A cells or incubated with exogenous glucagon. This effect was not associated with an increase in cell coupling but was attributed to the paracrine action of glucagon. It would appear that an optimal glucagon concentration in the vicinity of the B cell is necessary for insulin secretion. These studies provide direct evidence that the A cells play an important role in the regulation of insulin secretion from the B cells through a paracrine interaction. The evidence summarised here is consistent with this view, that endogenous glucagon is insulinotropic and plays an essential intermediary role in the stimulation of insulin secretion by certain secretagogues such as arginine.

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