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Studies on Glucagon Secretion Using Isolated Islets of Langerhans of the Rat

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Summary. Glucagon secretion and its control have been studied in perifused isolated islets of Langerhans of the rat. It was shown that a low concentration of glucose *per se* does not cause increased glucagon secretion, but that at low glucose concentrations the amino acid arginine stimulates a biphasic secretory response. Such amino acid stimulated glucagon secretion can be suppressed by increasing the glucose content of the perifused media from 1.67 to 5.5 or 16.7 mM; insulin secretion is also then increased. Since high concentrations of added porcine insulin (10 mU/ml) did not affect amino acid stimulated glucagon secretion at low glucose concentration, it was concluded that high concentrations of glucose and not insulin secreted in response to that glucose are probably responsible for suppression of glucagon secretion. At low concentrations of glucose, epinephrine (2.5×10^{-7} M) also stimulated glucagon secretion. It is concluded that isolated rat islets of Langerhans can be used for the study of glucagon secretion in vitro, and that substances appearing in the blood in vivo at low glucose concentrations are probably responsible for increased glucagon secretion under conditions associated with hypoglycemia.

Key words: Perifused islets, glucagon and insulin secretion, glucose, arginine, epinephrine.

At present the control of pancreatic glucagon secretion in vivo is poorly understood. This situation has arisen mainly because of the lack of specificity of plasma glucagon assays and the possibility that compensatory mechanisms may release factors which act directly or indirectly to influence pancreatic glucagon secretion. In an effort to overcome these problems, investigators have utilized in vitro techniques such as the perfused pancreas or have incubated pieces of pancreas from a variety of animals [1–5]. A few studies have been done using isolated islets of Langerhans, but the results have been extremely variable [6–11]. In order to explain discrepancies between results obtained using this latter technique and those from perfused pancreas experiments, it is suggested that collagenase, the enzyme used to prepare islets, digests or removes receptors and/or alpha cells from the islets. However, using a carefully controlled collagenase digestion procedure we have succeeded in preparing rat islets capable of responding to various stimuli in a manner similar to that reported for the perfused rat pancreas.

Materials and Methods

Animals. Male albino rats (250–300 g), fed ad libitum on Purina rat chow and with free access to water, were used for all experiments.

Isolation of Islets of Langerhans. Islets were isolated substantially by the method of Lacy and Kostianovsky [12], with the added modification that glucose (100 mg/100 ml) was added to the Hanks buffer. Fed rats were anaesthetized with sodium pentobarbital (50 mg/kg), intraperitoneally), the pancreas exposed, the common bile duct cannulated and the entry of the common bile duct into the duodenum clamped. Approximately 20 ml of buffered Hanks solution was injected through the cannula to distend the pancreas and disrupt the exocrine tissue. The pancreas was removed and chopped into small pieces with scissors. Siliconized glassware was used throughout the remainder of the isolation procedure and subsequent manipulation of islets so as to prevent islets adhering
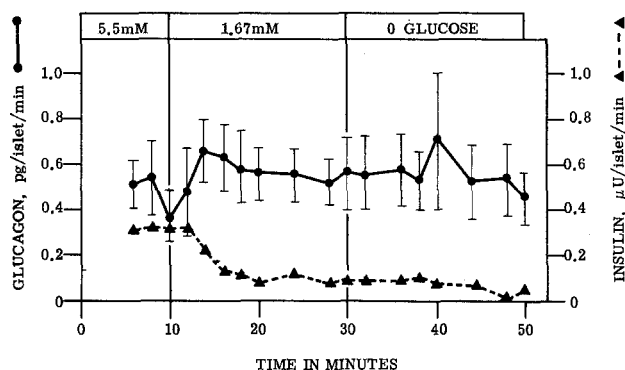
Fig. 1. The solid line (●———●) shows glucagon and the broken line (▲-----▲) insulin secretion rates from perifused isolated islets of Langerhans exposed to various concentrations of glucose. Each point represents the mean (± SEM) of six observations

to glass surfaces and possible lysis at the glass tissue interface. The slurry of tissue and fluid was poured into a glass tube (15×100 mm) and excess fluid removed from above the pancreas tissue. Collagenase (Type IV; Worthington Biochemicals, Freehold, New Jersey) was added to the slurry (6–12 mg collagenase powder per pancreas) and digestion carried out at 37° C with vigorous and continuous hand shaking for 7–10 min. Progress of the digestion was followed by viewing the digest under a stereomicroscope for quality and quantity of islets. Digestion was terminated using 4×10 ml buffer washes and centrifugation steps. After final centrifugation, the pellet of pancreas and islet tissue was poured into petri dishes and the islets removed with a glass loop whilst viewing under a stereomicroscope. The washing and centrifugation step as well as harvesting of islets was carried out at room temperature. The harvested islets were kept at 4° C in a small volume of Krebs bicarbonate medium (0.50 ml) and continuously gassed with 95%-O_2:5%-CO_2. Islets, approximately 150 in number, were transferred to a Swinnex 13 mm Millipore chamber (Bedford, Massachusetts) containing a 5 µm pore size membrane. The time elapsed between removal of the pancreas from the carcass and the beginning of perifusion was 45–60 min. The chamber containing islets was submerged in a water bath maintained at 37° C and perifused with Krebs bicarbonate buffer supplemented with 0.2 g/100 ml bovine plasma albumin (Fraction V Sigma Chemicals, St. Louis, Missouri). The pH of the perifusion medium was maintained at 7.4 by gassing with 95%-O_2:5%-CO_2.

Experimental Protocol. During each experiment two sets of islets were perifused simultaneously, one acting as a test and the other as control. The islets were perifused at a flow rate of 1 ml/min. An equilibration period of 15–20 min was allowed before agents under

investigation were added to the perifusion medium. Agents to be studied were introduced from reservoirs via three way valves which allowed rapid changes without affecting flow rate. Perifusate fractions (1.0 ml) were collected in the presence of Trasylol (500U, F.B.A. Pharmaceuticals, New York), immediately frozen and stored at −20° C until assayed for glucagon and insulin contents.

Radioimmunoassay Procedures

Glucagon. Aliquots of perifusate (0.20 ml) were assayed for glucagon using a double-antibody technique modified from the method of Pagliara et al. [2]. Porcine glucagon (Eli Lilly Co., Indianapolis, Indiana) was labelled with Iodine-125 (Amersham, Bucks, U. K.) to a specific activity of 400–600 µCi/µg, using the method of Hunter and Greenwood [13]. The diluent buffer was 0.05 M phosphate-saline, pH 7.0, containing 0.20 g/100 ml bovine plasma albumin. The final assay volume was 0.50 ml. Standards of porcine glucagon (0–1000 pg/ml) constituted in perifusion medium and unknowns in equal volumes (0.2 ml) were incubated for 48 h at 4° C with diluted guinea pig anti-glucagon serum (0.015 µl serum/0.1 ml buffer) kindly provided by Dr. A. Pagliara of St. Louis, Missouri. The tracer ^{125}I glucagon (in 0.2 ml buffer) was then added and the incubation allowed to proceed for another 36 h. Separation of bound from free hormone was accomplished by addition of goat anti-guinea pig gamma globulin serum (3.3 µl/0.1 ml buffer) and normal guinea pig serum (1.2 µl/0.1 ml buffer) as carrier. Sensitivity of the assay was 25 pg/ml.

Insulin. Aliquots of perifusate (0.10 ml) were assayed for insulin using the method of Makulu et al. [14] and standard curves were performed in the presence of an equal volume of perifusion medium, ^{125}I-insulin (New England Nuclear, Illinois) of specific activity 150–200 µCi/µg and guinea pig anti-porcine insulin serum prepared by the method of Wright et al. [15] were used for the assay. Separation of bound from free hormone was accomplished by precipitation with ethanol at a final concentration of 76%. Porcine insulin (Eli Lilly Co., Indianapolis, Indiana) was used as standard. Epinephrine and l-arginine hydrochloride were purchased from Sigma Chemicals, St. Louis, Missouri.

Results

Effect of Glucose Concentration on Glucagon Secretion Rate

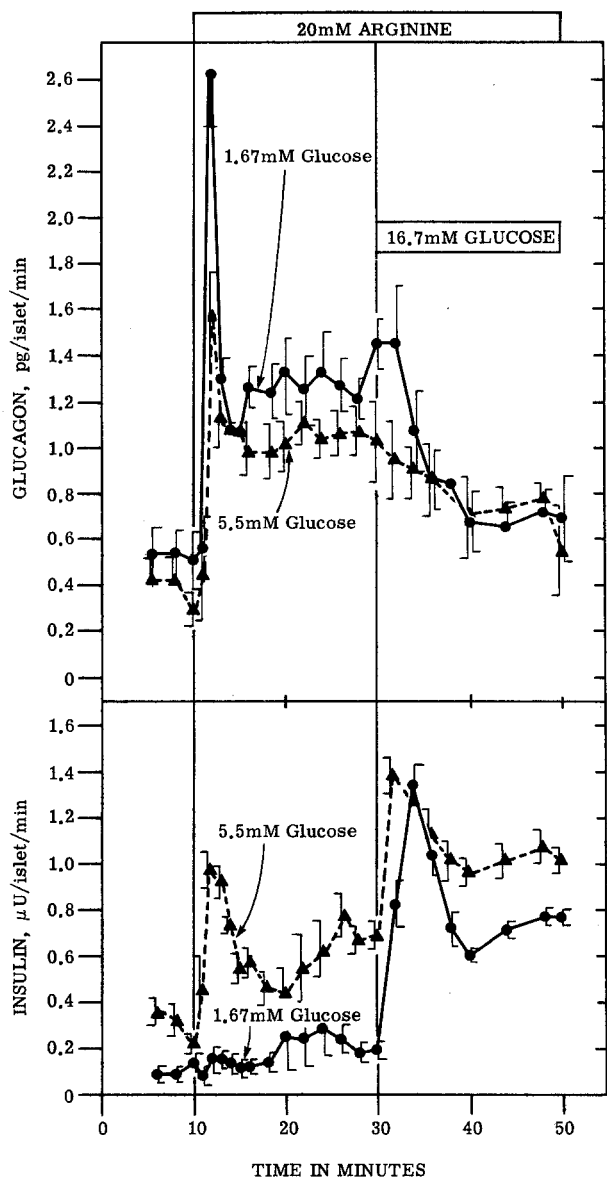As shown in Figure 1, decreasing glucose concentration in the perifusion media from 5.5 mM to zero in a

Fig. 2. Upper panel (A) shows the effect of various concentrations of glucose (1.67 mM, 5.5 mM and 16.7 mM) on glucagon secretion rates from perifused isolated islets of Langerhans stimulated with 20 mM arginine. Lower panel (B) shows insulin secretion rates from the same islets. Each point represents the mean (± SEM) of four observations

Fig. 3. Upper panel (A) shows the effect of various concentrations of glucose (1.67 mM, 5.5 mM and 16.7 mM) on glucagon secretion rates from perifused isolated islets of Langerhans stimulated with 5 mM arginine. Lower panel (B) shows insulin secretion rates from the same islets. Each point represents the mean (± SEM) of four observations

stepwise manner did not significantly alter glucagon secretion rates. Insulin secretion rates were higher at 5.5 mM glucose than at lower glucose concentrations.

Effect of Glucose Concentration on Arginine Stimulated Glucagon Secretion

Islets perifused in media containing 1.67 mM and 5.5 mM glucose demonstrated similar basal glucagon secretion rates (Fig. 2A, 0–10 min). The addition of arginine (20 mM) at 10 min stimulated glucagon se-
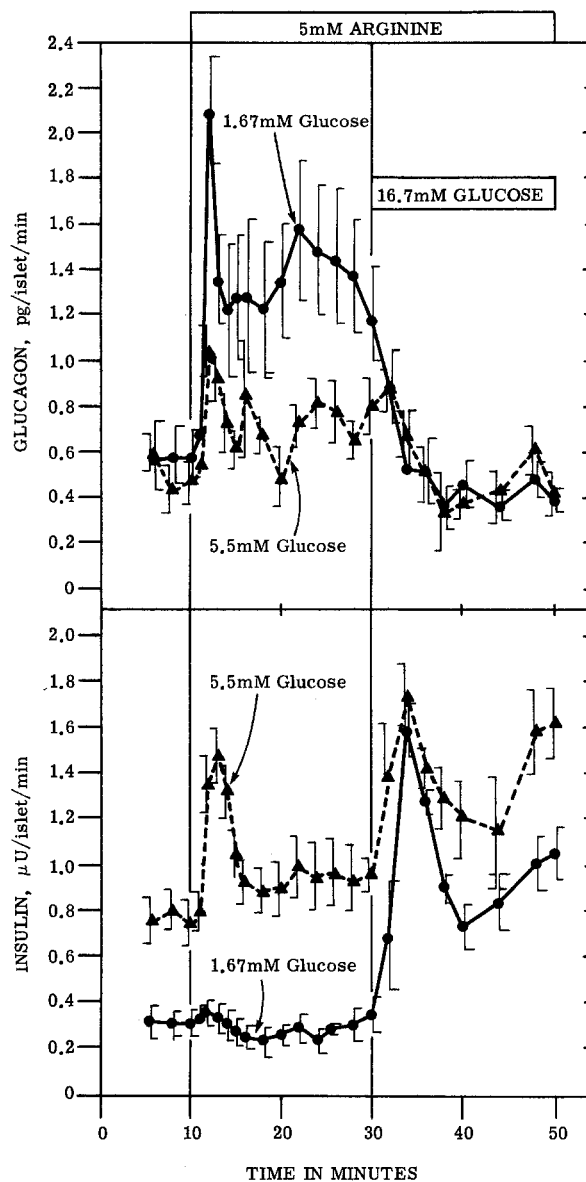
cretion from both sets of islets in a biphasic pattern. A sharp peak appeared 2 min after arginine addition, was followed by a nadir at 5–7 min and then by a second more persistant phase after approximately 10 min. The only distinguishing feature between the two responses was a significantly lower first peak ($p < 0.05$) for the islets perifused with medium containing 5.5 mM glucose. When the glucose concentration was increased to 16.7 mM at 30 min, glucagon secretion rates from both sets of islets decreased steadily. Insu-
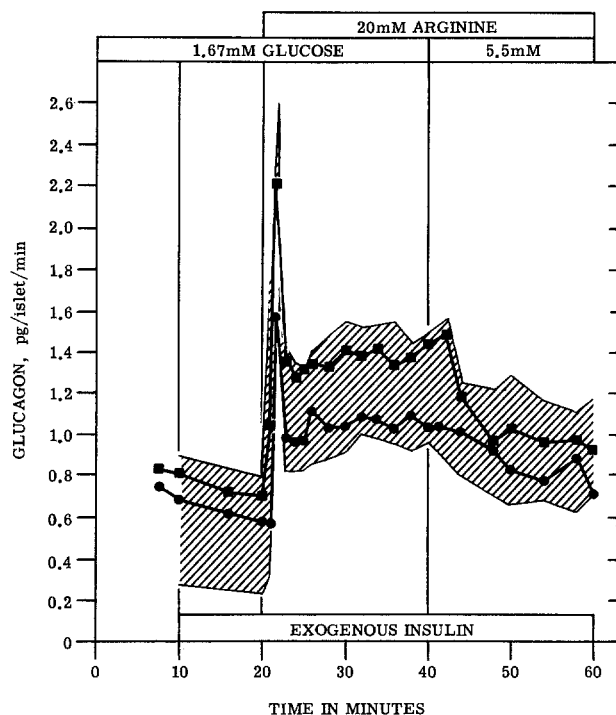
Fig. 4. Shows the response of islets to a 20 mM arginine stimulus in the presence of exogenous insulin 1000 μU/ml (●————●) or 10,000 μU/ml (■————■). The shaded area represents plus or minus one standard deviation from the mean values for the response of islets not exposed to exogenous insulin. Each point is the mean of 4–8 observations
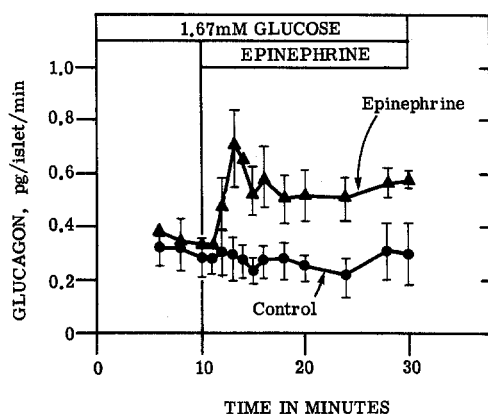

Fig. 5. The effect of epinephrine 2.7×10^{-7} M (▲————▲) on glucagon secretion from isolated islets of Langerhans. Each point represents the mean (± SEM) of four observations

lin secretion rates (Fig. 2B) were higher for islets perifused with 5.5 mM glucose and were further stimulated by arginine at this glucose concentration only.

Amino acid concentrations of 20 mM are non-physiological; therefore in order to demonstrate a possible physiological role of amino acids in controlling glucagon secretion during periods of glucose deprivation, a lower and more physiological concentra-

tion of arginine (5 mM) was used. As shown in Fig. 3A, a characteristic biphasic glucagon secretory response to 5 mM arginine was seen in islets perifused at low glucose concentration (1.67 mM). On applying the same arginine stimulus to islets perifused in the presence of 5.5 mM glucose a much smaller stimulant effect on glucagon secretion was induced. However, only the 12 min or initial peak of secretion was significantly greater ($p < 0.05$) than basal secretion rate. Subsequent increase in glucose concentration to 16.7 mM caused a prompt fall in glucagon secretion rates from both sets of islets. Throughout the perifusion, islets exposed to 5.5 mM glucose had higher insulin secretion rates than those for islets in 1.67 mM glucose (Fig. 3B). The addition of arginine caused an increase in insulin secretion only from those islets perifused with 5.5 mM glucose. Increasing the glucose concentration to 16.7 mM increased insulin secretion from both sets of islets.

Effect of Exogenous Insulin on Arginine Stimulated Glucagon Secretion

The suppression of amino acid stimulated glucagon secretion always accompanied increased insulin secretion at high glucose concentrations. In order to determine whether insulin inhibits glucagon secretion, exogenous insulin was added to the perifusion medium 10 min prior to an arginine stimulus. The results are shown in Fig. 4. Islets perifused with high concentrations of exogenous insulin (1 mU/ml or 10 mU/ml) demonstrated basal (10–20 min) and arginine stimulated (20–40 min) glucagon secretion rates which were not significantly different from those of islets perifused in the absence of exogenous insulin.

Effect of Epinephrine on Glucagon Secretion

Freshly diluted epinephrine was introduced into the perifusion medium at a final concentration of 2.7×10^{-7} M and islets perifused at low glucose concentration (1.67 mM). Glucagon secretion (Fig. 5) was seen to increase, although the response was small compared with that induced by amino acid in the previous studies.

Discussion

From the results presented it is evident that rat islets of Langerhans prepared by the collagenase digestion procedure can be used for studies of glucagon secretion. The finding that in the complete absence of amino-acid, glucagon secretion is not increased as the glucose in the medium is decreased perhaps favour the arguments of Johnson et al. [6] and Norfleet et al. [11].

They suggested that A-cells have membrane receptors for glucose which are destroyed by the collagenase, although the latter group was able to demonstrate a small inhibition, 18% by glucose, on glucagon secretion stimulated by arginine. However, destruction of these glucose receptors may still have occurred in their particular islet preparations, for Norfleet et al. [11] used approximately three to four times the amount of collagenase used in the present studies; the amounts used in the studies of Johnson et al. [6] were not specified. Another factor which may also be involved is the type of collagenase used. The preparation used here (Type IV) contains little trypsin, which may have been present in other collagenase preparations where the type of collagenase was not specified. Our findings clearly indicate that glucose can markedly alter arginine stimulated glucagon secretion, indicating that the receptors or systems by which glucose acts remain intact. Other groups using isolated islets from the rat [10] or guinea pig [8] have also been unable to demonstrate any effect of glucose on glucagon secretion. In studies using the perfused rat pancreas acute glucose deprivation failed to stimulate glucagon secretion [2]. Contrary to our findings and those of others, Ziegler et al. [16], Nonaka and Foa [17] using isolated rat islets, McChesney and Schofield [18] using mouse islets and Marliss et al. [19] using monolayer cultured rat islets have been able to demonstrate increased glucagon secretion with decreases in glucose concentration. In studies using the perfused canine pancreas, Christensen and Iversen [20] showed an increase in glucagon secretion as the glucose concentration in the perfusion medium was decreased. However, many investigators reporting increased secretion of glucagon in vitro at low glucose concentrations, used buffer systems which were supplemented with 5 mM glutamate [17, 18, 20]. Release of catecholamines from the perfused pancreas [20] during periods of glucose deprivation may also play an important role in secretion of glucagon by this preparation, for it has been shown that addition of epinephrine to perfusion or perifusion media stimulate glucagon secretion by isolated islets (Fig. 5), pancreas pieces [21] or the perfused pancreas [22] of the rat. Therefore it may not just be the absence of glucose which stimulates secretion of glucagon from the A-cell, but rather the presence of other agents in the blood during periods of hypoglycemia.

Glucose at concentrations of 5.5 mM or 16.7 mM suppressed arginine stimulated glucagon secretion in vitro, but such suppression was also associated with increased insulin secretion. Although the addition of insulin to the perifusion medium did not affect either basal or arginine stimulated glucagon secretion in the present studies, the highest concentration (10 mU/ml) may not have been sufficient to affect mechanisms

involved with the control of glucagon secretion. Pagliara et al. [23], using a concentration of insulin of 25 mU/ml in a perfused rat pancreas preparation, also demonstrated that amino acid stimulated glucagon secretion was not affected by exogenous insulin. Contrary to these findings are those of Harrison and Samols [24] claiming suppression of glucagon secretion from the perfused dog pancreas at an insulin concentration of only 500 μU/ml. The differences in findings may perhaps be related to differences in experimental design. Harrison and Samols [24] used a tolbutamide stimulus to increase the endogenous insulin secretion from the B-cell, whilst the studies reported here and those of Pagliara et al. [23] used added insulin. Therefore, it may be that insulin added to the perifusion medium is not able to get into the interstitial space between the A- and B-cells in sufficient concentration to decrease glucagon secretion.

In summary, our studies indicate that the perifused isolated rat islets of Langerhans can be used for the study of glucagon secretion. Furthermore, our results show that secretion of glucagon is not stimulated at low concentration or in the absence of glucose. Glucose in concentrations of 5.5 mM or greater appears to be the main suppressive factor on amino acid stimulated glucagon secretion. However, the exact mechanism for physiological control of glucagon secretion remains to be elucidated.

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