Comparison of Alpha- and Beta-cell Secretory Responses in Islets Isolated with Collagenase and in the Isolated Perfused Pancreas of Rats

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SUMMARY

The inhibitory actions of somatostatin (100 ng./ml.) on insulin release, stimulated by high glucose (20 mM), and on glucagon release, stimulated by arginine (15 mM), were studied with two in vitro systems: the isolated perifused rat islets prepared by the collagenase procedure and the isolated perfused rat pancreas. Suppression of arginine-induced glucagon release by glucose (20 mM) and glyceraldehyde (5 mM) was also assessed in both systems. With the perfused pancreas, somatostatin caused 32 per cent inhibition of glucose-mediated insulin release and inhibited arginine-induced glucagon release by 72 per cent. In the same system, glucose and glyceraldehyde were similarly potent inhibitors of arginine-induced glucagon secretion. In contrast to the isolated perfused pancreas, there was no significant somatostatin suppression of glucose-induced insulin release or arginine-induced glucagon release whether the inhibitor was present prior to or was added during stimulation by glucose or arginine. Furthermore, glucose was only minimally active and glyceraldehyde ineffective in inhibiting glucagon secretion due to arginine in the perifusion system.

The most plausible explanation for the difference in the endocrine response of islet cells in the two types of widely used in vitro systems is that the alpha and beta cells have lost inhibitory receptors in the plasma membrane as a result of the collagenase isolation technic. DIABETES *24*:961-70, November, 1975.

The two most widely used experimental systems for studying insulin and glucagon secretion in vitro consist of incubation or perifusion of pancreatic islets isolated with collagenase and perfusion of the intact isolated pancreas. These two major approaches are usually considered equivalent in basic chemoreceptor characteristics and fundamental metabolic responses. This seems to be generally true in regard to insulin release. However, agents that stimulate[1] or inhibit[2,3] glucagon release from the perfused pancreas were ineffective or only marginally active with isolated islets.

The present investigation is a direct comparison of the responses of the two experimental systems to major stimulators and inhibitors of insulin and glucagon secretion (e.g. glucose, arginine, glyceraldehyde, and somatostatin). Although perifused islets responded well to stimulants of insulin and glucagon release, they were relatively refractory to agents that are potent suppressors of alpha- and beta-cell hormone secretion in the isolated perfused pancreas.

MATERIALS AND METHODS

Isolation of Islets from Rat Pancreas and Perifusion Procedure

Adult male Sprague-Dawley rats (Holtzman Co., Madison, Wis.) weighing 300 to 400 gm. and fed ad libitum with Purina rat chow (Ralston Purina Co., St. Louis) were used in all experiments.

Rat pancreatic islets were prepared by the collagenase procedure of Lacy and Kostianovsky[4] with minor modifications. After an overnight fast (twelve to fourteen hours), the animals were given pilocarpine hydrochloride (12 mg. intraperitoneally) (Isopto Carpine, Alcon Laboratories, Fort Worth, Texas) to reduce the concentrations of exocrine pancreatic enzymes.[5] Two hours following this injection they were anesthetized with pentobarbital (50 mg./kg. intraperitoneally). The common bile duct was cannulated and distended with Hank's solution. The pancreas was then dissected from its attachments to the small intestine and minced with scissors and the acinar tissue digested by incubation and vigorous hand shaking with collagenase in a 38° water bath for approximately ten minutes. Forty milligrams collagenase dissolved in 3 ml. Hank's solution was used per pancreas. The two separate lots of collagenase used in all the studies were purchased from Worthington Biochemical, Freehold, New Jersey. Following diges-

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tion of the acinar tissue, one hundred islets were picked out in a period of thirty minutes and placed in a Millipore filter chamber containing a filter with a 1.2-μ pore size. The chamber was submerged in a 38° water bath, and the islets were perifused at a constant flow rate of 0.9 to 1.0 ml. per minute.

The perifusion medium was a Krebs bicarbonate buffer with the following composition: NaCl, 120 mM; KCl, 5.0 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 25 mM; and 0.05 per cent crystallized bovine plasma albumin (Metrix, Chicago). Additions of glucose (20 mM), arginine (15 mM), glyceraldehyde (5 mM), and somatostatin (100 ng./ml.) were made to the buffer as described in the following text or in the legends of the figures. The buffer was gassed with a mixture of O_2 and CO_2 (95:5), with a resulting pH of 7.4 to 7.6.

Two protocols were used. In the first, the islets were perifused for seventy minutes with buffer alone. Basal secretion rates were obtained during the final thirty minutes of this equilibration period. This basal period was followed by thirty-minute periods with added glucose, arginine, glyceraldehyde, and somatostatin or combinations of these agents. The final thirty minutes was a second control period with buffer alone. In the second protocol either glucose (20 mM) or somatostatin (100 ng./ml.) was added throughout the entire perifusion period, including the seventy-minute basal period, to ascertain any difference in effect on stimulated insulin and glucagon release that might occur by virtue of early introduction of these two agents. In most cases, and in both protocols, control and experimental perifusion chambers were run simultaneously with islets from the same preparation.

At suitable times, 0.5-ml. samples of the perifusate were briefly cooled on ice and frozen for storage at −20° C. until assayed. During the basal release period, samples were taken at five-minute intervals; after the switch to the medium containing the agent under study, samples were collected at one-minute intervals for five minutes, then every five minutes for the remaining twenty-five minutes. This same schedule of sampling was applied for every medium change. The transitions by switching from one circulation medium to the other were rapid and did not result in detectable changes in flow rate. The rates of insulin and glucagon release were calculated by multiplying the concentrations of the respective sample by the flow rate, which was measured at frequent intervals. Total release during a period of exposure to a stimulus was obtained by planimetry.

Perfusion of the Isolated Intact Rat Pancreas

The pancreas was isolated and perfused by the procedure described by Grodsky et al.[6] with minor modifications.[7] Induction of anesthesia, surgical preparation, and composition of the perfusion media have previously been described in detail.[8]

Immunoassay Procedures

Samples from the perfusion and perifusion systems were assayed for insulin and glucagon by double-antibody procedures.[8,9] Porcine insulin and glucagon served as standards. The highest concentrations of all substrates added to the infusion media were tested and found not to interfere with the immunoassay system.

RESULTS

Basal Insulin and Glucagon Secretion from Perifused Islets and the Perfused Pancreas

During the preperifusion period, insulin release from isolated islets gradually declined to rates varying between 0.2 and 0.4 μU. per minute per islet (figures 1 and 4), and glucagon secretion stabilized at rates ranging from 0.5 to 1.5 pg. per minute per islet (figures 3, 7 and 8A). On a weight basis, total basal insulin release was about ten times that of basal glucagon secretion.

With the perfused pancreas, stable basal insulin secretion was approximately 100 μU. per minute (figures 2, 5, and 12) and basal glucagon release less than 3 ng. per minute (figures 6, 10, and 14) during fifteen-minute equilibration periods. This is similar to results of previous studies.[8,10]

Effects of Glucose on Insulin and Glucagon Release

When challenged with 20 mM glucose (figure 1), insulin release rates from perifused islets were increased threefold above basal levels. Release increased to near maximum rates within two minutes of exposure to the stimulus and returned towards basal release rates upon complete removal of glucose. There is a striking difference between the rate of onset and of termination of the beta-cell response to addition and removal of glucose. The respective half times are approximately one minute and thirty minutes. A prompt beta-cell response occurred even after prolonged preperifusion in the absence of added glucose. It is noteworthy that in most comparable studies 2.5 to 5 mM basal glucose was always included in the preperifusion media.[11,12] In the perfused pancreas (figure 2) qualitatively similar insulin-release kinetics in response to 20 mM glucose was observed, while quantitatively a more marked (eight-to-tenfold) increase over basal rates of both phases was demonstrated.

As previously demonstrated in the perfused pancreas,[10] 20 mM glucose suppressed unstimulated

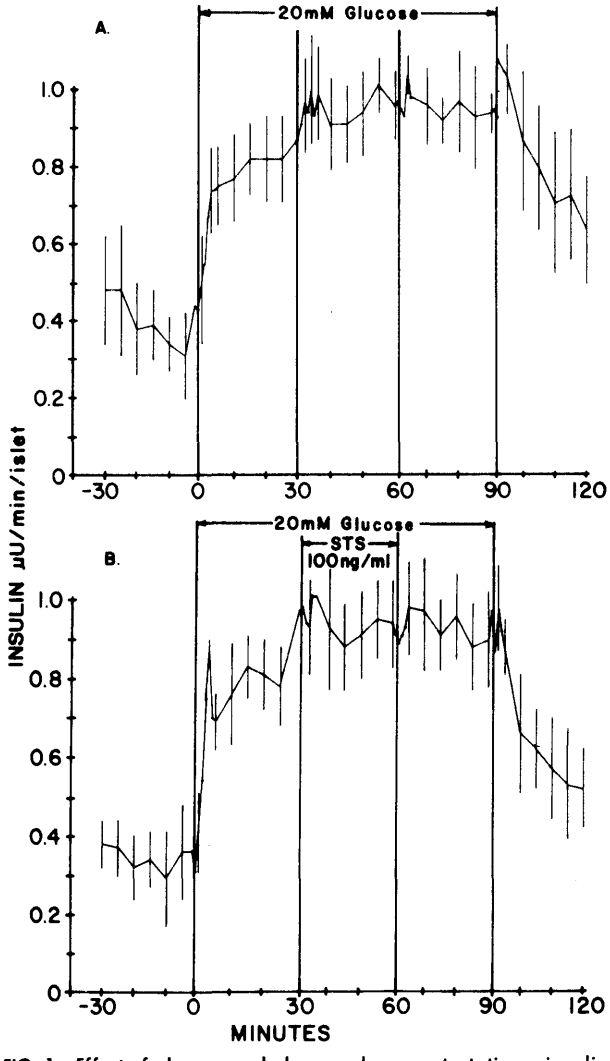

FIG. 1. Effect of glucose and glucose plus somatostatin on insulin release from isolated perifused islets. The islets were perifused for seventy minutes with substrate-free buffer. Basal release rates were obtained at five-minute intervals (t_{-30}-t_0). The islets were then perifused for ninety minutes (t_0-t_{90}) with a medium containing glucose (20 mM) and for the final thirty minutes (t_{90}-t_{120}) with substrate-free buffer to obtain the poststimulatory response. The buffer reservoir was switched at 0, 30, 60, and 90 minutes. Following each switch samples were obtained for one-minute intervals for five minutes and then at five-minute intervals for the remaining twenty-five minutes of each period. A: Insulin-release profile due to glucose (20 mM). B: Insulin-release profile due to glucose (20 mM) plus somatostatin (STS) (100 ng./ml.) perifused during time period t_{30} to t_{60}. For each condition, three experiments are recorded. Each value represents the mean ± S.E.M.

glucagon release by 80 per cent (data not shown). In contrast, there was no effect by the addition of glucose to the perfusate on basal glucagon release from perifused islets (figure 3).

Effect of Arginine Alone and in Combination with Glucose on Insulin Release

Arginine (15 mM) by itself had no effect on insulin

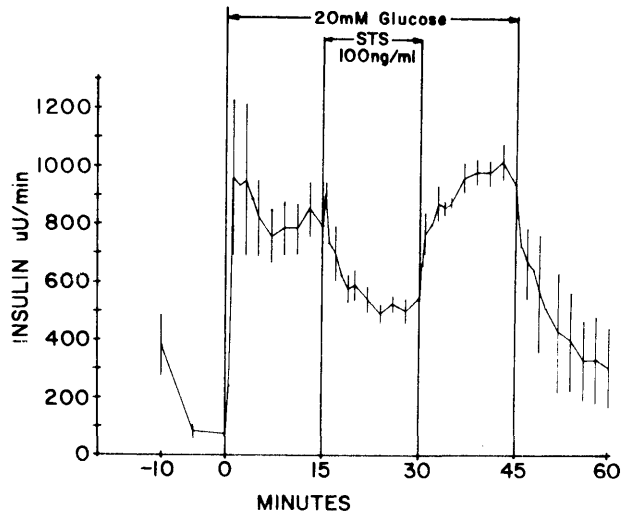

FIG. 2. Effect of glucose and glucose plus somatostatin on insulin release from the perfused pancreas. The pancreas was perfused for seventy-five minutes. After preperfusion for fifteen minutes (t_{-15}-t_0) with a substrate-free buffer, the circulation was switched to a solution containing glucose (20 mM) and then to glucose plus somatostatin (STS) (100 ng./ml.), as indicated. The poststimulatory responsiveness was tested by switching back to the preperfusion fluid (t_{45}-t_{60}). Samples were obtained at five-minute intervals during the preperfusion period; after the switch to the medium containing the stimulant or inhibitor under study, samples were collected at 0.5 minutes after the change, then every minute for five minutes, and finally every two minutes for the remaining ten minutes of each period. Three experiments are recorded. Each value represents the mean ± S.E.M.

release from the isolated islets (figure 4). However, in the perfused pancreas arginine alone clearly stimulated release, although not very markedly (figure 5). Upon addition of 20 mM glucose, peak insulin release from

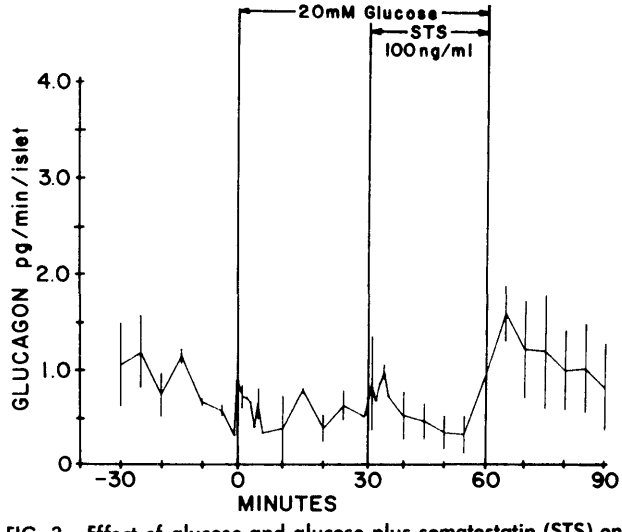

FIG. 3. Effect of glucose and glucose plus somatostatin (STS) on glucagon secretion from isolated perifused islets. The perifusion was performed as indicated in the legend to figure 1. Three experiments are recorded. Each value represents the mean ± S.E.M.

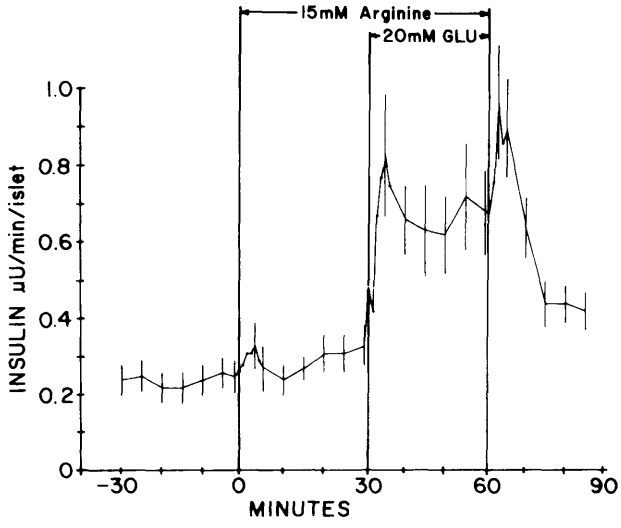

FIG. 4. Effect of arginine and arginine plus glucose (20 mM) (GLU) on insulin release from isolated perifused islets. The perifusion was performed as indicated in the legend to figure 1. Six experiments are recorded. Each value represents the mean ± S.E.M.

the perfused pancreas was greater than that observed with the 20 mM glucose stimulus alone (compare figure 2 with figure 5). The glucose and arginine effects were nearly additive. This is different from arginine-induced insulin secretion with threshold glucose (i.e. 2.5-5 mM), which leads to potentiation of arginine-induced insulin secretion.[8] In contrast to the perfused pancreas, the combination of high glucose and high arginine on isolated perifused islets did not result in a further increase in insulin release over that observed with 20 mM glucose alone (cf. figures 1 and 4).

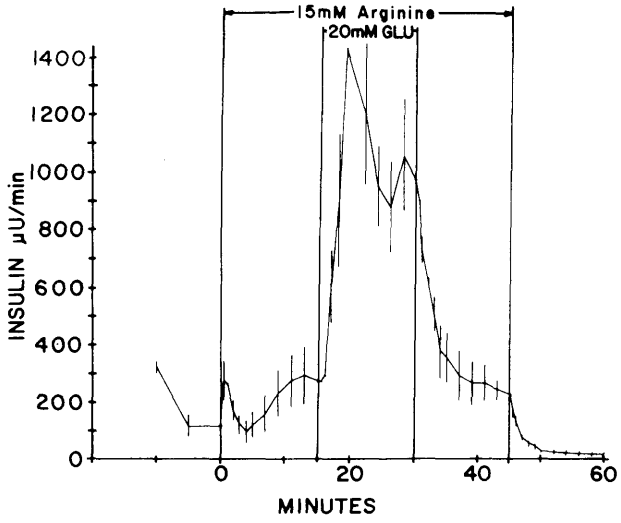

FIG. 5. Effect of arginine and arginine plus glucose (GLU) on insulin release from the perfused pancreas. The perfusion was performed as indicated in the legend to figure 2. Three experiments are recorded. Each value represents the mean ± S.E.M.

Effect of Arginine Alone and in Combination with Glucose on Glucagon Secretion

Arginine releases glucagon biphasically from the isolated perfused pancreas, and this release is rapidly inhibited to basal secretion rates upon addition of 20 mM glucose (figure 6). Removal of glucose with continued arginine perfusion results in a slow return of glucagon to maximal rates of secretion. Upon subsequent removal of the arginine stimulus, glucagon rapidly falls below basal secretion rates (figure 6).

Glucagon release from isolated perifused islets due to 15 mM arginine was consistently observed and was reversible upon removal of the stimulant, as occurred with the perfused pancreas (figure 7). Glucagon secretion occurred rapidly and attained maximum secretion rates three-to-fivefold higher than basal levels within one to two minutes of addition of the stimulus. Termination of release after removal of the stimulant was slower than initiation ($t_{1/2}$ ~ ten minutes). In marked contrast to the perfused pancreas, in which 20 mM glucose inhibited total arginine-stimulated glucagon secretion by 51 per cent, glucose had only a minor inhibitory effect (18 per cent) on arginine-stimulated glucagon release from isolated islets (table 1; compare figure 7A with 7B). A further indication that glucose was acting as an antagonist of arginine was the rapid surge of glucagon secretion following removal of both glucose and arginine from the infusion. This short burst was not observed when arginine was removed in the absence of glucose (cf. figures 7A and 7B, sixty

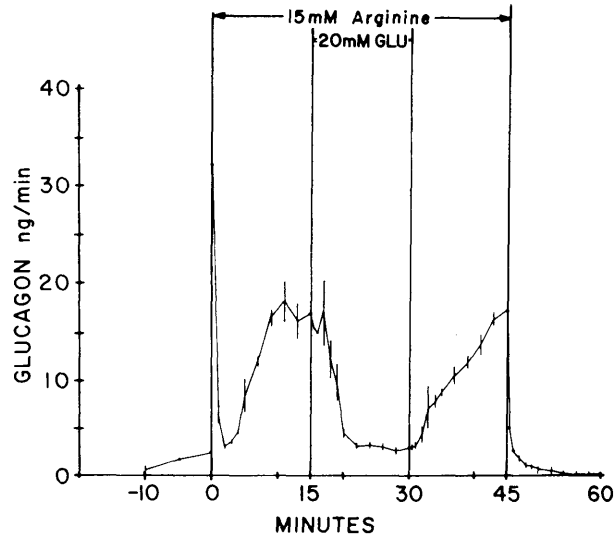

FIG. 6. Effect of arginine and arginine plus glucose (GLU) on glucagon release from the perfused pancreas. The perfusion was performed as indicated in the legend to figure 2. Three experiments are recorded. Each value represents the mean ± S.E.M.

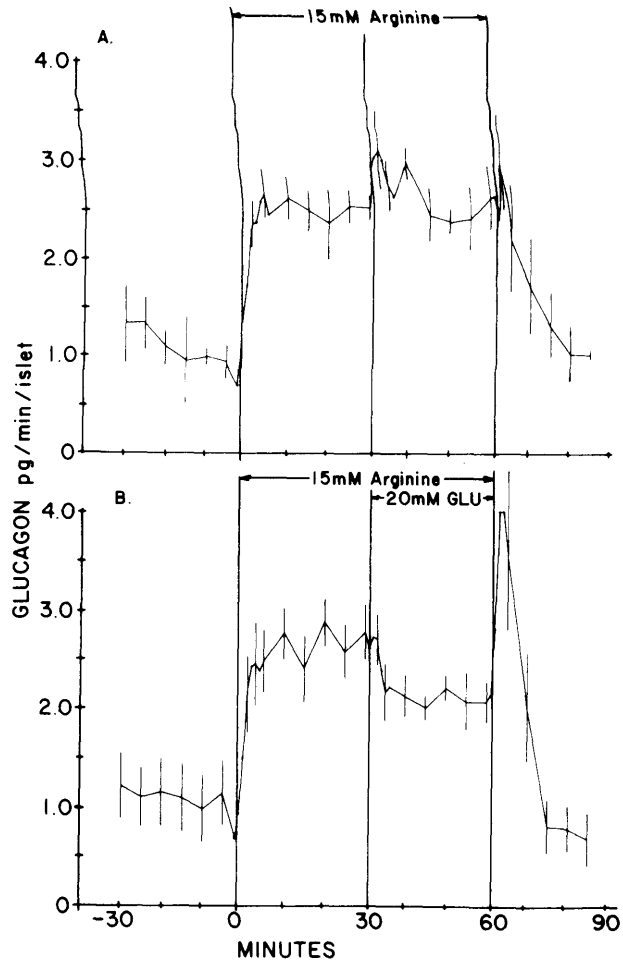

FIG. 7. Effect of arginine and arginine plus glucose (GLU) on glucagon release from isolated perifused islets. The perifusion was performed as indicated in the legend to figure 1. A: Glucagon release profile due to 15 mM arginine. B: Glucagon release profile due to arginine (15 mM) plus glucose (20 mM) perifused during time period t_{30} to t_{60}. For each condition, six experiments are recorded. Each value represents the mean ± S.E.M. ——→

to ninety minutes). This "off response" suggests derepression of glucagon release upon removal of glucose.

To exaggerate a possible inhibitory effect of glucose on glucagon secretion from isolated islets, 20 mM glucose was infused for seventy minutes before the addition of 15 mM arginine, since we have previously demonstrated that preperfusion of the isolated pancreas with as little as 5 mM glucose prior to stimulation with 20 mM arginine inhibited glucagon release by 80 per cent.[8] However, even when present throughout, glucose was still ineffective in inhibiting glucagon release (figure 8).

Effect of Somatostatin on Insulin and Glucagon Secretion

It is well documented that somatostatin inhibits insulin and glucagon secretion both in vivo[13] and in vitro.[14,15] In the present study, glucose-mediated insulin release in the perfused pancreas was reduced 32 per cent by a high dose of somatostatin (100 ng./ml.) (figure 2 and table 2). Insulin release rapidly returned to stimulated levels upon removal of somatostatin. In contrast, glucose-mediated insulin secretion from perifused islets was not affected either by addition of somatostatin in the middle of the perifusion (compare figures 1A and 1B, thirty to sixty minutes, and table 2) or by perifusion of somatostatin for seventy minutes

TABLE 1

Total integrated glucagon release due to arginine (15 mM) before and following addition of glucose, somatostatin and glyceraldehyde*

	Perifused Islets Glucagon†			Perfused Pancreas Glucagon†		
Condition	pg./30 min./islet	%Inhibition	p‡	ng./15 min.	%Inhibition	p‡
Glucose (20 mM) (n)				(n)		
−	80.2 ± 6.9			186 ± 12		
6		18	<0.01	3	51	<0.01
+	65.5 ± 5.2			91 ± 8		
Somatostatin (100 ng./ml.)						
−	86.5 ± 8.2			198 ± 5		
9		14	N.S.	3	72	<0.01
+	74.7 ± 8.0			55 ± 10		
Glyceraldehyde (5 mM)						
−	101.9 ± 12.4			164 ± 31		
3		6	N.S.	4	41	<0.05
+	95.4 ± 8.2			96 ± 14		

*The results presented in this table are from the experiments described in figures 6, 7, 10, 11, 14, and 15.

†The values represent the mean ± S.E.M. of the total integrated secretion rates of glucagon obtained by planimetry of the areas under the curves extending over the indicated period of time.

‡Degree of significance by paired *t* test comparing release before and following addition of somatostatin.

TABLE 2

Total integrated insulin release due to glucose (20 mM) before and following addition of somatostatin*

Condition Somatostatin (100 ng./ml.)	Perifused islets Insulin† (n)	mU./30 min./islet	% Inhibition	p‡	Perfused pancreas Insulin† (n)	mU./15 min.	% Inhibition	p‡
−	3	0.027 ± 0.005	0	N.S.	3	12.05 ± 1.97	32	<0.05
+		0.029 ± 0.004				8.15 ± 0.74		

*The results presented in this table are from the experiments described in figures 1 and 2.

†The values represent the mean ± S.E.M. of the total integrated secretion rates of insulin obtained by planimetry of the areas under the curves extending over the indicated period of time.

‡Degree of significance by paired *t* test comparing release before and following addition of somatostatin.

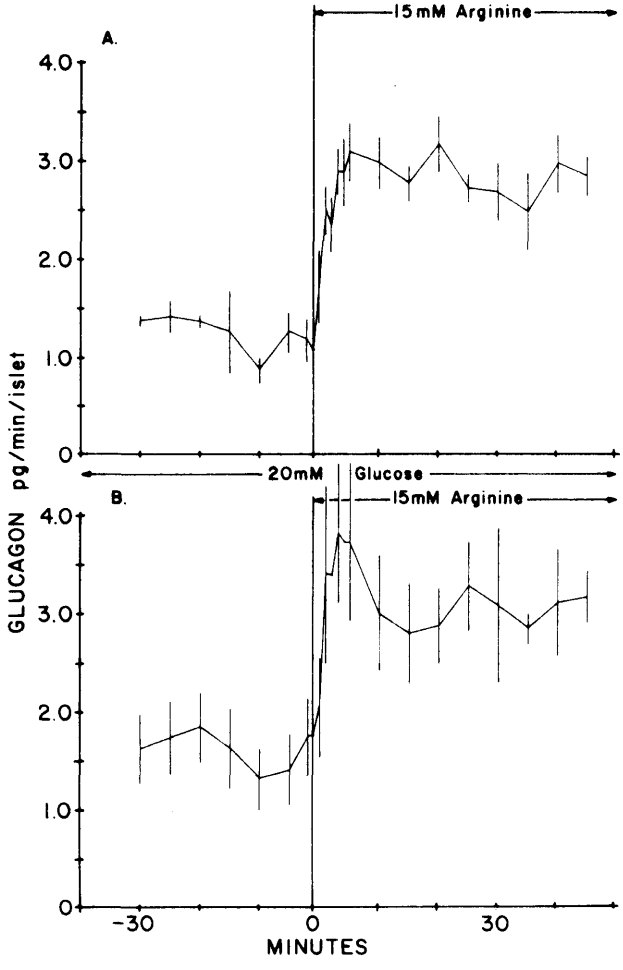

FIG. 8. Effect of glucose on inhibition of arginine-induced glucagon secretion. A: Glucagon-release profile from isolated islets perifused for seventy minutes with substrate-free buffer followed by perifusion with 15 mM arginine for forty-five minutes (t_0 - t_{45}). B: Glucagon-release profile from isolated islets perifused with glucose (20 mM) with addition of arginine (15 mM) for forty-five minutes (t_0 - t_{45}). For each condition three experiments are recorded. Each value represents the mean ± S.E.M.

prior to and during stimulation with glucose. Furthermore, somatostatin had no effect on basal insulin secretion (figure 9).

Arginine-stimulated glucagon release from the perfused pancreas was reduced to basal rates by 100 ng. per milliliter of somatostatin (figure 10 and table 1). Upon removal of somatostatin, glucagon release did not return to prestimulatory levels. Only 40 per cent of the maximum stimulated rate was restored. Perifused islets, however, showed little inhibition of stimulated glucagon release by the same concentration of somatostatin that produced suppression to basal secretion rates in the perfused system. Although a 14 per cent decrease in stimulated glucagon secretion was observed, it was not statistically significant (figure 11, table 1). Also, only a slight "off response" occurred upon removal of both arginine and somatostatin from the perifusion media (figure 11).

To further investigate this loss of suppressibility of isolated islets in the perifusion system, experiments were performed in which somatostatin was infused for seventy minutes prior to and during the stimulation with arginine. Again somatostatin was unable to prevent arginine-stimulated glucagon release in three paired experiments (total release 49.5 ± 7.0 versus 73.3 ± 11.2 pg. per thirty minutes per islet in perifusions with arginine alone versus arginine plus somatostatin, respectively, $p > 0.1$).

Effects of Glyceraldehyde on Arginine-induced Insulin and Glucagon Release

Glyceraldehyde has been shown to be a potent stimulus for insulin release from mouse islets obtained with collagenase[16] and from microdissected mouse islets.[17] Since this agent is also a potent inhibitor of amino-acid-induced glucagon secretion in the isolated perfused pancreas,* glyceraldehyde was used to compare alpha-cell suppressibility of the two systems.

Glyceraldehyde (5 mM) superimposed on arginine resulted in biphasic release of insulin both in the perfusion and perifusion systems (figures 12 and 13), similar to that observed with 20 mM glucose. This

*Unpublished data from our laboratory.

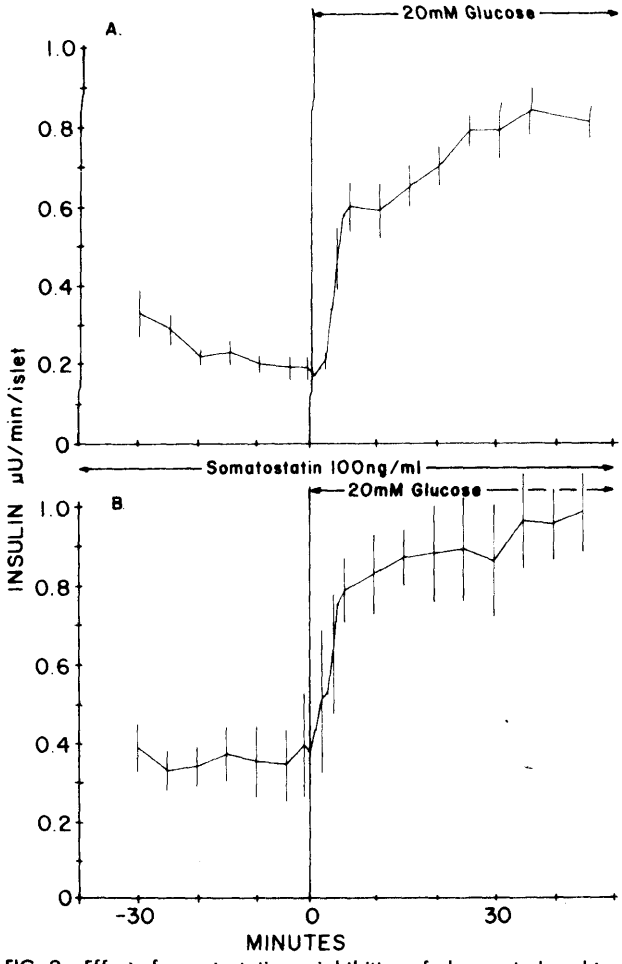

FIG. 9. Effect of somatostatin on inhibition of glucose-induced insulin release. A: Insulin-release profile from isolated islets perifused for seventy minutes with substrate-free buffer followed by perifusion with glucose (20 mM) for forty-five minutes (t_0-t_{45}). B: Insulin-release profile from isolated islets perifused with somatostatin (100 ng./ml.) with addition of glucose (20 mM) for forty-five minutes (t_0-t_{45}). For each condition three experiments are recorded. Each value represents the mean ± S.E.M.

agent was also a potent inhibitor of amino-acid-induced glucagon secretion in the perfusion system (figure 14 and table 1) again similar to that observed with 20 mM glucose. In contrast to the perfused pancreas, glyceraldehyde was incapable of inhibiting arginine-stimulated glucagon release from perifused isolated islets (figure 15 and table 1).

DISCUSSION

It was one of the objectives of the present investigation to develop a technic for studying the dynamics of insulin and glucagon secretion from perifused rat islets obtained with the collagenase procedure. Although we were able to demonstrate both insulin and glucagon release to appropriate stimuli, only glucose resulted in minimal but significant inhibition of glucagon secretion. Somatostatin did not significantly inhibit insulin or glucagon secretion, and glyceraldehyde, a potent inhibitor of glucagon secretion from the perfused pancreas, was ineffective in inhibiting glucagon release from the isolated islet.

There was also a marked quantitative difference between the alpha cell responses to arginine stimulation in the two systems and the glucose response of the beta-cell. The ratio of glucose-stimulated insulin release per unit time is approximately 1:800 when comparison is made between the secretory rate of the isolated islet and that of the perfused pancreas. When this same calculation is performed for glucagon secretion, the ratio is approximately 1:4,600, 17 per cent of the expected amount. This sixfold decrease in glucagon release might be explained by a greater susceptibility of alpha cells to damage in the course of the collagenase procedure.

Previous comparable studies from other laboratories have been less successful in simultaneously stimulating insulin as well as glucagon secretion from isolated perifused rat islets. Whereas insulin release can be demonstrated routinely with this system, we are not aware of comparable published data utilizing the perifused islet system to determine the dynamics of stimulated glucagon secretion from rat islets. For example, in a recent report, Johnson et al. were unable to elicit either arginine stimulation or glucose suppression of basal glucagon release from perifused rat islets.[15] Similarly, in other studies with batch-

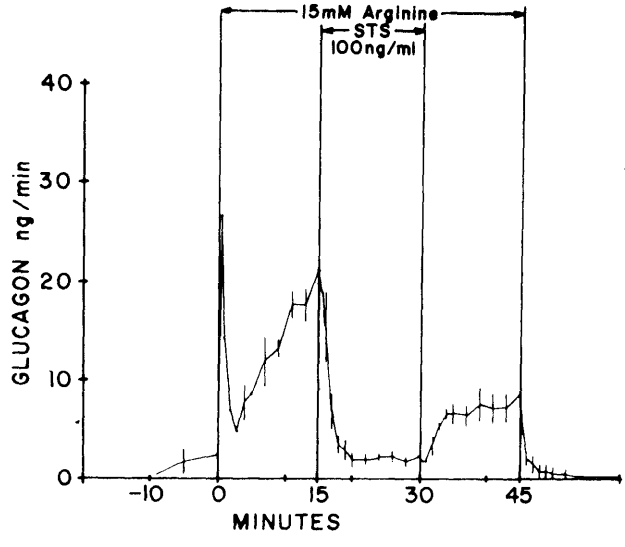

FIG. 10. Effect of arginine and arginine plus somatostatin (STS) on glucagon release from the perfused pancreas. The perfusion was performed as indicated in the legend to figure 2. Three experiments are recorded. Each value represents the mean ± S.E.M.

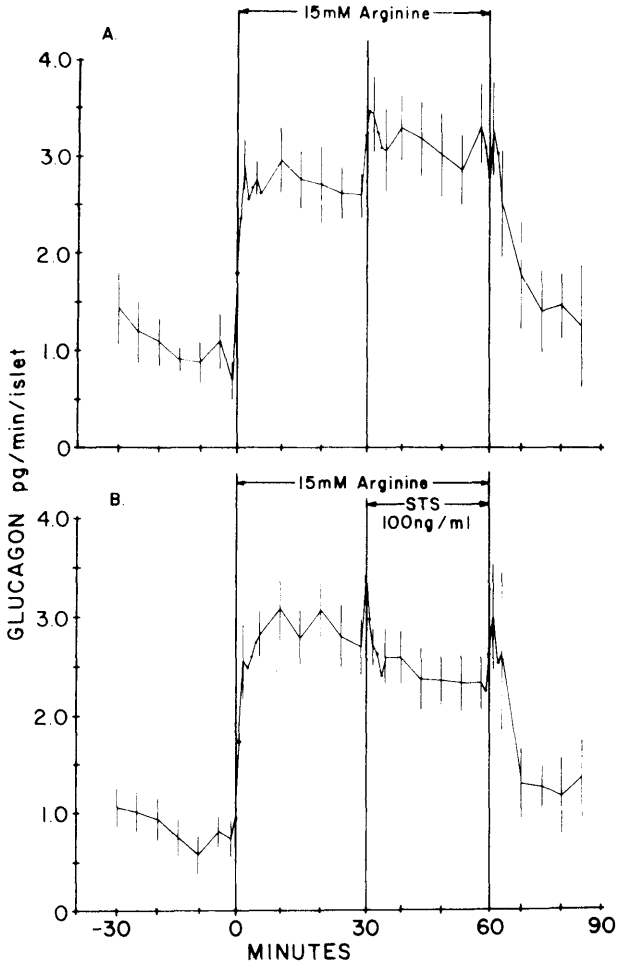

FIG. 11. Effect of arginine and arginine plus somatostatin from isolated perifused islets. The perifusion was performed as indicated in the legend to figure 1. A: Glucagon-release profile due to arginine (15 mM). Six experiments are recorded. B: Glucagon-release profile due to arginine plus somatostatin (100 ng./ml.) perifused during time period t_{30} to t_{60}. Nine experiments are recorded. Each value represents the mean ± S.E.M.

incubated isolated rat islets, suppression by high glucose of basal glucagon was either minimal or nonexistent.[2,3] An exception are the studies by Edwards and Taylor, who incubated batches of guinea pig islets and were able to obtain stimulation of glucagon release with arginine but failed to show inhibition of basal glucagon release with glucose.[18]

The differences in the results of the present study and those previously reported may well be due to preparation of the animals prior to islet isolation. In contrast to the other studies, islets were obtained from animals fasted overnight and pretreated with pilocarpine to decrease the zymogen content of the pancreas.[5] Kuo et al. have demonstrated that prior treatment of the rat with pilocarpine, a potent cholinomimetic

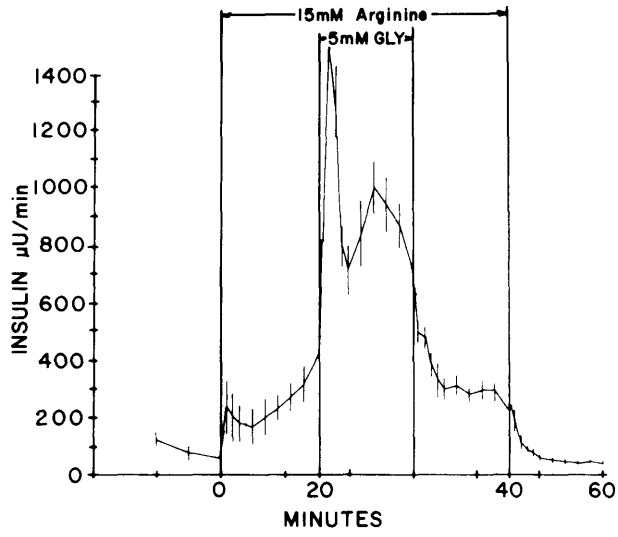

FIG. 12. Effect of arginine and arginine plus glyceraldehyde (GLY) on insulin release from the perfused pancreas. The perfusion was performed as indicated in the legend to figure 2. Four experiments are recorded. Each value represents the mean ± S.E.M.

agent, results in an increased yield of islets, together with a greater and more predictable response of adenyl cyclase activity to stimulation by a variety of hormones.[5] The use of pilocarpine resulted also in increased yields of islets in the present study. Kuo et al. suggested that both the increased yield and adenyl cyclase responsivity of their islet preparations may have been due to decreased interference during the isolation procedure by proteolytic enzymes that could destroy the islet plasma membrane structure,[5] analo-

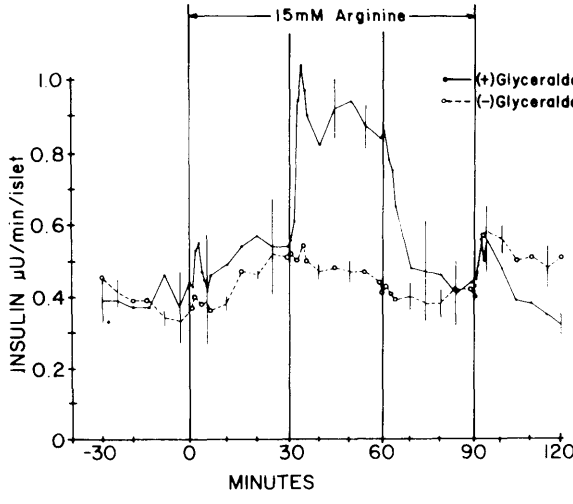

FIG. 13. Effect of arginine alone and arginine plus glyceraldehyde (GLY) on insulin release from isolated perifused islets. Glyceraldehyde (5 mM) was perifused for thirty minutes (t_{30}-t_{60}). For each condition three experiments are recorded. Each value represents the mean ± S.E.M.

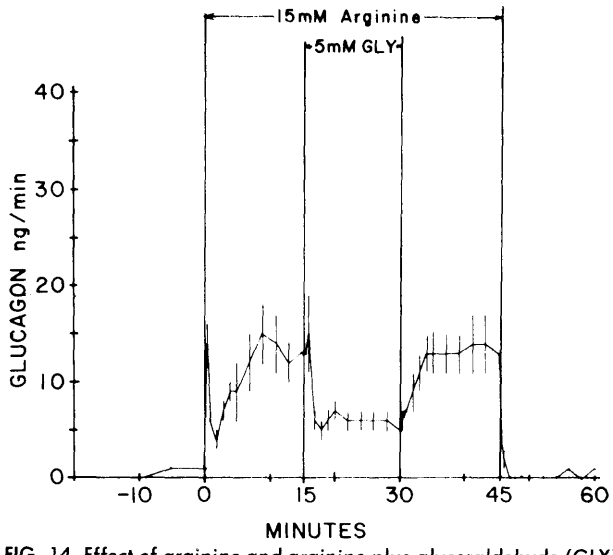

FIG. 14. Effect of arginine and arginine plus glyceraldehyde (GLY) on glucagon release from the perfused pancreas. The perfusion was performed as indicated in the legend to figure 2. *Four experiments are recorded. Each value represents* the mean ± S.E.M.

gous to the observations of Rodbell et al. with fat cell ghosts.[19] The fasting was probably responsible for the reduced insulin release due to glucose when compared with results obtained with islets from fed animals† but may have also helped to preserve, in an unknown manner, the alpha-cell responsiveness to arginine.

The alpha and beta cells of the isolated islets, however, when studied with the perifusion system exhibited drastic changes in their chemosensitivity, as judged by corresponding results in vivo[13] or with the isolated perfused pancreas in vitro.[8,14] Both insulin and glucagon release were not inhibited by a dose of somatostatin (100 ng./ml.), which was highly effective in comparable experiments with the intact perfused rat pancreas. Regarding insulin secretion, the present findings confirm two previous reports, one utilizing batch-incubated islets[20] and the other the perifused system,[15] which demonstrated the lack of somatostatin inhibition of glucose-stimulated insulin release. It remains to be elucidated through detailed dose-response studies whether this result of decreased somatostatin suppressibility seen here with one fixed dose is absolute or merely relative, i.e. the dose-response curve obtained with isolated islets may be shifted to the right when compared with the dose-response curve observed with the perfused pancreas. The possibility of relative resistance to inhibitory agents is underscored by the observations that in incubated[1] as well as perifused islets,[11] epinephrine, which inhibits insulin release through alpha-adrenergic receptor stimulation,[21] is effective in inhibiting insulin secretion only in concentrations 200-to-2,000-fold greater than circulating plasma levels. In contrast to isolated islets, epinephrine infused into the intact isolated pancreas is a potent inhibitor of insulin secretion at physiologic concentrations.[22]

There are a number of possible explanations for the loss of efficacy in inhibitors of glucagon and insulin release in isolated perifused islets: (1) the loss of inhibition by somatostatin could have been related to proteolytic enzymes contaminating the isolated islets. However, this would not explain the loss of inhibition due to glucose and glyceraldehyde; (2) the inhibitory action may be indirect and require nonendocrine factors (nerve cells or exocrine cells) that are lost or damaged during the isolation procedure; (3) the isolated islets might be unable to develop a state of hyperpolarization, which might be the basis of the inhibitory process. Such an inability to hyperpolarize may be due to membrane damage or metabolic impairment of the islets resulting from the isolation procedure; and finally (4) the alpha and beta cells may have lost inhibitory receptors.

It is currently not possible to clearly decide which, if any, of the four explanations is correct. Nevertheless, we feel that a decrease of specific receptors is the most likely cause of the results, as has been suggested for the inhibitory effects of somatostatin on insulin and glucagon release from monolayer cell cultures of newborn rat pancreas.[23] Such a working hypothesis is

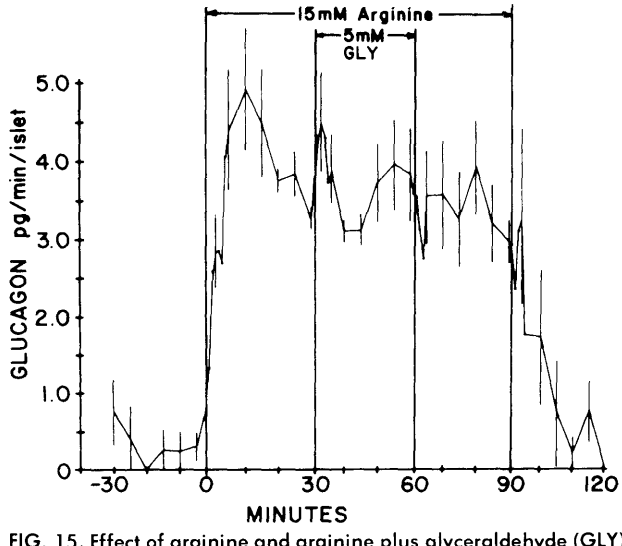

FIG. 15. Effect of arginine and arginine plus glyceraldehyde (GLY) on glucagon release from isolated perifused islets. The perifusion was performed as indicated in the legend to figure 1. Glyceraldehyde (5 mM) was perifused for thirty minutes (t_{30}-t_{60}). Three experiments are recorded. Each value represents the mean ± S.E.M.

†Unpublished data from our laboratory.

open to testing. An answer may be provided through detailed dose-dependency studies utilizing the present system of perifused isolated islets. It may also be feasible to restore the postulated receptor impairment or loss through development of in vitro organ cultures of isolated islets as has been accomplished with isolated fat cells for restoration of the insulin receptor[24] and with osteocytes for parathyroid hormone receptors.[25]

ADDENDUM

Since submission of this manuscript L.T. Lemay, A. Lemay, and P.E. Lacy (Biochem. Biophys. Res. Comm. *63*:1130, 1975) reported that somatostatin inhibition of glucose-stimulated insulin release was markedly increased from islets maintained in organ culture for forty-eight hours as compared with freshly isolated rat islets. They suggested that collagenase treatment resulted in damage to the cell surface, which was followed by the regeneration of the required surface components during the forty-eight-hour culture period.

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