The Independence of Insulin Release and Ambient Insulin In Vitro

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SUMMARY

The effect of insulin on its own secretion was tested in three independent experimental models using insulin concentrations that approached physiologic values. The collected secretions from glucose-stimulated islet tissue had no effect on insulin release from other islets. Perifused insulin had no effect on the release of endogenous insulin even when the assay was completely controlled for dilutional effects. Perifused insulin had no effect on the release of prelabeled insulin from glucose-stimulated islets. Similarly, proinsulin did not affect insulin release. Porcine insulin did not affect the function of porcine or canine islets. These studies strongly support the independence of glucose-stimulated insulin secretion and ambient insulin. DIABETES *32*:1162–1167, December 1983.

Feedback inhibition of insulin release by insulin itself has been suggested in perifused dog pancreas,[1,2] and isolated islets of hamsters[3] and mice.[4] However, the evidence for a feedback effect was either nonspecific for insulin or relied upon a difference between total immunoreactive insulin and the amount of the exogenous insulin added to the system. Generally, a large pharmacologic rather than physiologic concentration of insulin was used. Differentiation between the exogenous and endogenous insulin by radioactive pre-labeling is probably more precise although sample chromatography is cumbersome. Indeed, Shatz and Pfeiffer failed to demonstrate any direct feedback effect of exogenous insulin on the secretion of ^{3}H-leucine pre-labeled insulin in isolated rat islets.[5] Similar negative results were reported by Malaisse also in rat islets.[6] In all these experiments, the exogenous insulin was porcine and the importance of species-specific insulin is not clear.

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Received for publication 10 March 1983.

In vivo studies are also controversial. Prolonged, high circulating levels of exogenous bonito insulin inhibit insulin secretion in man.[7] Bonito insulin has biologic activity in humans but does not cross-react with antibodies to human insulin. Porcine insulin effects on insulin secretion have been assessed by measuring changes in plasma C-peptide immunoreactivity, and an inhibitory effect was suggested when pharmacologic concentrations of insulin (600–700 μU/ml) were infused for a prolonged period of time.[8] However, high physiologic concentrations of porcine insulin failed to suppress plasma C-peptide in otherwise similar conditions[9,10] but led to a 40% decrease in C-peptide secretion in euglycemic conditions.[11]

In order to better understand the mechanisms of feedback inhibition three in vitro models were examined. First, dispersed islet tissue from either dogs or pigs was divided into two equal parts and placed in series in perifusion chambers such that the effluent from the first chamber perifused cells in a second chamber and the output from each chamber could be sampled. Second, islets from dogs and pigs were labeled with ^{3}H-leucine and perifused with or without exogenous porcine insulin and ^{3}H-insulin output compared after column chromatography. Finally, islets were perifused with exogenous insulin with tracer amounts of ^{125}I-insulin while controls were perifused in parallel but the same labeled exogenous insulin was added to the effluent. The effect of exogenous proinsulin was also investigated in both canine and porcine islets.

METHODS

Cell preparation and culture. Dog and pig pancreatic endocrine cells were purified by ductal perfusion as previously described.[12] Pig pancreas was perfused via the pancreatic duct with 0.5% collagenase (type I, Sigma, St. Louis, Missouri) and 0.5% hyaluronidase (Sigma) while the protocol for dog pancreas used only 0.2% collagenase. Cells were cultured for 3 days to attain the highest insulin/amylase ratio (data not shown). Medium 199 (Gibco, Grand Island, New York) with 10% heat-inactivated calf serum, Hanks' Salts, and 25 mM hydroxyethyl piperazine ethane sulfonic acid

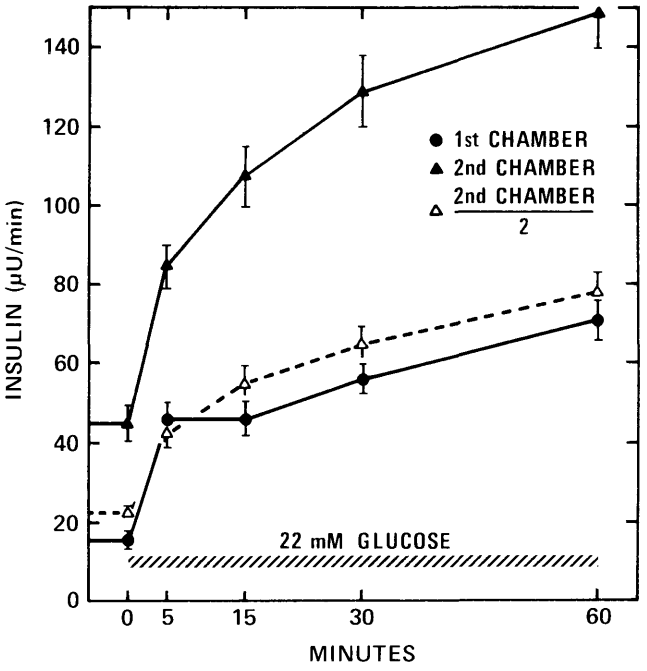

FIGURE 1. Insulin secretion data from the double chamber experiment. Double antibody RIA was employed. (●) = insulin from the first chamber, A; (▲) = insulin collected below the second chamber, B; (△) = ½ insulin collected below second chamber, B/2. Since A = B/2 no effect of the effluent of the first chamber on the insulin secretion by the second chamber can be assumed. Data are given as means ± SEM (N = 15).

(HEPES) was employed, and the cells were maintained at 25°C in spinner flasks (Bellco). Before each experiment the cells were washed three times in Hanks' Salts, counted, and checked for viability by trypan blue exclusion.

Perifusion. Cell isolation and culture yielded tissue functionally comparable to rat islets prepared by standard collagenase disruption. On the third day of culture, islet cell function was assessed according to the perifusion method of Lacy.[13] Approximately 1 × 10^6 cells were injected directly into a 2-ml, 25-mm diameter, Gelman filter holder containing a 1.2-μmillipore filter (Gelman) and a prefilter (Gelman Metrigard Superfine). Flow was maintained with a peristaltic pump (Buchler) at 1 ml/min. Perifusion was performed for 30 min at 37°C with 25 mM HEPES-buffered Hanks', pH 7.4, containing 2.75 mM glucose and 1% bovine serum albumin (RIA grade, Sigma). At the end of this period, a sample was taken for time zero. The cells were then challenged with medium 199, 25 mM HEPES, and 1% albumin containing 22.2 mM glucose for 60 min and 1-min samples were collected at 3, 5, 15, 30, and 60 min. There was no serum in the perifusion medium. These control perifusions in 21 preparations demonstrated a biphasic insulin response with little variation in quantitative function.

Double chamber experiment. Fifteen experiments were performed with canine tissue. At the third day of culture, identical cell masses (1 × 10^6 cells) were put into two perifusion chambers connected in series and perifused at a flow rate of 1 ml/min. Perifusate from the first chamber bathed the cells in the second chamber. Special attention kept the flow constant at all sampling ports because of the profound change in resistance when sampling below the first chamber with the second chamber disconnected. This difference in resistance can significantly affect flow resulting in a washout of insulin from the first chamber. Actually, flow rate has a profound effect on insulin output in perifusion, with higher flow rates giving greater insulin output by the same number of cells with the same stimulus. After 30 min of equilibration (2.75 mM glucose), a 60-min stimulus was given (22 mM glucose). The effluent from each chamber was collected at comparable time intervals (0, 5, 15, 30, and 60 min) and the secretion of insulin compared. In the absence of inhibition, i.e., no effect of the first chamber effluent on cells in the second chamber, the insulin concentration in the effluent collected below the second chamber should be twice that collected below the first. At the end of the experiment and after a 30-min perifusion with 2.75 mM glucose, the second

TABLE 1
Means and SEM of 10 paired experiments (six dogs, three pigs) addressing the effect of exogenous insulin marked with ^{125}I-insulin on endogenous insulin secretion in perifusion*

	1/1000	1/5000	1/10,000	1/20,000	1/30,000
Dogs (N = 7)					
Br	84.60 ± 2.05	67.37 ± 3.48	36.54 ± 2.59	25.93 ± 3.00	17.40 ± 2.24
Bi	58.87 ± 0.71‡	43.69 ± 6.31‡	23.06 ± 0.71‡	14.87 ± 1.84‡	11.14 ± 1.70‡
Bc	56.42 ± 2.05‡	47.37 ± 6.35‡	23.94 ± 1.50‡	14.70 ± 1.33‡	11.80 ± 1.60‡
			6.54 ± 1.07		
Pigs (N = 3)					
Br	75.67 ± 3.18	57.33 ± 0.67	39.33 ± 0.67	29.67 ± 0.88	21.33 ± 0.33
Bi	54.00 ± 4.51‡	31.67 ± 6.67	21.67 ± 1.67†	15.33 ± 0.88‡	13.33 ± 0.67‡
Bc	54.00 ± 7.50	29.00 ± 5.03	22.33 ± 0.33‡	14.00 ± 1.00‡	12.67 ± 1.86
Bx			4.47 ± 0.34		

*The data are expressed as percentages of radioactivity precipitated at various dilutions of the first antibody (anti-insulin). The same amount of exogenous radioactive insulin is involved in all data. Bx is the blank or nonspecific binding where no anti-insulin antibody has been added. Br is the reference binding where the radiolabeled insulin is precipitated with first antibody. In Bi the same amount of radioactive insulin is passed over islet tissue in perifusion to ascertain any inhibitory effect. In Bc a similar mass of islet tissue is perifused and the radioactive insulin is added after the perifusion to the collected sample.
†,‡ = P 0.01, 0.005, respectively, comparing Bi and Bc with Br. Clearly, cold insulin is introduced by the perifused cells as indicated by the drop in precipitated ^{125}I-insulin. However, there is no statistical difference between the cold insulin contributed by the islet tissue when perifused with potentially inhibitory insulin at 165 μU/ml (Bi) and when the same amount of insulin is added to the sample after the perifusion (Bc).

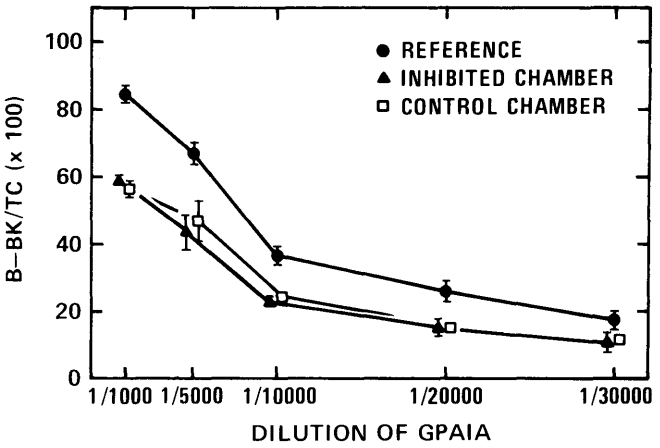

FIGURE 2. Means and SEM of seven paired experiments using 1 × 10^6 dog islet cells in perifusion with or without exogenous ^{125}I-insulin (165 μU/ml). Exogenous insulin was added to the perifusing medium above the "inhibited" chamber and to the effluent of the "control" chamber in the same amount with respect to radioactivity and insulin activity. B = counts bound to the anti-insulin antibody and precipitated in the RIA, Bk = nonspecific precipitation in the absence of anti-insulin antibody, and TC = the total number of counts added to the system. (●) = reference precipitation of the stock exogenous ^{125}I-insulin at various dilutions of the guinea pig anti-insulin antibody (GPAIA); (▲) = "inhibited" chamber demonstrating reduced radioactive precipitation correlating with release of endogenous insulin; (□) = control chamber with labeled insulin added to the effluent after perifusion. Exogenous insulin did not affect the release of endogenous in response to glucose in perifusion.

chamber was stimulated with 22 mM glucose. Samples were taken again at time 0, 1, 3, 5, 10, 15, 30, and 60 min. This control assessed the function of these cells in the second chamber without any suppressive effect of the first chamber. Insulin recovery from the perifusion apparatus in the presence of cells was evaluated by perifusing the chamber with medium containing ^{125}I-insulin. No significant difference in counts was found between the perfusion medium and perifusate; therefore, recovery of iodinated hormone was not affected greatly by the cells.

Insulin assay. Insulin concentrations were determined using the double-antibody radioimmunoassay described by Morgan and Lazarow.[14] Crystalline porcine insulin (lot 615-07J-256, Eli Lilly and Company, Indianapolis, Indiana) was prepared in standard concentrations. Guinea pig anti-insulin antibody (lot no. GP21, Miles Laboratories, Elkhart, Indiana) was used at 1:10,000 dilution, and precipitation was carried out with goat anti-guinea pig antibody (Linco lot 2011). ^{125}I-insulin (89 μCi/mg) was purchased from New England Nuclear (Boston, Massachusetts). One milliliter of 22.5% polyethylene glycol was added to each tube before precipitation to facilitate dilution and stabilize the pellet. This led to much less variability in the assay. The insulin buffer contained 1% bovine serum albumin (RIA grade, Sigma), 0.025 M EDTA, 0.025% thimerosal (Sigma), pH 7.6, in 0.05 M phosphate buffer, normal saline. All perifusion samples were run in triplicate and standards were prepared with perifusion medium in the unknowns to avoid error due to differences in nonspecific binding.

Exogenous insulin with ^{125}I-insulin marker. After 3 days of culture 1 × 10^6 cells were placed in each of two chambers and perifused in parallel with medium 199 containing 22 mM glucose, 1% bovine serum albumin (RIA grade), and 25 mM HEPES. After 30 min the second phase of insulin release was well established. In this experiment, the effect of exogenous insulin on the second phase of insulin release was tested. Flow was 20 ml/h in both chambers. ^{125}I-insulin (New England Nuclear, 89 μCi/μg) in isotonic buffer was added to the medium at a final concentration corresponding to 165 μU/ml and passed through the test chamber for 15 min. Medium diluted with the same volume of insulin buffer but without ^{125}I-insulin perifused the control. A 5-ml sample (15 min) was collected from both chambers. The sample from the insulin perifused chamber was counted in a Beckman gamma counter (Beckman Instruments, Fullerton, California), and diluted ^{125}I-insulin solution was added to the effluent from the control chamber to equalize the radioactivity and exogenous insulin in both samples. The very large amount of radioactivity used in the experiment permitted assay without dilution. Control and treated effluent volumes were kept equal by addition of insulin buffer. Aliquots of 0.3 ml from each sample were incubated for 24 h with 0.1 ml of various dilutions of guinea pig anti-porcine insulin antibody (Miles 1:1000, 1:5000, 1:10,000, 1:20,000, and 1:30,000). Perifusion medium diluted with appropriate volumes of insulin buffer and ^{125}I-insulin served as the reference. After 24-h incubation, 0.1 ml goat anti-guinea pig antibody (Linco) was added with a 1:70 dilution of normal guinea pig serum. After 3-h incubation, 1 ml of 22.5% polyethylene glycol was added to each tube, the samples were centrifuged, the supernatant aspirated, and the pellet counted. The percentages of ^{125}I-insulin counts precipitated in the pellets were compared. The ^{125}I-insulin counts precipitated in the reference tubes were considered maximal binding for each dilution of anti-insulin antibody. Release of insulin by the islets should result in fewer counts in the antibody pellet. To assess the equality of content of cells in the chambers, an effluent sample from each chamber was taken before adding exogenous insulin, and the insulin output was compared. The responsiveness of the cells in the chambers to glucose was demonstrated at the end of the experiment by performing a standard perifusion.

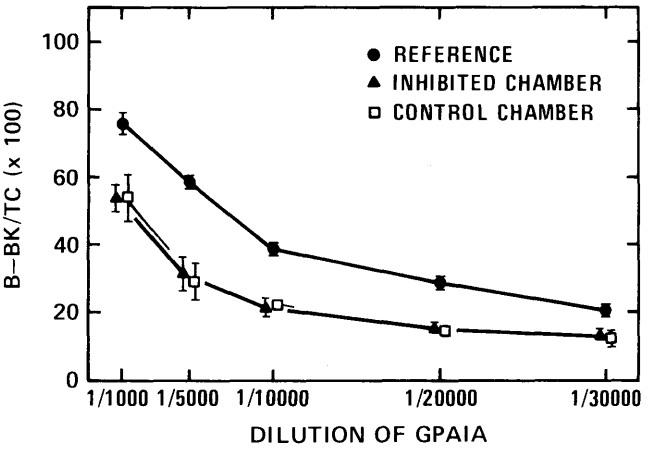

FIGURE 3. Means and SEM of three paired experiments with 1 × 10^6 pig cells examining the effect of exogenous insulin under the same experimental conditions as in Figure 5.

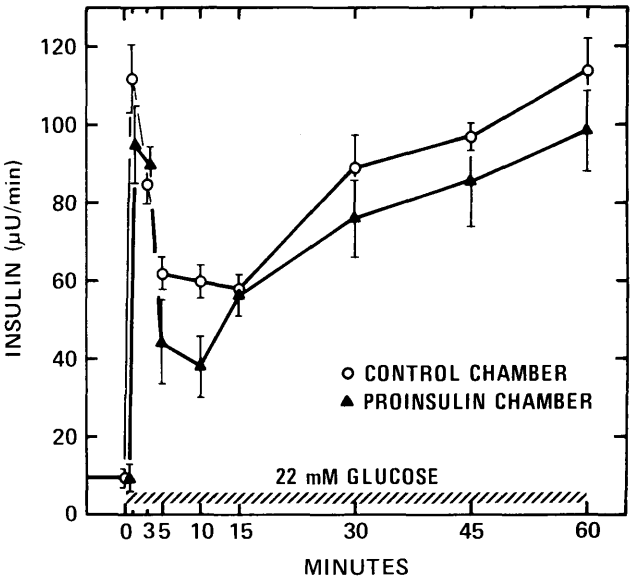

FIGURE 4. Means and SEM from seven perifusions with 1 × 10^6 porcine cells measuring insulin output in response to 22 mM glucose. (▲) = insulin output from cells perifused with 9000 pg/ml porcine insulin, equimolar to 150 μU/ml insulin; (○) = secretion in paired control chambers perifused without proinsulin.

Proinsulin study. In order to assess the potential suppressive activity of proinsulin, seven perifusions were performed with canine islet tissue adding a concentration of porcine purified proinsulin (Lilly) equimolar to 150 μU/ml of insulin (9000 pg proinsulin). Equimolar proinsulin and insulin standards were prepared and assayed simultaneously in each experiment to calculate the cross-reactivity of proinsulin and insulin. The insulin-like activity of proinsulin in our radioimmunoassay is about 20% that of insulin for each concentration studied (r = 0.999, intercept = 0, slope = 0.174; 17% of the theoretical slope of 1 for insulin).

Two chambers were perifused in parallel using 1 × 10^6 cells in each. The protocol was the same as for the exogenous insulin inhibition experiment. Therefore, the effect of proinsulin on the second phase of insulin secretion was evaluated. One chamber was perifused with the usual perifusing medium containing 9000 pg/ml proinsulin while the other chamber served as a control. One-minute samples taken from each chamber at 0, 1, 3, 5, 10, 15, 30, 45, and 60 min were assayed. Aliquots of the perifusing medium with proinsulin were run in the same radioimmunoassay and the resulting value was subtracted from the insulin concentration measured in samples from the chamber perifused with proinsulin. Thus, the actual insulin output could be compared for control and proinsulin-treated chambers.

Effect of exogenous insulin on the secretion of ^{3}H-insulin. After 2 days of culture 1 × 10^8 cells were washed. Labeling with ^{3}H-leucine followed the procedure of Shatz et al.[15] The cells were incubated overnight at 37°C in 20 ml of leucine-free MEM (Gibco), 10% calf serum, 0.5 mCi of l-leucine 4,5-^{3}H (56.5 mCi/mmol, NEN Laboratories), and 22 mM glucose. After incubation, the cells were washed and perifused. The flow was approximately 33 ml/h (100 ml/3 h), and perifusion was performed at 37°C with HEPES-buffered medium 199 containing 22 mM glucose, 1 mg/ml bovine serum albumin with and without 150 μU/ml of porcine insulin (Lilly). Perifusion fluid was collected for 3 h (100 ml) and precipitated in 100 ml of cold 20% trichloroacetic acid. The precipitate was washed three times with 5% trichloroacetic acid, extracted in 10 ml of 1 M acetic acid, and chromatographed on Sephadex G-50 (fine) equilibrated with 1 M acetic acid. The column was standardized for the chromatographic position of insulin using ^{125}I-insulin. Fractions were counted in liquid scintillation for ^{3}H and in a gamma counter for ^{125}I. This experiment tested the effect of exogenous insulin on the sum of both phases of glucose-stimulated insulin secretion. The filters containing the cells were extracted in 2 ml of 1 M acetic acid containing 0.1 ml 2% saponin for 24 h and 0.5 ml of the solution to determine comparable ^{3}H labeling of the islet tissue aliquots.

Statistical analysis. Paired and nonpaired *t* tests were used. Experiments were designed to avoid type II error (acceptance of the null hypothesis when it is actually false). The power of the test was calculated accepting a probability of type II error (beta) of less than 0.10 as the confidence limit for the acceptance of the null hypothesis. This confidence limit assumes an actual difference not higher than 25% between controls and cells exposed to exogenous insulin. Pearson correlation and linear regression analysis were used to predict the insulin-like activity of proinsulin in our RIA system at various concentrations of the hormones. All data are given as mean ± SEM.

RESULTS

Double chamber experiment. Figure 1 shows the means and SEM of 15 experiments. The concentration of insulin coming from the first chamber (A), the second chamber (B), and the concentration of insulin coming from the second chamber divided by 2 (B/2) are plotted. All the numbers are significantly different at the 0.005 level when A is compared with B. However, this calculation only says there is not complete inhibition of insulin output by the second chamber due to suppression by the first chamber. One can also evaluate the hypothesis A = B/2. In this case, no effect of the first chamber on the second chamber is assumed. However, if A is greater than B/2, inhibition of the second chamber is implied; if A is less than B/2, stimulation is implied. In fact, no significant difference could be found between A and B/2. Thus, no difference in the insulin release by the two chambers can be demonstrated. Although A and B/2 are not significantly different, this does not necessarily prove that they are the same. In order to avoid type II error (false negative) the power of the test must be calculated. The probability of this kind of error depends upon four factors: (1) the level of significance chosen to reject the hypothesis: A = B/2, (2) the actual difference existing between A and B/2 (delta), (3) variability of the statistic, and (4) sample size. Assuming that a difference of 10%, 25%, or 50% actually exists between the secretions of the two chambers the power of the test in these experiments is 28.8%, 99.1%, and 99.9%, respectively. Therefore, the likelihood of type II error for the statement, A = B/2, is 71.23% if the actual difference was 10%, 0.87% if it was 25%, and less than 0.00001 if it was 50%. From this analysis we can say with confidence that ambient islet secretions do not impart any major inhibitory effect on insulin release in response to glucose.

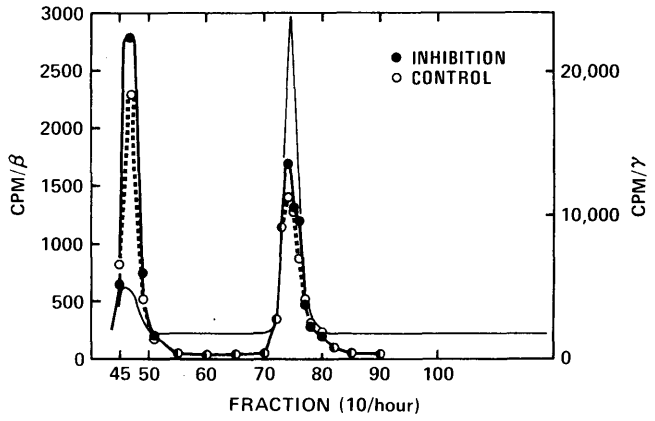

FIGURE 5. Porcine islet cells were labeled with H^3-leucine overnight, washed, and perifused with 22 mM glucose. The effluent was collected for 180 min and precipitated with 10% cold trichloroacetic acid. The precipitate was washed and extracted with 1.0 M acetic acid. This was run over a G-50 column and the chromatographed fractions were counted for in liquid scintillation for ^{3}H-leucine and in gamma counter to follow an ^{125}I-insulin marker. (●) = perifusion with standard medium plus 150 μU/ml porcine insulin; (○) = perifusion with standard medium without exogenous insulin. No evidence for feedback inhibition can be seen. Means of four experiments.

The data in Figure 1 do not reflect the first phase of insulin secretion due to the delay in sampling to avoid excessive manipulation of the perifusion apparatus. Otherwise, double chamber circuit perifusion was identical to standard perifusion. Even though the secretion rates from the second chamber appear slightly higher than the rates from the first chamber, none of the numbers was statistically significant at the 0.05 level.

After each double chamber perifusion the cells from the second chamber were perifused alone for 30 min with 2.75 mM glucose and then with 22 mM glucose for 60 min in order to assess comparability to the first chamber. The secretion of insulin rose from a baseline of 21.03 ± 3.52 μU/min to a first peak of 59.04 ± 3.81 μU/min at t_5. The second phase reached a secretory rate of 62.80 ± 3.75 μU/min at t_{30} and 87.96 ± 5.96 at t_{60}. These results are comparable to secretion from the first chamber during double chamber perifusion.

Exogenous insulin with ^{125}I-insulin marker. Data for canine and porcine cells are given in Table 1. The nonspecific binding was 5.92 ± 0.80% of total counts (N = 10). In both species the best sensitivity of the radioimmunoassay (RIA) was at a first antibody dilution of 1:10,000. The binding in the reference tubes was 36.54 ± 2.59% (N = 7) in the canine experiments and 39.33 ± 0.67% (N = 3) in the porcine experiments. This binding percentage offers a wide range of RIA sensitivity for the insulin concentrations expected from perifusion. Insulin secretion ranged between 50 and 60 μU/min with no evidence of inhibition by exogenous insulin. The precipitation of ^{125}I-insulin in treated and control samples is compared. Figures 2 and 3 show that the insulin-treated and control values are essentially coincident. A difference of 25% between treated and control chambers was the smallest difference we expected to identify in this experiment. The values at 1:10,000 first antibody were compared for statistical significance. If we assume a P_α of less than 0.05 and a P_β of less than 0.10, exogenous insulin failed to suppress the second phase of insulin secretion in any significant way.

Proinsulin study. A brisk biphasic insulin response to glucose was elicited in the presence or absence of proinsulin. Baseline insulin secretion was the same in both chambers (9.61 ± 2.00 μU/min in the control compared with 9.86 ± 2.63 μU/min from the chamber perifused with proinsulin). A first peak occurred in the first minute of perifusion at 111.99 ± 9.30 μU/min in the control compared with 95.25 ± 11.13 μU/min in the proinsulin chamber. At t_{10} the secretion was 59.52 ± 4.20 μU/min and 38.82 ± 8.18 μU/min, respectively. A steady rise followed with a secretion rate of 89.22 ± 7.74 μU/min in the control and 75.62 ± 10.25 μU/min in the experimental chamber at t_{30} and 114.02 ± 8.06 μU/min compared with 98.52 ± 10.45 μU/min at the t_{60} min (Figure 4). The slightly lower values in the proinsulin-treated chambers were not statistically significant.

Effect of exogenous insulin on the secretion of ^{3}H-insulin. The tritium counts in protein were extracted from the filters that held the islet cells. The total radioactivity extracted from the filters after perifusion was about 10 times the radioactivity collected in 3 h of perifusion and about 100 times the chromatographic peak corresponding to insulin. A radioactive peak between void volume and the insulin peak corresponding to proinsulin was never found in either species. When the chamber was perifused with exogenous insulin, the mean insulin peak in four experiments performed with porcine cells was 1700 ± 1000 cpm/ml while 1400 ± 350 cpm/ml were recovered in the insulin peak from the control chamber (Figure 5). In three experiments with canine islets, the insulin peak from insulin-treated chamber was 4500 ± 900 cpm/ml while 4400 ± 1500 cpm/ml were recovered from controls (Figure 6). These experiments fail to demonstrate an effect of exogenous insulin on the release of ^{3}H-leucine labeled insulin.

DISCUSSION

Islets of Langerhans function as a tightly integrated endocrine community regulated by the secretions and secretagogues of the component cells, cell-cell interactions, innervation, and, perhaps, acinar islet interactions. Understanding the mechanisms of integrated function has not been straightforward due to the difficulties of separating islet tissue from acinar tissue and the inability to separate component cells of islets in order to study component function. It is known that purified islets do not secrete particularly

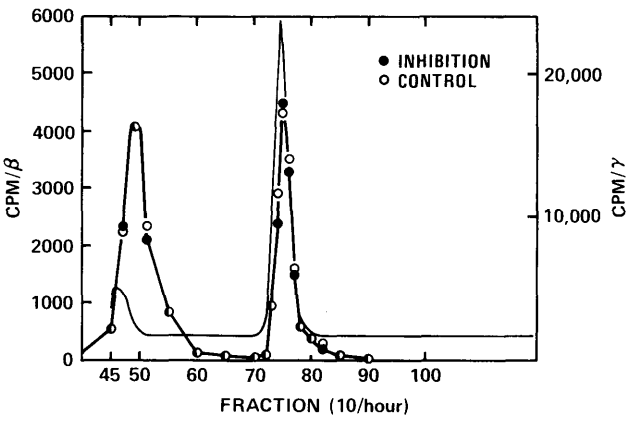

FIGURE 6. Data for three experiments performed as in Figure 5 but with canine cells rather than porcine cells.

well when incubated as a pellet in a stimulating concentration of glucose.[4] Perifusion abolishes local gradients of secreted products and leads to a striking secretion of insulin in the well-recognized biphasic pattern. The observed improvement of in vitro function when gradients are abolished suggests that beta-cells are affected by some form of feedback inhibition.

In the simplest mechanism of feedback inhibition, ambient insulin could suppress the output of additional insulin by stimulated beta-cells. This possibility has been surprisingly difficult to study. The use of pharmacologic concentrations of exogenous insulin has been troublesome because of the dilutions necessary to perform the radioimmunoassay. If a small difference between the high exogenous insulin concentration and an expected value constitutes the expected endogenous response, dilution to bring the insulin concentration into a conveniently assayable range at a given antibody dilution will obscure the endogenous contribution. Finally, there is the possibility that species specificity could be important in feedback inhibition such that exogenous insulin of a different species would not have the expected biologic effect.

The double chamber experiment described resolves some of these difficulties (Figure 1). Clearly, baseline insulin secretion was not affected by exposure of the second chamber to the output of the first. Expected differences in insulin concentration were not difficult to clarify in our radioimmunoassay. Stimulation with 22 mM glucose led to a brisk response from both chambers and the secretion of insulin from the two chambers was not significantly different. Since cells of the same species were compared in this experiment, there should be no error due to species specificity. The double chamber experiment must be controlled for flow at each sampling port because over a considerable range insulin output is directly proportional to flow.

Generally, when exogenous insulin has been used as a potential inhibitor, the same exogenous insulin has been a major contaminant of the radioimmunoassay system in antibody competition with endogenous insulin and a ^{125}I-insulin standard. Correction for exogenous insulin gave an indirect value subject to some error especially when the exogenous hormone exceeded the expected value of insulin secretion by a factor of two or three. To avoid this problem we used the same ^{125}I-insulin as a potential suppressor and as the competing ligand in the radioimmunoassay. This study failed to demonstrate any feedback mechanism on insulin secretion by islet secretions or insulin itself or proinsulin.

These experiments addressed the second phase of insulin secretion as the most likely period for a feedback loop. The first phase of insulin secretion is self-limiting in perifusion where no endogenous inhibiting factor could be present in the perifusion medium.[14,16] Furthermore, most previous studies on insulin secretion that suggested a short feedback loop dealt with the second phase of insulin release, specifically those studies performed in vivo.[8,11] The discrepancy between our data and those studies that support insulin feedback inhibition could be explained by the use of pharmacologic insulin concentrations in other studies[1,2] while we used a normal to high physiologic concentration (150–165 μU/ml). Dunbar[3] reported that recirculating the perifusion medium led to an inhibition of insulin secretion. Our double chamber model is similar to that model but does not allow accumulation of metabolites that could lead to nonspecific suppression. Although the same author did not demonstrate an inhibitory effect of insulin on its own secretion in perifusion, proinsulin had a modest inhibitory effect.[3] In that experiment the assay antibody reacted equally with insulin and proinsulin antibody while in our study the antibody was cross-reactive with proinsulin to a lesser extent (about 20%). With less interference in the radioimmunoassay we failed to demonstrate a significant reduction in insulin secretion due to exogenous proinsulin. Our results are in agreement with most studies performed in vivo where inhibition of the second phase of insulin secretion has not been demonstrated.[8–10]

In summary, three in vitro systems fail to support a role for ambient insulin in regulating insulin release. Paracrine regulation is not addressed in these experiments and may certainly play a powerful regulatory role.

ACKNOWLEDGMENTS

The assistance of Peggi Polen is greatly appreciated. This work was supported by grants from the March of Dimes and Kroc Foundation. Dr. Merrell was supported by a Research Career Development Award from the NIH.

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