Inhibition of Insulin Secretion by Exogenous Insulin in Normal Man as Demonstrated by C-peptide Assay

John E. Liljenquist, M.D., David L. Horwitz, M.D., Ph.D., Anthony S. Jennings, M.D., Jean-Louis Chiasson, M.D., Ulrich Keller, M.D., and Arthur H. Rubenstein, M.D., Nashville and Chicago

SUMMARY

To investigate whether insulin exerts feedback regulation of its own secretion, paired studies were performed in normal men in two separate protocols. In the first protocol, four men were studied on two occasions. On one occasion, insulin was infused at 5 mU. per kilogram per minute for 120 minutes, achieving arterial insulin levels of 600 to 700 μU./ml. With use of a variable glucose infusion, the plasma glucose concentration was maintained at fasting levels for 60 minutes and then raised abruptly to 165 mg./dl. and was maintained at that level for the remaining 60 minutes. On a second occasion, the study was repeated except that saline was infused instead of insulin. The plasma glucose concentration remained at fasting levels until the last 60 minutes, when it was similarly raised to 165 mg./dl. and maintained at that level until the end of the study. Connecting peptide reactivity (CPR) was measured as an index of endogenous insulin secretion in the presence of exogenous insulin. During the initial 60 minutes of the study, with euglycemia maintained, there was a significant (46 per cent) decline ($p < 0.01$) in the levels of CPR in the insulin-treated subjects. At 60 minutes, when hyperglycemia was induced, CPR rose in both groups. The rise of CPR in the insulin-treated group, however, was significantly less ($p < 0.01$) than in the saline control group.

To investigate whether insulin inhibition of insulin secretion during hyperglycemia would occur without prolonged insulin pretreatment, paired studies were performed in three additional men. In this protocol, insulin (5 mU. per kilogram per minute) or saline was infused for 70 minutes. Euglycemia was maintained for just 10 minutes. Thereafter, the plasma glucose concentration was raised to 170 mg./dl. in both groups. This acute induction of hyperglycemia without prolonged insulin pretreatment resulted in similar increases in CPR in both insulin- and saline-treated groups.

From these data we conclude that (1) exogenous insulin administration, with maintenance of euglycemia, results in significant inhibition of basal insulin secretion; (2) the administration of exogenous insulin for 60 minutes before and for 60 minutes after the acute induction of hyperglycemia results in significant inhibition of glucose-stimulated insulin secretion; and (3) exogenous insulin administered for just 10 minutes before and during the acute induction of hyperglycemia, however, does not result in inhibition of the insulin response to the hyperglycemic stimulus. DIABETES *27*:563-70, May, 1978.

The possible existence of feedback inhibition of insulin secretion by insulin itself has been the subject of several studies and considerable controversy. Data have been reported on a variety of techniques and different animal species that have suggested that insulin does exert feedback inhibition of its own secretion. Using the isolated, perfused, canine pancreas technique, Iversen and Miles have demonstrated 25 to 78 per cent inhibition of insulin secretion with perfusate insulin levels as low as 180 μU./ml.[1] Pancreatic islets from normal mice have been shown to secrete more insulin when perfused than when they were incubated and insulin was allowed to accumulate in the medium.[2] When insulin was added to the incubation medium at a concentration of 250 μU./ml., the secretory activity of these normal islets was almost completely suppressed.[2] Marked degranulation[3,4] and increased insulinogenic activity of pancreatic islets[5] have been produced by incubation with anti-insulin serum. The hyperinsulinism produced in the Syrian hamster by a transplantable islet cell tumor results in a decrease in the amount of insulin extractable from the

This paper was presented at the Thirty-fifth Annual Meeting of the American Diabetes Association held in New York on June 16, 1975.

From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, and the Department of Medicine, University of Chicago, Pritzker School of Medicine, Chicago, Illinois.

Accepted for publication December 2, 1977.

pancreas and in the amount of insulin released by the beta cell both in vivo and in vitro.[6] Rappaport et al. noted a marked decline in insulin output during the infusion of physiologic amounts of insulin into an in situ isolated portion of canine pancreas.[7]

While there is considerable evidence to suggest insulin feedback inhibition of insulin secretion, such inhibition has not been universally demonstrable. Using the isolated perfused rat pancreas, Grodsky noted no inhibition of insulin secretion with the use of high concentrations of fish insulin.[8] Similarly, Malaisse et al. were unable to show either inhibition of insulin secretion by employing insulin levels of 600 to 1,000 μU./ml. or increased insulin secretion with anti-insulin serum in an isolated islet preparation. Furthermore, infusion of bonito insulin into the pancreaticoduodenal artery of the dog had no effect on the concentration of dog insulin in the pancreatic vein.[10]

In man, few data are available regarding feedback inhibition of insulin secretion. Ohgawara et al. administered prolonged infusions (three to eight hours) of bonito insulin to normal men and then noted diminished insulin responses to glucose loading. A similar infusion of bonito insulin for one hour was without effect.[11]

One of the problems inherent in such studies as those outlined above concerns the difficulty of measuring endogenous insulin secretion in the presence of exogenous insulin. Therefore, investigations have turned to bonito insulin, which differs from mammalian insulin in immunologic specificity but possesses comparable biologic activity.[10] Recently, however, a radioimmunoassay for the determination of human C-peptide reactivity (CPR) has been developed.[12] C-peptide is cleaved from proinsulin in the conversion of proinsulin to insulin and is secreted by the beta cell in equimolar amounts with insulin. Its measurement in circulating plasma, therefore, serves as an accurate index of endogenous insulin secretion even in the presence of exogenous insulin.[13] The availability of this assay prompted us to investigate the question of insulin inhibition of insulin secretion in normal man.

METHODS

Subjects. Seven normal male volunteers participated in this study. All but one were screened prior to study and fulfilled the following criteria: There was no personal history of diabetes mellitus or any other endocrine or major disease; a three-hour glucose tolerance test was normal (40 gm. of glucose per square meter of body surface);[14] hepatic function, assessed by serum oxaloacetic transaminase, serum bilirubin, and alkaline phosphatase, was normal; renal function, as assessed by serum urea nitrogen and urinalysis, was normal; complete blood counts were also normal.

The subjects were placed on a 300-gm. carbohydrate diet, to which they adhered for three days prior to study, and were studied in the morning after a 12-to-14-hour fast.

Procedures. All seven subjects underwent paired studies as outpatients on the Clinical Research Center of Vanderbilt University Hospital. A Teflon catheter was inserted percutaneously in a brachial artery. A continuous pump-driven saline infusion maintained the patency of this catheter without added anticoagulant. In the opposite arm, a Teflon catheter was inserted into an antecubital vein through which saline, insulin, glucose, and potassium chloride were administered as necessary. All blood sampling was from the arterial catheter. Two specific protocols were carried out.

The first protocol comprised two paired studies in four normal men. In one study, monocomponent pork insulin was infused at the rate of 5 mU. per kilogram per minute for two hours to each of four subjects. During the first hour, euglycemia was maintained by a variable glucose infusion according to the method of Andres et al.[15] After 60 minutes of insulin administration, the glucose infusion rate was increased to raise the plasma glucose level abruptly to 165 mg./dl., at which level it was maintained for the remaining hour of the study. Potassium chloride was infused for two hours at the rate of 20 to 25 mEq. per hour in order to prevent hypokalemia. In the other study of the pair, saline was infused instead of insulin and no glucose or potassium chloride was infused until the second hour of the saline infusion, when the plasma glucose level was similarly raised to and maintained at 165 mg./dl. Potassium chloride was infused at 15 mEq. per hour only during the hyperglycemic period. Studies were randomized as to order of performance.

The second protocol comprised two paired studies in three normal men. In this protocol, saline or insulin (5 mU. per kilogram per minute) was infused for 70 minutes. Euglycemia was maintained in both groups (by infusing glucose in the insulin-treated group) for only 10 minutes before acutely inducing hyperglycemia of 170 mg./dl., which was maintained until 70 minutes. Potassium chloride again was infused at 15 to 25 mEq. per hour to maintain normokalemia.

Arterial plasma samples were obtained every five

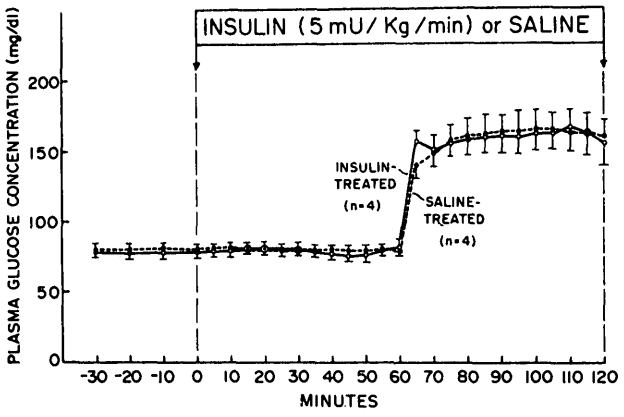

FIG. 1. The arterial plasma glucose concentrations (mean ± S.E.) achieved in four normal subjects studied on two occasions during the infusion of regular pork insulin or saline. For 60 minutes the plasma glucose concentration was maintained at fasting levels in the insulin-treated group by a variable glucose infusion. At 60 minutes, sufficient glucose was infused in both groups to raise the arterial glucose concentration to 160-165 mg./dl., at which level it was maintained for the duration of the study.

minutes throughout the study and were assayed for plasma glucose, immunoreactive insulin, C-peptide reactivity, and potassium ion.

Analytic methods. Plasma glucose was measured immediately after drawing arterial blood samples by the glucose oxidase method of the Beckman glucose analyzer. Immunoreactive insulin was measured by a double-antibody method.[16] C-peptide reactivity was assayed as previously described.[12] Plasma potassium ion concentration was measured by flame photometry by the Vanderbilt Hospital Clinical Laboratory.

Statistical methods. Data from the euglycemic and hyperglycemic periods of the first protocol and from the hyperglycemic period of the second protocol were separately analyzed by two-way replicated analysis of variance, with time of sampling and type of infusion (saline or insulin) taken as independent factors. Because no interaction between these factors was demonstrated, statistical significance was determined from the F-ratio of the mean square for each factor to that of the residual variance.[17]

RESULTS

In the first experimental series, four normal men underwent paired studies. After a 30-minute basal period, saline or insulin at the rate of 5 mU. per kilogram per minute was infused for two hours (figure 1). The plasma glucose concentration in the insulin-treated subjects was held at basal levels for the initial 60 minutes. This was accomplished by infusing glucose at a variable rate. After 60 minutes, the plasma glucose concentration was abruptly raised to 165 mg./dl. and maintained at that level for the second and final hour of the study. The insulin levels obtained in these paired studies are shown in table 1. In the subjects receiving exogenous insulin, plasma immunoreactive insulin levels rose rapidly and plateaued between 600 and 700 μU./ml. In the subjects receiving saline alone, insulin rose to levels of 30 μU./ml. The C-peptide immunoreactivity data corresponding

TABLE 1

Effect of insulin (5 mU. per kilogram per minute) and saline infusions on plasma insulin immunoreactivity during euglycemia and hyperglycemia in paired studies of four normal men

	Minutes															
	−30	−20	−10	0*	10	20	30	40	50	60†	70	80	90	100	110	120
	(μU./ml.)															
Insulin-treated																
A.A.	8	4	5	4	384	431	486	453	560	514	556	573	578	484	631	551
B.B.	2	2	3	2	427	440	518	548	609	620	662	559	592	612	668	612
C.C.	3	6	6	9	470	483	511	565	566	715	688	605	803	648	740	825
D.D.	9	8	9	6	478	537	669	636	679	686	665	762	857	821	891	808
Mean	5.5	5.0	5.8	5.3	440	473	546	551	604	634	643	625	708	641	723	699
S.E.	1.8	1.3	1.3	1.5	22	24	42	38	27	45	29	47	72	69	57	69
Saline-treated																
A.A.	6	7	11	6	5	7	6	5	7	6	25	27	30	27	34	30
B.B.	5	5	6	4	4	5	4	2	3	2	—	24	—	35	—	37
C.C.	7	6	9	7	12	16	3	5	4	5	19	23	22	22	31	25
D.D.	5	6	6	6	6	7	6	4	6	6	35	38	32	36	36	36
Mean	5.8	6.0	8.0	5.8	6.8	8.8	4.8	4.0	5.0	4.8	26	28	28	30	34	32
S.E.	0.5	0.4	1.2	0.6	1.8	2.5	0.7	0.7	0.9	0.9	5	3.4	3	3	1	3

*From zero to 120 minutes, saline or insulin at 5 mU. per kilogram per minute was infused. Sufficient glucose was infused from zero to 60 minutes to maintain euglycemia in the insulin-treated subjects.

†From 60 to 120 minutes, sufficient glucose was infused in both groups to raise plasma glucose levels to 165 mg./dl.

TABLE 2

Effect of insulin (5 mU. per kilogram per minute) and saline infusion on circulating C-peptide reactivity during euglycemia and hyperglycemia in paired studies of four normal men

	Minutes															
	−30	−20	−10	0*	5	10	15	20	25	30	35	40	45	50	55	60†
	(ng./ml.)															
Insulin-treated																
A.A.	1.1	0.6	0.6	0.5	0.8	0.8	0.5	0.3	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0
B.B	1.56	1.73	1.66	2.13	2.28	1.49	1.06	1.53	1.41	1.7	1.64	1.66	1.49	1.51	0.77	1.1
C.C.	2.2	1.5	1.3	1.9	1.4	1.75	1.9	1.6	1.6	0.9	1.2	1.15	1.3	1.1	1.1	1.2
D.D.	2.6	1.8	2.1	1.8	1.6	2.3	2.0	1.7	1.5	1.8	1.6	1.1	1.5	1.3	1.7	1.1
Mean	1.87	1.41	1.42	1.58	1.52	1.59	1.37	1.28	1.15	1.15	1.16	1.09	1.12	1.03	0.94	0.85
S.E.	0.33	0.28	0.32	0.37	0.30	0.31	0.36	0.33	0.35	0.38	0.33	0.35	0.31	0.29	0.31	0.28
Saline-treated																
A.A.	1.0	1.0	1.1	1.0	1.2	1.1	1.1	0.7	0.8	0.7	1.0	0.7	1.2	1.2	0.6	1.1
B.B.	1.36	1.32	1.8	2.17	2.1	1.53	1.71	1.26	1.65	1.96	1.95	1.89	1.59	1.22	1.36	1.63
C.C.	2.2	1.7	2.1	1.7	1.75	1.8	1.6	1.4	1.35	1.3	1.35	1.4	1.25	1.1	1.15	1.2
D.D.	3.1	2.7	2.9	2.4	2.8	2.3	2.9	2.5	3.0	2.6	2.3	2.0	2.5	2.2	2.5	2.3
Mean	1.92	1.68	1.98	1.82	1.96	1.68	1.83	1.47	1.70	1.64	1.65	1.50	1.64	1.43	1.40	1.56
S.E.	0.47	0.37	0.37	0.31	0.33	0.25	0.38	0.38	0.47	0.41	0.29	0.30	0.30	0.26	0.40	0.27

*From zero to 120 minutes, saline or insulin at 5 mU. per kilogram per minute was infused. Sufficient glucose was infused from zero to 60 minutes to maintain euglycemia in the insulin-treated subjects.

†From 60 to 120 minutes, sufficient glucose was infused in both groups to raise plasma glucose levels to 165 mg./dl.

to these subjects are found in table 2. In the saline-treated subjects, there was a gradual 14 per cent decline in CPR during the first 60 minutes of the study. In the insulin-treated subjects, CPR declined by 46 per cent during the 60-minute euglycemic period. Statistical evaluation of these data by analysis of variance indicated that the fall in CPR in the insulin-treated subjects was significant at a $p < 0.01$.

At 60 minutes, the plasma glucose concentration was raised abruptly to a plateau of 165 mg./dl., as noted in figure 1. In response to this acute rise in plasma glucose, CPR increased in both groups. The rise in CPR in the insulin-treated subjects was significantly less ($p < 0.01$) than in the saline-treated group. This level of significance was achieved in the analysis of variance both when the actual data in the two groups were analyzed and when the delta rise in CPR for each subject was compared.

To investigate whether insulin inhibition of CPR secretion during hyperglycemia would occur without prolonged insulin pretreatment, we performed paired studies in three additional normal men. In this protocol, insulin (5 mU. per kilogram per minute) or saline was infused for 70 minutes. Euglycemia was maintained for just 10 minutes in both groups. Thereafter the plasma glucose concentration was raised to 170 mg./dl., as noted in figure 2. Similar insulin levels to those of the first protocol were achieved (table 3). After 10 minutes of insulin or saline administration, arterial CPR was nearly identical in both groups. At that point, the acute induction of hyperglycemia resulted in prompt increases in CPR in both groups that were not, however, significantly different from each other (table 4).

As the circulating potassium concentration may af-

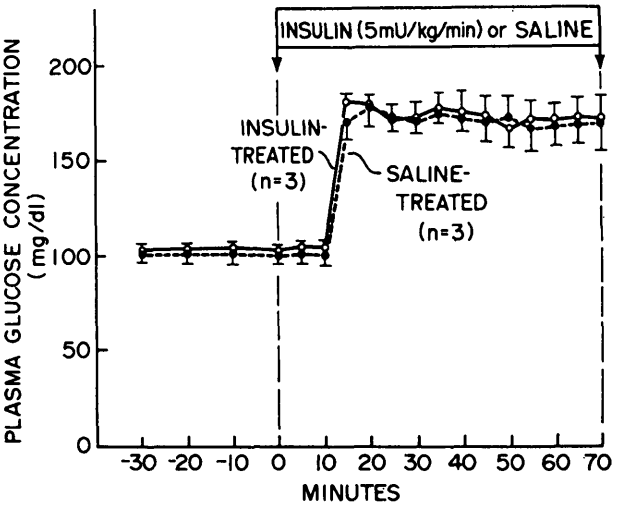

FIG. 2. The arterial glucose concentrations (mean ± S.E.) achieved in three normal subjects studied on two occasions during the infusion of regular pork insulin or saline. The plasma glucose concentration was maintained at fasting levels for 10 minutes in the insulin-treated group; this was achieved by infusing glucose at a variable rate. At 10 minutes, sufficient glucose was infused in both groups to raise the arterial glucose concentration to 170 mg./dl., at which level it was maintained for the duration of the study.

TABLE 2

Continued

	Minutes											
	65	70	75	80	85	90	95	100	105	110	115	120
	(ng./ml.)											
Insulin-treated												
A.A.	1.7	1.1	1.0	1.1	1.7	1.9	1.7	1.5	2.1	2.0	2.0	1.7
B.B.	3.0	2.85	2.4	1.98	2.64	2.27	3.25	2.86	3.65	3.51	3.42	2.8
C.C.	2.7	2.3	1.8	2.6	3.6	4.5	3.8	3.5	4.2	4.0	4.6	4.7
D.D.	4.3	4.0	5.0	4.7	5.4	5.8	5.7	5.7	5.1	5.9	6.3	5.5
Mean	2.93	2.56	2.55	2.60	3.34	3.62	3.61	3.39	3.76	3.85	4.08	3.68
S.E.	0.54	0.60	0.87	0.77	0.79	0.93	0.83	0.88	0.63	0.80	0.91	0.87
Saline-treated												
A.A.	4.1	2.6	2.7	2.4	3.6	3.0	3.2	3.1	3.6	4.6	4.9	3.7
B.B.	3.92	3.29	3.60	4.42	3.86	3.72	4.4	4.23	4.6	5.02	4.48	4.73
C.C.	3.1	3.7	3.4	2.4	4.0	4.2	4.4	4.3	5.3	5.7	5.6	4.1
D.D.	6.9	6.3	6.8	6.8	6.3	6.6	8.6	8.8	8.5	7.5	9.3	9.5
Mean	4.51	3.97	4.13	4.01	4.44	4.38	5.09	5.11	5.5	5.71	6.07	5.51
S.E.	0.83	0.81	0.91	1.05	0.63	0.78	1.20	1.26	1.06	0.64	1.1	1.85

fect insulin secretion,[18] potassium chloride was infused to maintain normokalemia. The greatest variance in any individual subject in plasma potassium concentration, which was measured every 20 minutes, was 0.5 mEq./L., with most subjects varying by only 0.2 to 0.3 mEq./L.

DISCUSSION

The measurement of C-peptide reactivity (CPR) in this study provides an accurate index of endogenous insulin secretion in the presence of exogenous insulin for the following reasons: (1) C-peptide is secreted from the beta cell in equimolar amounts to insulin;[13] (2) the per cent extraction of C-peptide during its initial passage through the liver is far less than for insulin, thus allowing more C-peptide to enter the systemic circulation; therefore, changes in arterial CPR may reflect more precisely changes in beta cell secretory rates than the measurement of arterial insulin itself;[19] and (3) the radioimmunoassay for CPR is specific for human C-peptide and does not cross-react with any porcine C-peptide or proinsulin that might be exogenously administered.[13]

The present study was designed to determine whether exogenously administered insulin could in-

TABLE 3

Effect of insulin (5 mU. per kilogram per minute) and saline on mean plasma insulin immunoreactivity in paired studies of three normal men

	Minutes										
	−30	−20	−10	0*	10†	20	30	40	50	60	70
	(μU./ml.)										
Insulin-treated											
E.E.	7	5	7	7	500	612	597	584	701	555	632
F.F.	8	12	13	12	395	615	599	664	749	—	699
G.G.	14	9	9	10	553	797	902	—	869	1,136	893
Mean	9.7	8.7	9.7	9.7	483	675	699	624	773	846	741
S.E.	2.1	2.0	1.8	1.5	46	61	101	40	50	290	78
Saline-treated											
E.E.	5	4	2	4	3	20	13	16	10	8	9
F.F.	8	3	10	9	9	27	22	32	29	30	12
G.G.	7	7	9	6	8	39	45	41	40	41	42
Mean	6.7	4.7	7.0	6.3	6.7	29	27	30	26	26	21
S.E.	0.9	1.2	2.5	1.5	1.9	6	10	7	9	9.7	11

*From zero to 70 minutes, saline or insulin at 5 mU. per kilogram per minute was infused. Sufficient glucose was infused from zero to 10 minutes to maintain euglycemia in the insulin-treated subjects.

†From 10 to 70 minutes, sufficient glucose was infused in both groups to raise plasma glucose levels to 170 mg./dl.

TABLE 4

Effect of insulin (5 mU. per kilogram per minute) and saline on circulating C-peptide reactivity in paired studies of three normal men

	Minutes																	
	−30	−20	−10	0*	5	10†	15	20	25	30	35	40	45	50	55	60	65	70
	(ng./ml.)																	
Insulin-treated																		
E.E.	1.88	1.57	2.41	1.66	2.02	2.04	3.54	3.42	2.56	2.48	3.00	2.92	3.36	3.6	3.03	2.77	2.98	2.98
F.F.	3.28	3.11	2.77	2.72	2.93	2.44	4.34	3.83	3.26	4.27	4.82	4.27	3.89	3.52	4.56	4.32	4.92	5.86
G.G.	2.97	3.09	2.71	2.12	3.62	2.71	4.6	5.22	4.63	4.43	3.96	5.27	4.6	4.93	5.52	4.61	4.72	4.54
Mean	2.71	2.59	2.63	2.17	2.86	2.40	4.16	4.16	3.48	3.73	3.93	4.15	3.95	4.02	4.37	3.9	4.21	4.46
S.E.	0.42	0.51	0.11	0.31	0.46	0.19	0.32	0.54	0.61	0.62	0.53	0.68	0.36	0.46	0.73	0.57	0.62	0.83
Saline-treated																		
E.E.	1.84	1.99	2.65	2.14	2.52	1.54	3.33	3.54	3.96	3.0	3.56	3.60	3.7	3.74	3.47	2.85	3.53	3.95
F.F.	1.67	1.90	2.02	1.84	2.43	2.06	5.10	4.31	3.14	3.08	4.0	4.13	3.86	3.63	4.3	4.34	4.8	6.1
G.G.	2.12	2.47	2.63	2.2	2.71	3.78	4.53	4.59	5.22	5.39	6.30	5.39	5.56	5.39	5.77	5.83	6.1	6.42
Mean	1.88	2.12	2.43	2.06	2.55	2.46	4.32	4.15	4.11	3.82	4.62	4.37	4.37	4.25	4.51	4.34	4.81	5.49
S.E.	0.13	0.18	0.21	0.11	0.08	0.68	0.52	0.31	0.60	0.78	0.85	0.53	0.60	0.57	0.67	0.86	0.74	0.78

*From zero to 70 minutes, saline or insulin at 5 mU. per kilogram per minute was infused. Sufficient glucose was infused from zero to 10 minutes to maintain euglycemia in the insulin treated subjects.

†From 10 to 70 minutes, sufficient glucose was infused in both groups to raise plasma glucose levels to 170 mg./dl.

hibit basal and glucose-stimulated insulin secretion. With the use of the glucose clamp technique of Andres et al.,[15] it was possible to maintain euglycemia during insulin administration as well as to achieve and maintain a hyperglycemic plateau during administration of insulin or saline (figures 1 and 2). Thus, the only difference between the two paired studies in both protocols as far as their performance was concerned was the administration of insulin and appropriate amounts of glucose in the one study and the administration of saline and appropriate amounts of glucose in the other. Thus, the CPR response noted in table 2 should reflect solely the addition of insulin in one study of the pair. These data suggest that at fasting glucose concentrations the infusion of pork insulin so as to achieve circulating insulin concentrations of 600 to 700 μU./ml. does result in significant inhibition of basal insulin secretion, as indicated by the fall in CPR during the euglycemia period. Furthermore, when hyperglycemia was induced, after one hour of insulin pretreatment (table 2) significant inhibition of the rise in CPR was also noted in those subjects receiving exogenous insulin, indicating inhibition by exogenous insulin of glucose-stimulated insulin secretion.

These data differ somewhat from those of Ohgawara et al., who infused bonito insulin in man for one to eight hours.[11] The infusion rate used (12 mU. per minute or 0.17 mU. per kilogram per minute in a 70-kg. man) was far less than the 5 mU. per kilogram per minute pork insulin infusion rate employed in the present study. At this low infusion rate of bonito insulin, no decrease in the basal levels of human insulin was noted, indicating no suppression of basal insulin secretion. However, after three and eight hours of such an infusion of bonito insulin, the insulin response to glucose loading was significantly less in the insulin-treated subjects. On the other hand, if the glucose loading occurred after only one hour of bonito insulin infusion, the insulin response to the glucose load was not decreased. In the present study, much larger amounts of insulin were administered and did result in significant inhibition of basal insulin secretion and of glucose-stimulated insulin secretion when insulin pretreatment was administered for 60 minutes. However, when insulin pretreatment was administered for only 10 minutes (table 4), no inhibition of glucose-stimulated insulin secretion was noted. Thus, the amount of insulin administered and the length of insulin administration appear to be important factors influencing the ability of insulin to inhibit its own secretion.

The mechanism by which insulin inhibits its own secretion in vivo cannot be ascertained by the present study. Insulin administration does result in decreases in the circulating levels of such insulin secretagogues as glucagon[20] and the branched-chain amino acids leucine and isoleucine.[21] As potassium depletion states have also been associated with altered insulin secretion, potassium chloride was administered during the present study to minimize this variable. However, Gordon et al. have shown that acute changes in

serum potassium levels are not associated with altered insulin secretion.[18] Data from several in vitro studies suggest a direct effect of insulin on the beta cell as the explanation of this phenomenon.[1,2,5]

A 100-fold increase in arterial insulin concentration occurred in the present studies during insulin administration, an increment four to five times greater than that observed under physiologic circumstances. However, this may not represent a large increase in the insulin concentration in the fluid bathing the beta cell. It is conceivable, therefore, that, had we administered significantly more insulin, an even more pronounced inhibition of insulin secretion might have been produced. On the other hand, the administration of insulin in man and dog in much smaller amounts, with euglycemia also maintained, has resulted in significant decreases in circulating glucagon levels, suggesting that the arterial insulin concentration does play an important role in the regulation of islet secretory function.[20,22] It should be pointed out, however, that negative feedback systems that employed a trophic hormone, such as adrenocortical-stimulating hormone, are much more sensitive and are capable of exerting negative feedback inhibition, with only one-to-twofold changes in the circulating concentration of the primary hormone.[23]

The present study thus presents evidence to suggest that physiologic changes in circulating arterial insulin levels can significantly inhibit both basal and glucose-stimulated insulin release in man. While the phenomenon of insulin inhibition of insulin secretion in man has been demonstrated in the present study, the physiologic revelance of this finding remains unclear.

ACKNOWLEDGMENTS

The authors would like to acknowledge the excellent technical assistance of Angelita Cruz. We are most grateful for the excellent secretarial skills of Lissa Hancock, Joyce Dawson, and Carolyn Sielbeck.

This investigation was supported by Vanderbilt University Diabetes-Endocrinology Center grant AM 17026, the General Clinical Research Center grant 5M01 RR95, U.S.P.H.S. grants AM 19206 and AM 13941, and the University of Chicago Diabetes-Endocrinology Center grant AM 17046.

Dr. Liljenquist is an investigator of the Howard Hughes Medical Institute. Dr. Horwitz is a recipient of a U.S.P.H.S. Research Career Development Award (AM 00181).

REFERENCES

[1]Iversen, J., and Miles, D. W.: Evidence for a feedback inhibition of insulin secretion in the isolated perfused canine pancreas. Diabetes *20*:1-9, 1971.

[2]Loreti, L., Dunbar, J. C., Chen, S., and Foa, P. P.: The autoregulation of insulin secretion in the isolated pancreatic islets of lean (obOb) and obese-hyperglycemic (obob) mice. Diabetologia *10*:309-15, 1974.

[3]Morgan, C. R., Carpenter, A. M., and Lazarow, A.: Effect of insulin antibodies on pancreatic islet insulin and beta cell granule content. Anat. Rec. *153*:49-54, 1965.

[4]Logothetopoulos, J., Davidson, J. K., Haist, R. E., and Best, C. H.: Degranulation of beta cells and loss of pancreatic insulin after infusions of insulin antibody or glucose. Diabetes *14*:493-500, 1965.

[5]Ziegler, M., Hahn, H. J., and Klatt, D.: Influence of isolated insulin antibodies on the insulin secretion of the islets of Langerhans *in vitro*. Diabetologia *8*:148-49, 1972.

[6]Sodoyez, J. C., Sodoyez-Goffaux, F., Rossen, R. M., and Foa, P. P.: Function of the pancreatic b-cells in hamsters bearing a transplantable islet cell tumor. Metabolism *18*:433-38, 1969.

[7]Rappaport, A. M., Ohira, S., Coddling, J. A., Empey, G., Kalnins, A., Lin, B. J., and Haist, R. E.: Effects on insulin output and on pancreatic blood flow of exogenous insulin infusion into an in situ isolated portion of the pancreas. Endocrinology *9*:168-76, 1972.

[8]Grodsky, G. M.: Insulin secretion from the perfused pancreas. Acta Diabetol. Lat. *5*:239, 1968.

[9]Malaisse, W. J., Malaisse-Lagae, F., Lacy, P. E., and Wright, P. H.: Insulin secretion by isolated islets in presence of glucose, insulin and anti-insulin serum. Proc. Soc. Exp. Biol. Med. *124*:497, 1967.

[10]Sando, H., Kanazawa, Y., and Kuzuya, T.: Effect of bonito insulin on endogenous insulin secretion in dogs. Am. J. Physiol. *218*:1357-62, 1970.

[11]Ohgawara, H., Suzuki, K., and Kosaka, K.: Effect of prior infusion of exogenous bonito insulin on glucose-induced insulin release in man. Endocrinol. Jpn. *20*:307-14, 1973.

[12]Melani, F., Rubenstein, A. H., Oyer, P. E., and Steiner, D. F.: Identification of proinsulin and C-peptide in human serum by specific immunoassay. Proc. Natl. Acad. Sci. U.S.A. *67*:148-55, 1970.

[13]Rubenstein, A. H., Clark J. L., Melani, F., and Steiner, D. F.: Secretion of proinsulin C-peptide by pancreatic beta cells and its circulation in blood. Nature London *224*:697-99, 1969.

[14]Klimt, C. R., Prout, T. E., Bradley, R. F., Dolger, H., Fisher, G., Gastineau, C. F., Marks, H., Meinert, C. L., and Schumacher, O. P.: Standardization of the oral glucose tolerance test. Diabetes *18*:299-310, 1969.

[15]Andres, R., Swerdloff, R. S., Pozefsky, T., and Coleman, D.: Manual feedback technique for control of blood glucose concentration. *In* Automation in Analytical Chemistry. Skeggs, L. T., Ed. New York, Mediad, 1966, pp. 486-91.

[16]Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Plasma levels of normal, subdiabetic and diabetic rats. Diabetes *12*:115-26, 1963.

[17]Cooper, B. E.: Statistics for Experimenters. Oxford, Pergamon Press, 1969, pp. 144-76.

[18]Gorden, P.: Glucose tolerance with hypokalemia. Failure of short-term potassium depletion in normal subjects to reproduce

the glucose and insulin abnormalities of clinical hypokalemia. Diabetes *22*:544-51, 1973.

[19]Horwitz, D. L., Kuzuya, H., and Rubenstein, A. H.: Circulating serum C-peptide. A brief review of diagnostic implications. N. Engl. J. Med. *295*:207-09, 1976.

[20]Chiasson, J. L., Liljenquist, J. E., Finger, F. E., and Lacy, W. W.: Differential sensitivity of glycogenolysis and gluconeogenesis to insulin infusions in dogs. Diabetes *25*:283-91, 1976.

[21]Adibi, S. A., Morse, E. L., and Amin, P. M.: Role of insulin and glucose in the induction of hypoaminoacidemia in man: studies in normal, juvenile diabetic and insulinoma patients. J. Lab. Clin. Med. *86*:395-409, 1975.

[22]Raskin, P., Fujita, Y., and Unger, R. H.: Effect of insulin-glucose infusions on plasma glucagon levels in fasting diabetics and nondiabetics. J. Clin. Invest. *56*:1132-38, 1975.

[23]Liddle, G. W., Island, D., and Meador, C. K.: Normal and abnormal regulation of corticotropin secretion in man. Recent Prog. Horm. Res. *18*:125-66, 1962.