Absence of Glucose Response to Physiologic Levels of Serum Insulin

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SUMMARY

Insulin was given by intravenous injection and infusion into a peripheral vein of dogs to simulate the serum insulin levels obtained following pancreozymin or an intravenous glucose load. A significant change in free fatty acids was obtained without any change in serum glucose. A similar result was obtained by portal vein instillation of insulin. Pancreozymin plus intravenous glucose caused a much higher serum insulin concentration than glucose alone. However, the glucose turnover was the same. It is suggested that the causal relationship of insulin and glucose should be re-evaluated. Hyperinsulinemia in response to hyperglycemia may not be directly related to glucose homeostasis. DIABETES *18*:397-401, June, 1969.

Pancreozymin given intravenously in dogs has been shown to produce higher serum insulin levels than obtained following an intravenous glucose load.[1] This hyperinsulinemia is accompanied by a fall in free fatty acids, FFA, but no change in blood glucose. It was suggested that the duration of hyperinsulinemia was inadequate to lower blood glucose. Multiple pancreozymin injections prolong the hyperinsulinemia however, without affecting blood glucose. Unger and co-workers have noted similar insulin secretion, without hypoglycemia, following secretin, gastrin and pancreozymin.[2,3] They also found pancreozymin to be the most potent betatropin of the three hormones. With prolonged infusion of pancreozymin at approximately ten times the dose we had used, they noted an abrupt rise in pancreaticoduodenal vein glucagon and hyperglycemia occurred soon thereafter. The absence of glucose response after smaller doses of pancreozymin might therefore be due to the simultaneous release of insulin and glucagon.

A review of the literature revealed no studies in which the effects of physiologic levels of exogenous insulin have knowingly been determined in vivo. Insulin was therefore given by intravenous injection and infusion to simulate the serum insulin levels obtained in response to pancreozymin and glucose.

MATERIALS AND METHODS

Mongrel dogs weighing 12 to 20 kg. were vaccinated against distemper and hepatitis and dewormed with a broad spectrum parasiticide. They were fed ken-r-biskit supplemented with raw beef liver and ground cooked beef trimmings. They were maintained for at least fifteen days prior to any studies. Unless otherwise noted, all animals were fasted twenty to twenty-four hours prior to study.

For peripheral infusion, cannulas were placed in both hind paws thirty minutes in advance of studies. For portal vein infusion, the silastic rubber cannula was placed in the portal vein four days prior to the first study. It was brought out through a subcutaneous tunnel in the back and secured. The cannula was flushed with 0.01 per cent heparin at surgery and daily thereafter. No heparin was used within two hours of any study.

All dogs were awake and unanesthetized for study and secured in a modified Pavlov frame. This procedure has proven satisfactory and produces no discomfort to the animals.

To simulate the insulin response to pancreozymin, it was desired to produce a peak insulin level between 50 and 100 μU. per ml. With assumption of a blood volume 70 ml. per kg., a glucagon-free insulin injection of 5,000 μU. per kg. was given and found to approximate the desired concentration. After twenty min-

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utes, insulin was also infused at a rate of 0.0025 U. per minute per kg. for the remainder of one hour. To simulate the insulin response from a glucose load, an injection of 0.005 U. per kg. was followed immediately by constant infusion of 0.0005 U. per kg. per minute for twenty minutes. For most studies, the infusion pump speed was then reduced to one half for the remainder of one hour.

Bovine insulin which assayed less than 0.01 per cent glucagon by immunoassay was used. It was injected in a peripheral vein to bypass the degradation by the liver. It was also injected into the portal vein in the same animal fasted and nonfasted.

To investigate the effect of pancreozymin upon glucose disappearance rate and turnover, animals received either intravenous glucose alone or glucose plus pancreozymin. Two dogs received intravenous glucose, 0.5 gm. per kg., plus C-14-glucose tracer and two received the glucose plus pancreozymin, 1 unit per kg. The following day, the study was repeated with the same animals but the groups reversed. Plasma glucose, C-14-glucose, insulin and free fatty acid were determined. Serum glucose was determined by the glucose oxidase method using the Glucostat procedure of Worthington Biochemical Corp., Freehold, New Jersey. The serum radioactivity was counted in a liquid scintillation counter to a counting error of less than 1 per cent. The K value for glucose disappearance was calculated according to the method of Ikkos and Rolf.[4] Free fatty acids were measured using the method of Dole.[5] Immunoassayable insulin was determined using a modification of the Yalow and Berson technic.[6]

The possibility that pancreozymin was an insulin inhibitor was studied in vitro by rat diaphragm assay. Pancreozymin, 0.14 U. per ml. was added to 1,000 μU. of insulin and compared to 1,000 μU. without pancreozymin. The quantity of C-14-glucose incorporated into glycogen was determined.

RESULTS

To simulate the serum insulin response to pancreozymin, insulin was given as a single injection, 0.005 U. per kg., into a paw vein followed in twenty minutes by an infusion of 0.0025 U. per minute per kg. The average response of insulin, glucose and FFA in four such studies is shown in table 1, along with the average of six pancreozymin studies previously reported.[1] The insulin values are similar and produce a fall in FFA with no change in serum glucose.

A greater and more prolonged insulin elevation was produced by simultaneous injection and constant infusion. The average of five such studies is compared to the results obtained following an intravenous glucose load in table 2. During insulin infusion, serum glucose did not decrease more than 5 mg. per 100 ml. in any animal and average values are unchanged. The changes in insulin and FFA are quite comparable to the values following a glucose load.

In some animals, higher insulin concentrations were obtained by more rapid infusion with resultant hypoglycemia. The insulin, glucose and FFA of four such studies are shown in table 3. It would appear that serum insulin levels between 70 and 80 μU. per ml. will decrease blood glucose levels. With much higher values, hypoglycemia was obtained. The FFA decrease was less marked than with lower serum insulin concentrations. The last FFA value rises probably as a result of catecholamine release during hypoglycemia.

TABLE 1

Simulation of serum insulin response to pancreozymin by insulin injection and infusion*

	Insulin injection paw vein Peripheral serum studied				Pancreozymin 0.5 U./kg. Hepatic vein serum studied			
	Exogenous insulin	Glucose	Free fatty acids	P†	Endogenous insulin	Glucose	Free fatty acids	P†
Control	6	96	1,069		5	90	915	
1 min.	71	97	1,082	N.S.	60	92	933	N.S.
3 min.	46	96	1,068	N.S.	61	93	920	N.S.
5 min.	32	98	980	N.S.	44	96	720	<.05
10 min.	23	98	786	N.S.	4	92	510	<.01
20 min.	18	99	691	<.05	1	96	539	<.01
30 min.	20	102	702	<.01	0	91		
45 min.	19	101	676	<.05				
60 min.	17	100	643	<.05				

*0.005 U./kg. priming injection followed in twenty minutes by 0.00025 U./min./kg.
†Probability of no difference from control FFA.

TABLE 2

Simulation of serum insulin levels during intravenous glucose load by insulin injection and infusion*

	Insulin infusion peripheral vein. Peripheral serum studied (average of five studies)				Intravenous glucose 0.5 gm./kg. Peripheral serum studied (average of four studies)			
	Insulin μU./ml.	Glucose mg./100 ml.	Free fatty acid μEq./L.	P†	Insulin μU./ml.	Glucose mg./100 ml.	Free fatty acid μEq./L.	P†
Control 1	7	88	1,001		15	106	886	
Control 2	8	92	962		17	99	941	
1 min.	90	93	1,108	N.S.	91	344	737	N.S.
3 min.	61	92	1,083	N.S.	79	307	700	N.S.
5 min.	45	93	1,016	N.S.	69	264	646	<.05
10 min.	33	93	770	<.05	55	220	590	<.05
20 min.	27	94	713	<.05	41	164	590	<.05
30 min.	22	94	729	<.05	29	148	422	<.01
45 min.	20	93	705	<.05	—	—	—	—
60 min.	21	91	636	.07	19	117	438	<.01

*0.005 U./kg. priming injection plus 0.0005 U./min./kg. for twenty minutes then 0.00025 U./min/kg.
†Probability of no difference from control FFA.

Insulin was infused into the portal vein of one animal following only a two-hour fast. Two days later, infusion at the same rate was accomplished after a twenty-four-hour fast. The results shown in table 4 suggest that insulin degradation by the liver is much less in the nonfasted animal. Peripheral serum insulin values above 100 μU. per ml. were accompanied by a slight rise in serum glucose and a decrease in FFA. Following twenty-four-hour fast, the same amount of insulin produced about one fifth as great an elevation of serum levels and this amount of insulin did not affect the glucose or FFA.

The insulin response to glucose alone compared to glucose plus pancreozymin is shown in table 5. In spite of the much higher serum insulin levels when animals received glucose plus pancreozymin, the glucose disappearance rate is the same in both groups. The C-14 values were also equal in both groups. Glucosazones were done on only a few samples and are therefore not reported.

Rat diaphragm studies to determine the effect of pancreozymin upon the amount of C-14-glucose incorporated into glycogen averaged 25.3 μg. per 10 mg. wet weight for 1,000 μU. of insulin alone and 26.1 μg. per 10 mg. when pancreozymin was added at a concentration of 0.14 units per ml. This study would appear to rule out the possibility that pancreozymin both stimulates insulin secretion and inhibits its activity.

TABLE 3

Insulin infusions which produced hypoglycemia* (average of four studies)

	Insulin μU./ml.	Glucose mg./100 ml.	Free fatty acid μEq./L.	P†
Control	14	94	1,072	
Control	14	95	1,070	
5 min.	47	92	1,104	
10 min.	72	91	1,109	
20 min.	79	78	980	
30 min.	83	69	911	
40 min.	212	58	917	
50 min.	204	51	986	
60 min.	221	49	1,109	

*0.005 U./kg. priming injection plus 0.0005 U./min./kg. for sixty minutes.
†No significant difference from control.

DISCUSSION

The dissociation of insulin and blood glucose, as demonstrated in these studies, was not unexpected. In recent years, several studies have demonstrated insulin secretion in response to secretin, gastrin and pancreozymin without any hypoglycemic effect in the normoglycemic animal.[1-3,7,8] White and Dupre[8] observed that secretin produced an immediate hyperinsulinemia in man with no glucose effect in normoglycemic individuals. A 7 mg. per 100 ml. change in glucose was obtained in hyperglycemic individuals.

Elevated serum insulin without glucose response has also been observed following the administration of fatty acids,[9] the C-terminal tetrapeptide of gastrin,[10] and xylitol.[11] The notable exception is protein and amino acid stimulation in which hyperinsulinemia is associated with hypoglycemia.[12,13] Zierler and Rabinowitz[14] infused insulin, 10 μU. per minute, in the forearm and observed a decrease in FFA without any effect upon glucose uptake. Blood insulin levels were not determined. Sokal and co-workers[15] were able to produce a 50 per cent decrease in FFA by injecting 0.02 U. per kg. in dogs.

TABLE 4

Portal vein infusion of insulin following two and twenty-four-hour fast*
(peripheral serum studied)

	2-hr. fast			24-hr. fast		
	Insulin μU./ml.	Glucose mg./100 ml.	Free fatty acid μEq./L.	Insulin μU./ml.	Glucose mg./100 ml.	Free fatty acid μEq./L.
Control 1	31	111	754	1	71	1,212
Control 2		108		4	70	1,198
1 min.	54	110	556	25	71	1,206
3 min.	82	112	462	25	72	1,350
5 min.	77	114	432	22	70	1,322
10 min.	108	112	498	22	68	1,270
20 min.	134	118	420	24	67	1,278
30 min.	124	120	396	23	74	1,278
45 min.	78	121	350	26	70	1,173
60 min.	100	127	398	23	76	1,261

*0.005 U./kg. priming dose plus 0.0005 U./min./kg. for sixty minutes.

TABLE 5

Comparison of glucose or glucose plus pancreozymin
(average of four animals studied)

	Serum insulin		Serum glucose	
	Glucose	Glucose plus pancreozymin	Glucose	Glucose plus pancreozymin
Control	22	21	104	107
1 min.	86	166	334	352
3 min.	101	380	300	344
5 min.	78	234	282	292
10 min.	49	149	230	248
20 min.	35	110	177	203
30 min.	24	105	159	167
60 min.	15	110	124	139
			K (20-60 min.) = .0082/min.	K (20-60 min.) = .0087/min.

The in vitro pancreozymin studies using the rat diaphragm do not show any insulin inhibitory effect. Also, the studies of Greenwood, Landon and Stamp[16] would suggest that cortisol and growth hormone are not involved in the lack of glucose response. They found an insulin dose of 0.025 to 0.050 U. per kg. (five to ten times the dose used in this study) was necessary to produce a minimal increase in these hormones which did not occur before thirty minutes.

The relationship of the nutritional state to the lack of glucose response was not obvious to us. A review of the original data has shown no correlation between the level of FFA and the presence or absence of a response to insulin. In addition, the portal vein infusion after a two-hour fast, table 4, produced high serum insulin levels and a slight rise in serum glucose. Preliminary studies in humans after an overnight fast indicate that a glucose lowering response may be obtained at blood levels of 40 μU. per ml. Insulin from the same vial has no effect in dogs at twice this concentration. This would suggest a species difference. However, this would not explain the lack of response to endogenous insulin, table 5.

Perhaps the best explanation for the results of the present study was supplied by Soskin and co-workers in 1934.[17] They showed that a depancreatized dog maintained by a constant infusion of insulin and glucose yielded a normal glucose tolerance curve but if prior glucose was withheld, a diabetic curve resulted. These results were incompatible with the thesis that the pancreatic response to glucose was responsible for normal glucose tolerance. They concluded that in the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas, the normal liver responds to a glucose load to maintain glucose homeostasis.

Although adequate amounts of insulin are necessary for normal glucose metabolism, it may be questioned

whether the prompt secretion of insulin into the circulation in response to glucose enters directly into the mechanism of glucose homeostasis. Until the relationship between serum insulin and glucose is better established, it may be improper to equate the blood glucose response during a tolerance test to the levels of serum insulin obtained. A lack of relationship may explain the finding of both increased and decreased insulin response in the glucose intolerant individual. It might also be questioned whether the observation of increased serum insulin in response to a glucose load is properly termed insulin resistance. The greater insulin in response to glucose plus pancreozymin shown in table 5 would be insulin resistance by such a definition. Since pancreozymin does not appear to directly affect glucose metabolism, this may only represent a glucose-insulin dissociation or possibly the release of biologically inactive insulin in response to pancreozymin.

Since glucose appears to be the major stimulus for insulin secretion,[18] a direct correlation between serum insulin and glucose levels could be expected at any blood glucose concentration. To use these data to demonstrate that insulin regulates blood glucose seems unwise at present. These data may merely demonstrate that glucose regulates the serum insulin concentration at different serum insulin levels in health and disease.

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