Effect of Blood Glucose on Glucagon Secretion in Anesthetized Dogs

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SUMMARY

Insulin induced hypoglycemia and tolbutamide administration accompanied by much smaller falls in blood glucose were associated with increased pancreatic vein IRG concentrations in laparotomized dogs under barbiturate anesthesia. Hyperglycemia may have diminished pancreatic IRG release. Changes in IRG levels in peripheral and jejunal veins during hypoglycemia were unremarkable. In dogs, pancreatectomy was associated with no change in IRG release into a jejunal vein. Very small doses of glucagon administered intraportally during hyperglycemia were effective in promoting IRI release.

It is concluded that a fall in blood glucose from the basal state is a stimulus, and hyperglycemia is an inhibitor, of pancreatic IRG release. It is possible that glucagon may act in the basal state to deliver repeated small pulses of glucose and insulin into the circulation. DIABETES *18*:11-18, January, 1969.

Since glucagon is a powerful stimulator of hepatic glycogenolysis and gluconeogenesis, it is possible that glucagon may function to maintain blood glucose in times of glucose deficiency.[1] The cross-circulation experiments of Foa and associates[2-4] demonstrated the presence of a hyperglycemic material in the pancreaticoduodenal blood of dogs with insulin-induced hypoglycemia. Subsequently, the development of a sensitive immunoassay for glucagon[5] made possible the direct measurement of glucagon during hypoglycemic states, and Unger et al.[6] showed an increase in glucagon secretion when the blood glucose concentration fell in dogs. This demonstration of glucagon release in response to hypoglycemia lends support to the proposed physiological role of glucagon as a hormone of substrate need.

More recently, it has been shown that glucagon stimulates the releases of insulin[7-9] and that oral glucose ingestion is associated with a rise in immunoreactive glucagon concentrations in the peripheral blood.[10,11] Both of these observations tend to conflict with the classical concept of the role of glucagon as a provider of glucose. Furthermore, it has been shown that there is a substance in the gut which cross-reacts with the glucagon immunoassay,[12,13] and this substance is probably released into the circulation where it is measured as immunoreactive glucagon along with the circulating glucagon released from the pancreas.[14,15] Since peripheral circulating IRG levels may represent material released from at least two sources, it is difficult to interpret changes in concentration of circulating IRG. Also, it has been recently shown that glucagon is degraded in the immunoassay[16,17] and with storage in the freezer[17] unless the proteolytic enzyme inhibitor, Trasylol, is added to the plasma samples. Consequently, we have reassessed the effect of changing the blood glucose concentration on glucagon released into gut and pancreatic veins, employing technics to minimize the degradation of circulating hormone.

METHODS AND MATERIALS

The studies were performed on overnight-fasted mongrel dogs (20-30 kg.) with laparotomy performed under barbiturate anesthesia. Needles were placed in the cranial pancreaticoduodenal vein, a large jejunal vein, and into a peripheral vein and kept patent by flushing with isotonic saline. Care was taken not to obstruct the pancreatic vein during the experiments by using a 21-gauge thin-walled needle, inserted in the direction of blood flow. At the time of blood sampling, the pancreatic vein at its entry into the portal vein

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was occluded with a simple thread snare to prevent reflux from the portal vein.

Blood was collected in heparinized vessels surrounded with ice and the plasma was separated within thirty minutes. Plasma for glucose and immunoreactive insulin (IRI) measurements was stored frozen. Plasma for immunoreactive glucagon (IRG) assay was stored frozen in Trasylol (Bayer Ltd.), a proteolytic enzyme inhibitor (1,000 Kallikrein Inhibitor Units, KIU, per 250 μl plasma).

Plasma glucose was measured with a ferricyanide reagent, using a Technicon AutoAnalyzer. IRI was measured by a double antibody radioimmunoassay[18] and IRG by a similar technic developed in this laboratory.[17] The precision of the glucagon assay for measuring plasma samples is 0.216 mμg./ml. and the sensitivity (the minimum measurable change with 95 per cent confidence) is 0.310 mμg./ml.[15]

RESULTS

Insulin infusions

Insulin (0.5-0.7 U. beef pork Regular Insulin/kg.) was rapidly injected into a peripheral vein in five dogs. The effect on plasma glucagon and glucose concentrations is shown in figure 1; fuller details, including

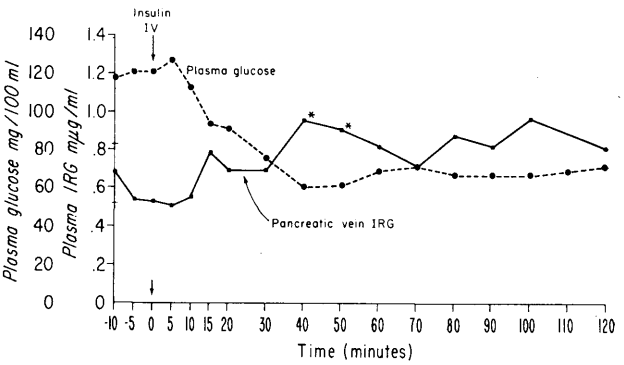

FIG. 1. Hypoglycemia was induced in five dogs by insulin (0.5-0.7 U./kg.) injected into a peripheral vein. The asterisks represent statistically significant rises in mean pancreatic IRG levels over basal.

individual pancreatic IRG, peripheral vein plasma glucose values, and the mean values for jejunal and peripheral vein IRG levels are presented in table 1. Pancreatic vein IRG levels began to rise concomitantly with the fall in plasma glucose. The IRG levels (mμg. $\pm$ S.E.M.) at forty minutes (.96 $\pm$ .18) and fifty minutes (.81 $\pm$ .23) after the insulin injection were significantly greater (paired t test) than the immediate preinjection levels (.53 $\pm$ .17, $p < .05$). The peak

TABLE 1

Acute insulin hypoglycemia

	Control period		Minutes after insulin intravenously					
Dog No.§	—10	—5	0	5	10	15	20	30
27 PV IRG†	.44	.32	.20	.52	.40	.68	.32	.32
PG‡	138	140	143	145	151	—	144	108
28 PV IRG	.34	.52	.64	.32	.36	.30	.48	.36
PG	101	101	100	98	92	82	78	66
29 PV IRG	.52	.30	.32	.31	.32	.30	.40	.52
PG	106	106	99	119	114	93	80	54
30 PV IRG	1.18	.80	.86	.66	.80	1.48	1.40	1.40
PG	128	128	132	141	121	121	121	87
31 PV IRG	.96	.78	.64	.74	.88	1.18	.86	.86
PG	118	131	131	132	89	81	66	—
Pancreatic vein IRG (mμg./ml.) (mean $\pm$ S.E.M.)	.69$\pm$.17	.54$\pm$.11	.53$\pm$.17	.51$\pm$.09	.55$\pm$.12	.79$\pm$.24	.69$\pm$.20	.69$\pm$.20
Jejunal vein IRG (mμg./ml.) (mean $\pm$ S.E.M.)	.39$\pm$.03	.41$\pm$.05	.37$\pm$.04	.38$\pm$.05	.38$\pm$.05	.41$\pm$.05	.39$\pm$.05	.37$\pm$.08
Peripheral vein IRG (mμg./ml.) (mean $\pm$ S.E.M.)	.32$\pm$.04	.29$\pm$.02	.30$\pm$.03	.36$\pm$.04	.34$\pm$.02	.32$\pm$.04	.32$\pm$.04	.27$\pm$.02
Peripheral vein plasma glucose (mg./100 ml.) (mean $\pm$ S.E.M.)	118$\pm$7	121$\pm$8	121$\pm$9	127$\pm$9	113$\pm$11	94$\pm$9	91$\pm$14	76$\pm$12

*Rise over basal $p \leq .05$. †PV IRG pancreatic vein IRG (mμg./ml.) ‡PG plasma glucose mg./100 ml. (peripheral vein). §In five dogs, insulin (0.5-0.7 U./kg.) was given at 0 time into a peripheral vein.

in the IRG levels occurred at forty minutes, which coincided with the nadir of the plasma glucose values. IRG levels were higher in the pancreatic vein, in both basal and hypoglycemic states, than in the jejunal or peripheral veins (table 1). The hypoglycemia did not cause a significant change in plasma IRG levels in either peripheral or jejunal veins.

At the conclusion of the above experiments, a pancreatectomy was performed in the same five animals. Pancreatectomy resulted in no change in peripheral vein glucose or jejunal vein glucagon concentrations. The effect of pancreatectomy on plasma IRI concentrations was not ascertained in these experiments. The same dose of insulin as before pancreatectomy was then rapidly injected into a peripheral vein. Despite a fall in the plasma glucose to levels lower than those in the experiments in the intact animal, IRG levels in a jejunal vein remained unchanged (figure 2).

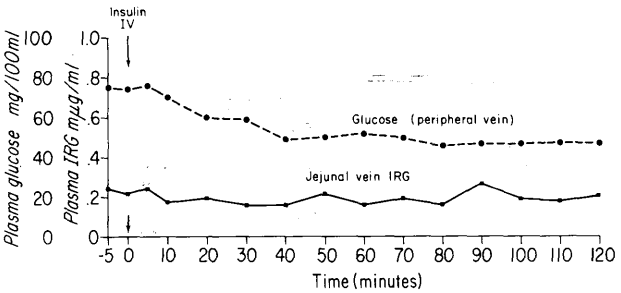

FIG. 2. Following pancreatectomy in five dogs, insulin (0.5-0.7 U./kg.) induced hypoglycemia had no effect on mean IRG levels in a jejunal vein.

Tolbutamide infusion

The production of hypoglycemia with large doses of insulin can scarcely be described as physiologic. Therefore, tolbutamide, which promotes hypoglycemia by stimulating insulin release, was given to four dogs, and the effect on glucagon release was studied. This study also provided an opportunity to correlate endogenous IRG and IRI secretion. Tolbutamide (Upjohn), 1 gm. was injected over a one-minute period into a peripheral vein. The results are shown in table 2, and include the individual values for IRG in the pancreatic vein, IRI and plasma glucose in a peripheral vein, and the mean IRG values for jejunal and peripheral veins. The relationship between pancreatic vein IRG levels and peripheral vein IRI and glucose values is illustrated in figure 3. During the one-hour control period prior to injection of tolbutamide, there was no significant

TABLE 1 (Continued)

Acute insulin hypoglycemia

	Minutes after insulin intravenously							
Dog No.§	40	50	60	70	80	90	100	120
27 PV IRG†	.47	.48	.28	.32	.32	.76	.52	.52
PG‡	100	108	100	110	110	96	82	86
28 PV IRG	.58	.58	.76	.72	.72	.36	.32	.44
PG	52	52	52	56	56	68	75	81
29 PV IRG	.96	.96	.48	.40	.40	.35	.42	.74
PG	41	41	51	52	52	56	60	59
30 PV IRG	1.30	1.42	1.58	1.44	2.16	1.84	3.00	1.71
PG	68	66	74	71	75	71	72	84
31 PV IRG	1.44	1.09	.97	.68	.76	.77	.55	.60
PG	46	44	—	—	44	44	44	44
Pancreatic vein IRG (mμg./ml.) (mean ± S.E.M.)	.96±.18*	.91±.17*	.81±.23	.71±.20	.87±.34	.81±.28	.96±.52	.80±.24
Jejunal vein IRG (mμg./ml.) (mean ± S.E.M.)	.45±.17	.31±.08	.30±.16	.37±.07	.38±.12	.30±.08	.32±.05	.27±.07
Peripheral vein IRG (mμg./ml.) (mean ± S.E.M.)	.42±.10	.39±.07	.33±.06	.28±.03	.28±.03	.31±.06	.29±.05	.33±.06
Peripheral vein plasma glucose (mg./100 ml.) (mean ± S.E.M.)	61±11	62±12	69±11	72±13	67±12	67±9	67±7	71±8

*Rise over basal $p \leq .05$. †PV IRG pancreatic vein IRG (mμg./ml.) ‡PG plasma glucose mg./100 ml. (peripheral vein). §In five dogs, insulin (0.5-0.7 U./kg.) was given at 0 time into a peripheral vein.

TABLE 2

Tolbutamide effect

Dog No.	Minutes of control period			Minutes after 1 gm. tolbutamide intravenously			
	50	55	60	2	5	10	20
32 PV IRG§	.32	.36	.32	.28	.32	.32	.36
IRI‖	15	19	20	25	31	49	45
PG**	123	122	124	123	122	121	116
33 PV IRG	.48	.48	.36	.32	.20	.32	.32
IRI	15	15	11	26	—	24	24
PG	114	112	110	112	—	108	100
35 PV IRG	.36	.36	.36	.26	.28	.26	.52
IRI	20	19	20	24	22	24	29
PG	113	111	111	131	119	117	108
36 PV IRG	.52	.68	.68	1.08	.88	.80	.88
IRI	10	11	10	23	16	14	13
PG	108	111	111	111	98	95	93
Pancreatic vein IRG (mμg./ml.) (mean ± S.E.M.)	.42±.05	.47±.07	.43±.08	.48±.20	.42±.15	.42±.12	.52±.13
Jejunal vein IRG (mμg./ml.) (mean ± S.E.M.)	.35±.05	.34±.05	.35±.05	.29±.03	.35±.08	.37±.06	.75±.37
Peripheral vein IRG (mμg./ml.) (mean ± S.E.M.)	.28±.02	.24±.02	.24±.02	.25±.007	.26±.04	.24±.03	.31±.01
Peripheral vein IRI (μU./ml.) (mean ± S.E.M.)	15±2	16±2	15±3	25±1	23±4	28±7	26±7
Peripheral vein plasma glucose (mg./100 ml.)	115±3	114±3	114±3	119±5	113±8	110±6	104±5

Dog No.	Minutes after 1 gm. tolbutamide intravenously						
	30	40	50	60	70	80	90
32 PV IRG§	.44	.72	.64	.52	.64	.72	.54
IRI‖	40	43	60	65	57	61	61
PG**	112	110	106	103	100	97	93
33 PV IRG	.32	.86	.64	.56	.56	.88	.64
IRI	27	21	24	24	29	23	23
PG	92	90	90	86	87	89	89
35 PV IRG	.72	.66	.78	.20	.58	.54	.60
IRI	9	13	13	22	33	39	44
PG	108	107	107	107	115	101	96
36 PV IRG	1.04	1.58	1.82	1.72	1.60	2.00	2.30
IRI	16	20	13	16	13	12	10
PG	91	88	97	87	84	85	79
Pancreatic vein IRG (mμg./ml.) (mean ± S.E.M.)	.63±.16	.95±.21*	.97±.29*	.75±.33	.84±.25†	1.0±.33‡	1.04±.43
Jejunal vein IRG (mμg./ml.) (mean ± S.E.M.)	.55±.16	70±.30	.52±.13	.44±.10	.46±.07	.46±.10	.45±.07
Peripheral vein IRG (mμg./ml.) (mean ± S.E.M.)	.32±.02	35±.03	.32±.02	.28±.02	.28±.04	.25±.01	.23±.02
Peripheral vein IRI (μU./ml.) (mean ± S.E.M.)	23±7	24±6	28±11	32±11	33±9	34±11	35±11
Peripheral vein plasma glucose (mg./100 ml.)	101±3	99±6	100±4	96±5	97±7	93±4	89±4

*Rise over basal $p < .05$. †Rise over basal $p < .02$. ‡Rise over basal $p < .01$. §PV IRG pancreatic vein IRG (mμg./ml.). ‖IRI IRI (peripheral vein) μunits/ml. **PG plasma glucose (peripheral vein) mg./100 ml. The complete values for the one-hour control period are not listed.

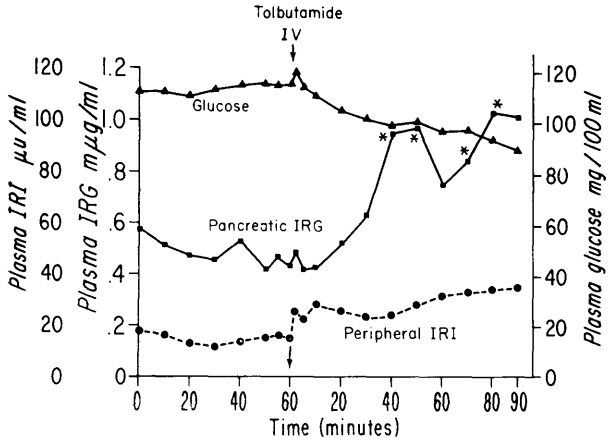

FIG. 3. Tolbutamide, 1 gm., was injected into a peripheral vein. Significant rises in pancreatic IRG levels over basal are represented by asterisks. Each point represents the mean of four dogs.

change in the concentrations of IRG, IRI, or glucose.

Following the tolbutamide injection, there was only a modest rise in peripheral IRI levels, associated with a gradual decrease of the plasma glucose concentrations. Pancreatic vein IRG levels began to rise twenty to thirty minutes after the tolbutamide, and the rise above the control value immediately prior to injection was significant at 40, 50, 70 and 80 minutes ($p < .05$, .05, .05, .01, respectively). The IRG levels peaked initially at fifty minutes and then again at eighty minutes. As in the insulin infusion studies, changes in the jejunal and peripheral veins were less than that observed in the pancreatic vein, although inconsistent and significant rises in the jejunal vein levels occurred between twenty and fifty minutes following tolbutamide

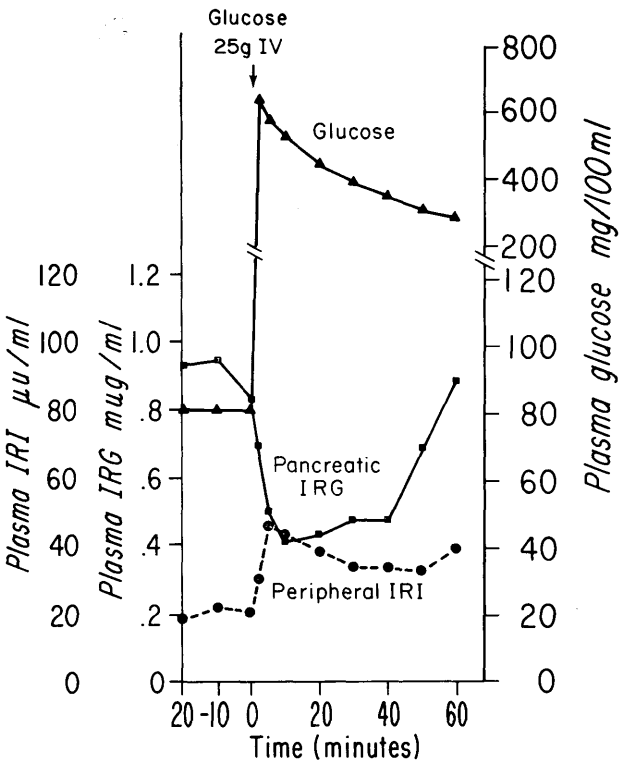

FIG. 4. Glucose, 25 gm., was given to four dogs two hours after tolbutamide administration and the effect on the mean plasma IRI, IRG, and glucose is illustrated.

(table 2).

Glucose infusion

In the same four dogs, two hours after the tolbutamide injection, 25 gm. of glucose was infused over four minutes (figure 3, table 3). At this time, pancreatic vein IRG levels were still elevated over the basal

TABLE 3

Effect of 25 gm. intravenous glucose on IRG levels

Dog No.	Control period*		Minutes after intravenous glucose								
	—20	—10	0	2	5	10	20	30	40	50	60
32 PV IRG†	.68	.68	.64	.48	.32	.24	.24	.36	.32	1.04	2.32
IRI‡	34	45	42	26	38	35	22	24	24	28	35
PG§	80	81	80	600	600	528	414	366	315	300	285
33 PV IRG	.56	.56	.52	.32	.12	.08	.12	.24	.20	.28	.32
IRI	9	10	10	16	20	20	19	20	22	26	43
PG	85	85	85	600	600	600	600	582	537	453	441
35 PV IRG	.66	.48	.48	.42	.50	.44	.52	.30	.42	.40	.30
IRI	25	25	25	65	97	99	97	74	67	57	60
PG	86	86	86	732	620	568	440	352	312	252	224
36 PV IRG	1.88	2.12	1.72	1.60	1.08	.92	.88	1.04	1.00	1.08	.64
IRI	8	8	8	15	27	24	16	18	23	20	21
PG	73	73	74	640	496	436	340	292	264	224	208

*Control period refers to time 100-120 minutes following tolbutamide. †PV IRG pancreatic vein IRG mμg./ml. ‡IRI (peripheral vein) μU./ml. §PG plasma glucose (peripheral vein) mg./100 ml.

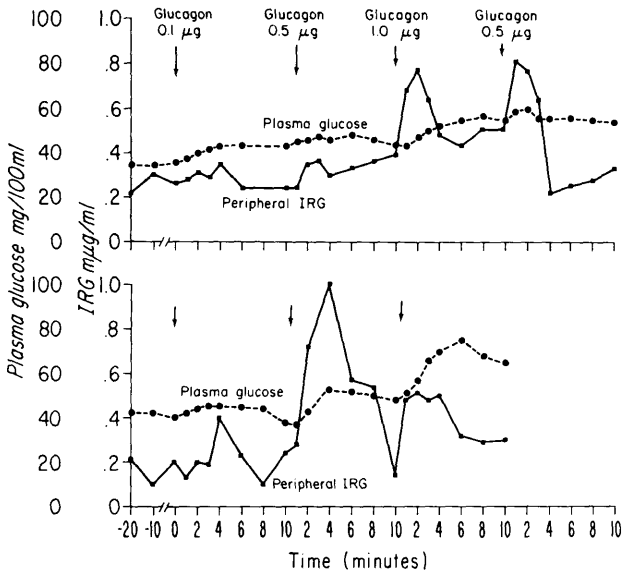

FIG. 5. In two pancreatectomized dogs, two hours following intravenous insulin (0.5-0.7 U./kg.), the effect of small rapid intraportal infusions of crystalline glucagon on plasma glucose and peripheral vein IRG levels is shown.

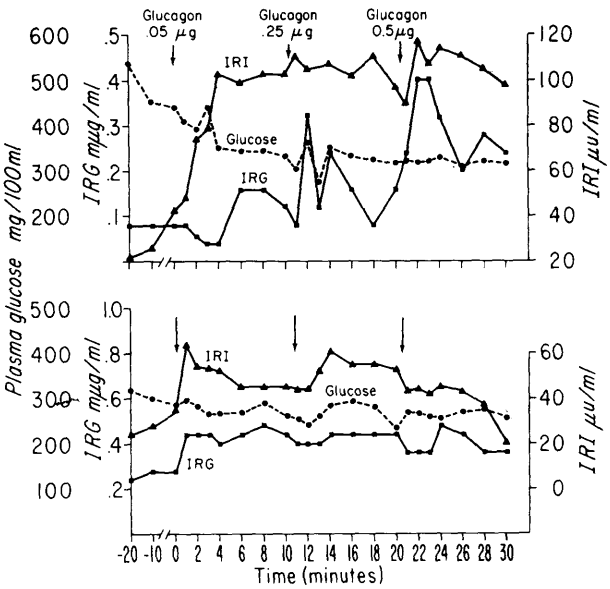

FIG. 6. In two intact dogs, sixty minutes after the infusion of 25 gm. glucose intravenously, the effect of small, rapid intraportal infusions of crystalline glucagon on peripheral vein IRI and IRG levels is shown.

and had reached a steady state for twenty minutes in all four dogs. The ensuing hyperglycemia resulted in a rise in IRI levels but a fall in pancreatic vein IRG levels. The IRG levels subsequently rose in one dog (dog 32) at fifty and sixty minutes after glucose but remained depressed in the other three.

Glucagon infusions

In order to ascertain the effects of small amounts of glucagon under the present experimental conditions, and to determine the ability of the glucagon assay to detect, in the peripheral blood, changes in pancreatic IRG secretion, the following experiments were performed. Small amounts of beef-pork glucagon (Eli Lilly) were injected into the portal vein of two of the pancreatectomized dogs, 120 minutes after the injection of insulin (figure 5). Glucagon, in a dose of 0.1 μg., caused increases in the peripheral glucose concentration without reflecting changes in circulating IRG. Doses of glucagon of 0.5-1 μg. were, however, detectable in the peripheral blood.

Two dogs with an intact pancreas that had received tolbutamide and glucose infusions were given glucagon intraportally sixty minutes after the glucose injections, when hyperglycemia was still present (figure 6). Glucagon, in a dose of 0.05 μg., was sufficient to produce rises in circulating IRI levels in both dogs, and additional, larger glucagon infusions (0.25-0.5 μg.) failed to produce further IRI secretion. Neither dog showed distinct rises in peripheral circulating IRG levels after 0.05 μg. glucagon intraportally, although in one dog there was a distinct change after 0.25 and 0.5 μg.

Thus, under the conditions of the above experiments, intraportal infusions of glucagon, in amounts which could not be detected in the peripheral blood by immunoassay, produced a rise in circulating IRI and glucose levels. The physiologic levels of portal vein glucagon in a dog may thus be less than 0.25 μg., and possibly in the range 0.05-0.1 μg. or even less.

DISCUSSION

These studies are in agreement with the in vivo findings of Unger et al.[6,19] of a rise in pancreatic vein IRG during insulin induced hypoglycemia and are also in agreement with the in vitro studies of Vance et al.,[20] who showed increased IRG release from isolated rat islets of Langerhans incubated in a low glucose concentration as compared with a high glucose concentration. Certain qualitative differences, however, exist between the data of Unger et al. and the present results. Unger et al. showed a slow steady rise in pancreatic IRG which did not become significant until two hours after the insulin infusion and was still rising at three hours, whereas we demonstrated peak levels which coincided with the nadir of the glucose values at forty

minutes, and thereafter there was no further significant increase. The negative effect of hypoglycemia on IRG secretion reported by Lawrence[11] and Samols et al.[21] is probably due to the fact that glucagon concentrations were measured in peripheral blood. We were unable to show changes in IRG concentration in the peripheral vein in the present experiments, despite a rise in pancreatic vein levels.

The absolute rise in IRG concentrations above control values was greater after tolbutamide than after the injection of insulin despite a small decrement in blood glucose concentrations after tolbutamide (tables 1 and 2). Therefore, it seems likely that tolbutamide may have stimulated IRG release from the pancreas directly as well as possibly indirectly through the slight fall in blood glucose.[22] Our data are consistent with the reports[6,19] that hyperglycemia depresses pancreatic IRG secretion. Pancreatic vein glucagon levels fell consistently in each of four dogs following injection of glucose into a peripheral vein. The subsequent rise in glucagon levels in one dog (Dog 32) in association with continued hyperglycemia cannot be explained. It might be postulated that glucagon release is stimulated by slight decreases and inhibited by small increases in circulating glucose concentrations and thus contributes to blood glucose homeostasis. The data are consistent with glucagon as a hormone of glucose need as originally proposed by Foa and co-workers[2-4] and with the established glycogenolytic and gluconeogenic[5] properties of glucagon.[1]

We have confirmed the insulin stimulating properties of glucagon[7-9] and our results agree with those of Ketterer et al.[23] who showed that intraportal doses of glucagon which could barely be detected in the peripheral blood by immunoassay were nevertheless associated with insulin secretion. The insulin secretion promoted by glucagon in the present experiments was produced at a high glucose concentration and several workers have reported enhancement of glucagon-stimulated insulin secretion by glucose.[24-26] Since in the present experiments and in those of Unger et al.[6] hyperglycemia inhibited endogenous pancreatic glucagon release, it would seem that if pancreatic glucagon serves as a physiologic stimulus for insulin secretion, it must do so only when the blood glucose concentration is in the fasting range.

It is difficult to provide a unified concept of pancreatic glucagon action at the present time. Its release mechanisms appear to be sensitive to a rapid fall in the blood glucose. At the same time as glucagon provides glucose by breakdown of liver glycogen it may also help promote insulin secretion to utilize the released glucose. A steady state can be envisaged where glucagon acts to deliver repeated small pulses of glucose and insulin into the circulation, but such an action probably only occurs within the range of fasting blood glucose levels, and glucagon action ceases as the blood glucose level rises.

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