Altered Ability of the Liver to Produce Glucose Following a Period of Glucagon Deficiency

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SUMMARY

It is known that the effectiveness of a physiologic increment in glucagon to stimulate glucose production wanes with time even when counterregulatory insulin secretion is prevented. The aim of the present study was to establish whether the converse is true: does glucagon become a more effective stimulus of glucose production following a period of acute hypoglucagonemia?

To determine this, somatostatin was infused along with basal replacement amounts of glucagon (0.65 ng/kg · min) and insulin (228 μU/kg · min) into five postabsorptive conscious dogs. After 1.5 h of basal hormone replacement, the glucagon infusion was terminated and a selective fall in the plasma glucagon level (75 ± 6 to 30 ± 4 pg/ml) occurred. This resulted in a drop in tracer (^{3}H-glucose)-determined glucose production from 3.0 ± 0.4 to 1.5 ± 0.3 mg/kg · min. The plasma insulin level remained unchanged at 10 ± 1 μU/ml throughout the experiment. Euglycemia (110 ± 4 mg/dl) was maintained by exogenous glucose infusion. After 3 h of glucagon lack, restoration of the glucagon infusion returned the IRG level to control values (78 ± 6 pg/ml) but restored glucose output only partially (42 ± 9%), necessitating continued glucose infusion to preserve euglycemia. Repetition of the experiment in dogs whose pancreatic glucoregulatory feedback loops were broken surgically (two-stage pancreatectomy) rather than pharmacologically resulted in similar findings. It is concluded that glucagon deficiency of 3-h duration leads to a decrease, rather than an increase, in hepatic sensitivity to glucagon. DIABETES *30*:490–495, June 1981.

Physiologic increments in plasma glucagon have been shown to increase hepatic glucose production under a wide variety of circumstances.[1,2] It has been noted, however, that even when insulin is held constant, the increase in glucose production resulting from a constant elevation in glucagon is not sustained beyond 2–3 h.[3,4] If such a desensitization mechanism is an important component of acute homeostatic control, then a parallel resensitization mechanism should exist to restore normal glucagon sensitivity. The present study was designed to investigate whether a 3-h period of selective glucagon deficiency would lead to a sensitization of the liver such that restoration of the basal glucagon level would result in an overproduction of glucose.

METHODS

Animals and surgical procedures. Experiments were carried out on nine mongrel dogs (19–25 kg) of either sex, which had been fed a high protein diet (Wayne Dog Chow, Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois) for 2–3 wk before their use. Nine days before each experiment, a laparotomy was performed under general anesthesia (sodium pentobarbitol, 25 mg/kg i.v.) and a Silastic catheter (Dow Corning Corp., Midland, Michigan) was inserted into a tributary of the splenic vein. The tip of the catheter was advanced until it was located at the junction of the splenic and portal veins. A similar catheter was inserted into the femoral artery following a cut-down over the left inguinal area, and its tip was positioned in the left iliac artery. After their insertion, both catheters were filled with saline containing heparin (200 U/ml; Abbott Labs., North Chicago, Illinois). Their free ends were then knotted and placed in a subcutaneous pocket so that complete closure of both skin incisions was possible.

One day before each experiment, blood was withdrawn to determine the leukocyte count and hematocrit of the animal. Only animals having (1) a leukocyte count below 16,000/mm^3, (2) a hematocrit above 38%, (3) a good appetite (consuming all of their daily rations), and (4) normal stools were used. All animals were deprived of food for 16 h before an experiment.

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Received for publication 19 May 1980 and in revised form 27 January 1981.

On the day of an experiment, the subcutaneous ends of the catheters were exteriorized through a small skin incision made under local anesthesia (2% Lidocaine, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts).The contents of each catheter were aspirated, and saline was infused through them at a slow rate (0.1 ml/min) until the experiment was begun. The splenic vein catheter was used for hormone infusion and the arterial catheter for blood sampling. Angiocaths (18 gauge; Abbott Labs., North Chicago, Illinois) were inserted percutaneously into the two cephalic veins as well as the right saphenous vein. The right cephalic vein was used for (^{3}H-3)-glucose infusion and the left cephalic vein for nonradioactive glucose infusion, while the saphenous vein was used for somatostatin infusion. Following completion of pre-experimental preparations, the conscious dog was allowed to stand calmly in a Pavlov harness for 20–30 min before the start of the experiment.

Three experiments were carried out on depancreatized dogs to control for the use of somatostatin in the main group of dogs. Thus, in three animals, a partial pancreatectomy was performed at the time of catheter placement, as described elsewhere.[5] In summary, 9 days before the experiment, two-thirds of the dog's pancreas was excised under general anesthesia and the remaining third, the uncinate process, was relocated in a subcutaneous abdominal pocket. This pancreatic autograft was capable of secreting enough insulin to prevent glycosuria and weight loss throughout the period between surgery and experimentation. The postoperative care of the animal has been described.[6] On the day of the experiment, the pancreatic autograft was removed under local anesthesia (2% Lidocaine, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts) and the percutaneous cannulations described for the main group of dogs were carried out. Coincident with completion of the pancreatectomy, intraportal infusions of insulin (300 μU/kg · min) and glucagon (0.65 ng/kg · min) were started. The dog was then transferred to the Pavlov harness and allowed to rest for approximately 30 min before the start of the experiment.

Experimental design. Each experiment consisted of an 80- or 90-min tracer equilibration period during which hormone titration occurred. This was followed by a 30- or 40-min control period (−40 or −30 to 0 min), and a 255- to 270-min period of hormone manipulation (0 to 255 or 270 min). The restoration period was lengthened by 15 min in the depancreatized group in an attempt to clarify whether or not a temporal resensitization was occurring. The primed constant infusion of (3-^{3}H)-glucose (0.6 μCi/min) was started at −120 min and continued throughout the study. The priming dose of (3-^{3}H)-glucose equaled the amount of tracer infused in 140 min.

Somatostatin (SRIF; 0.8 μg/kg · min), when required, was infused from −90 to 255 min to inhibit endogenous insulin and glucagon secretion. Glucagon was infused at a basal rate (0.65 ng/kg · min) from −90 to 0 min in the somatostatin group and from −120 min to 0 min in the depancreatized group. It was discontinued from 0 to 180 min, and restored (0.65 ng/kg · min) for the remainder of the study. Insulin was infused at a rate of 300 μU/kg · min initially, but was increased or decreased as required to maintain euglycemia. The plasma glucose level was monitored every 5 min during the period of hormone titration, until a stable blood sugar had been achieved. In the pancreatectomized animals, the final insulin infusion rate was 297 μU/kg · min, while in the somatostatin-infused dogs, it averaged 228 μU/kg · min. The last alteration in the rate was made at least 10 min before the start of the control period.

Nonradioactive glucose (5 g/dl; Abbott Laboratories, North Chicago, Illinois) was infused at a variable rate (0.1–1.3 g/min; see Figures 1 and 2) during the period of glucagon deficiency or restoration as required to maintain euglycemia. In one dog glucose was not infused during the latter period so as to allow determination of the extent to which hypoglycemia would occur if the glucose level was not clamped.

Blood samples were drawn every 10 min throughout the control period and every 15 min thereafter.

Processing of blood samples. The collection and immediate processing of blood samples have been described.[7] The radioactivity of ^{3}H-glucose in plasma samples was determined using previously described techniques[8] and established liquid scintillation counting procedures. Plasma glucose concentrations were determined using the glucose-oxidase method in a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, California).

Immunoreactive glucagon (IRG) was assayed (in plasma samples to which 500 U of Trasylol/ml of plasma had been added), using 30K antiserum obtained from the University of Texas Southwestern Medical School.[9] Immunoreactive insulin (IRI) was measured by the Sephadex bound-antibody procedure.[10]

Materials. (3-^{3}H)-Glucose (New England Nuclear, Boston, Massachusetts) was used as the glucose tracer (500 μCi/0.005 mg). Insulin and glucagon were purchased from Eli Lilly and Company (Indianapolis, Indiana), Phadebas Insulin Radioimmunoassay Kit was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, New Jersey), and Trasylol was obtained from FBA Pharmaceuticals, Inc. (New York). Glucagon 30K antiserum was obtained from Dr. Roger Unger (Dallas, Texas), and the standard glucagon and ^{125}I-glucagon were obtained from Novo Research Institute (Copenhagen, Denmark). All solutions were prepared with normal saline and contained 0.3% bovine serum albumin. The (3-^{3}H)-glucose solution contained added glucose such that its final concentration was 1 mg/ml. Cyclic somatostatin was obtained from Bachem., Inc. (Marina Del Rey, California).

Tracer methods and calculations. The rate of endogenous glucose production ("rate of appearance") and utilization ("rate of disappearance") were determined by the method of primed tracer infusion. Calculation of the rates was carried out as described previously.[11]

Statistical comparisons were carried out using the paired and unpaired Student's *t* tests according to Snedecor et al.[12] Means ± SEM are given.

RESULTS

Insulin and glucagon levels. Insulin levels remained unchanged throughout the experiment at approximately 10 μU/ml in the somatostatin group and 13 μU/ml in the depancreatized group (Figures 1 and 2). The mean control period plasma glucagon concentrations in the two groups were 75 ± 6 and 130 ± 15 pg/ml, respectively, and fell by about 60% during the period of glucagon deficiency. Resumption

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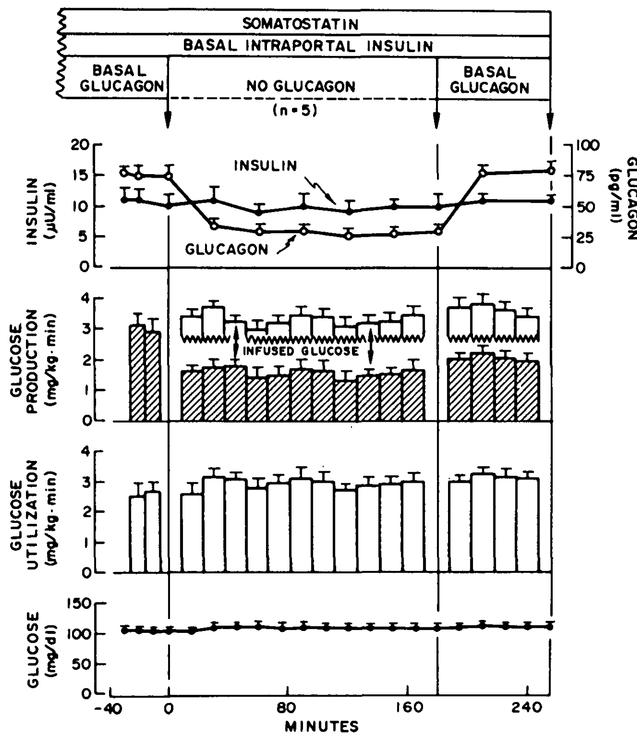

FIGURE 1. The ability of a preceding period of glucagon deficiency to alter the production rate of glucose in response to basal (B) intraportal glucagon infusions (0.65 ng/kg · min) in normal overnight-fasted conscious dogs maintained on somatostatin (0.8 μg/kg · min) and basal (B) intraportal infusions of insulin (228 μU/kg · min). Values are mean ± SE. Glucose was infused to maintain euglycemia throughout the experiment at a rate equivalent to the difference between the height of the open and hatched bars in panel 2.

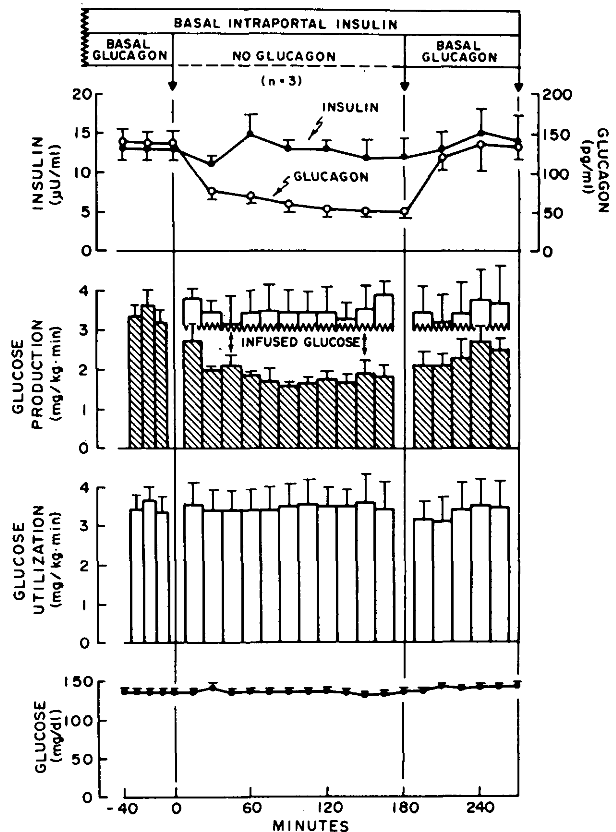

FIGURE 2. The ability of a preceding period of glucagon deficiency to alter the production of glucose in response to basal (B) intraportal glucagon infusions (0.65 ng/kg · min) in depancreatized overnight-fasted conscious dogs maintained on basal intraportal infusions of insulin (297 μU/kg · min). Glucose was infused to maintain euglycemia throughout the experiment at a rate equivalent to the difference between the height of the open and hatched bars in panel 2.

of basal glucagon infusion resulted in restoration of the initial level of the hormone (78 ± 6 and 131 ± 16 pg/ml, respectively).

Glucose levels and production rates. Endogenous glucose production dropped by 51 ± 8% (from 3.0 ± 0.4 to 1.5 ± 0.3 mg/kg · min, $P < 0.01$, Figure 1) when glucagon deficiency was induced in the somatostatin group. In the depancreatized dogs, glucagon lack was associated with a 45 ± 7% fall in hepatic glucose output (3.4 ± 0.4 to 1.8 ± 0.3 mg/kg · min, $P < 0.01$; Figure 2). Reestablishment of the basal glucagon level after a 3-h deficiency period caused increments of only 0.6 ± 0.1 and 0.5 ± 0.1 mg/kg · min in the SRIF and depancreatized groups, respectively. While the new rates were significantly above those evident in the period of glucagon deficiency ($P < 0.05$), they were significantly below those apparent in the control period ($P < 0.01$). Thus the hormone was only one-third as effective during the restoration period as it was during the initial control period (Table 1). The amount of exogenous glucose that was infused to maintain euglycemia is displayed as the difference between the rates of endogenous glucose production (shaded bars) and total appearance (open bars) in the second panel of Figures 1 and 2. The decreased effectiveness of the hormone was reflected in the fact that it was necessary to continue the glucose infusion during the glucagon restoration period to maintain euglycemia. The importance of this glucose infusion was clearly demonstrated in one experiment in which exogenous glucose was not supplied during the period of glucagon restoration (Figure 3). In that instance, the attenuated output of glucose was associated with a fall in the plasma glucose level from 101 to 60 mg/dl. Individual data illustrating the deficient hepatic response to the restored glucagon level in each dog are displayed in Table 1.

Since euglycemia was maintained throughout each experiment, and since the plasma insulin level did not vary, glucose utilization remained constant (Figures 1 and 2).

TABLE 1
Restoration of the control glucose production rate (GP) relative to restoration of the control plasma glucagon concentration

Dogs (N = 5)	(A) % Restoration of GP*	(B) % Restoration of glucagon*	A/B
1	61	142	0.43
2	24	87	0.28
3	44	97	0.45
4	18	79	0.23
5	63	183	0.34
6†	38	123	0.31
7†	31	75	0.41
8†	26	112	0.23
x	38	112	0.34
SEM	6	13	0.03

* The % restoration was calculated by dividing the increment in IRG or GP that occurred when glucagon was restored by the decrement in IRG or GP that occurred when glucagon was made deficient.
† Pancreatectomized animals.

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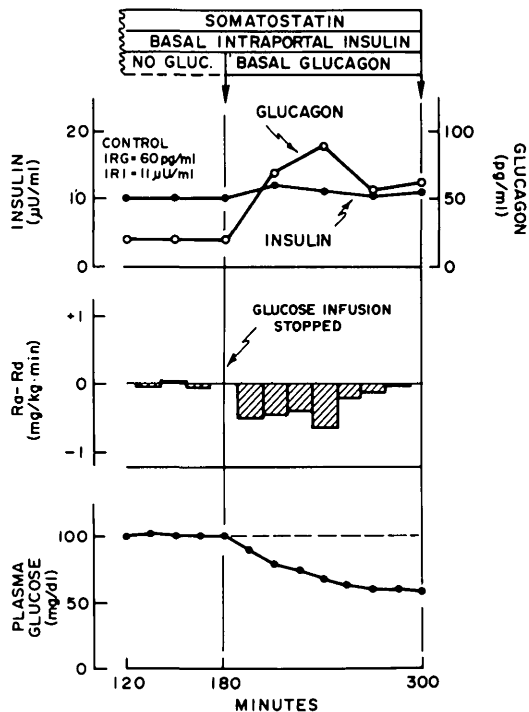

FIGURE 3. The failure of a basal intraportal glucagon infusion (0.65 ng/kg · min) to maintain glucose production and therefore the plasma glucose level following a 3-h period of glucagon deficiency in a normal overnight-fasted conscious dog maintained on somatostatin (0.8 μg/kg · min) and a basal intraportal replacement infusion of insulin (280 μU/kg · min). The experiment was similar to those shown in Figure 1 except that the glucose infusion was terminated when the basal glucagon level was restored (t = 180).

DISCUSSION

The present study demonstrated that a 3-h period of glucagon deficiency did not result in a sensitization of the liver to glucagon. In fact, it resulted in a moderate decline in hepatic sensitivity to the hormone, the evidence for which is twofold. First, and perhaps most striking, is the observation that glucagon was only 23–45% as effective in stimulating hepatic glucose production following a period of glucagon deficiency as it was before the deficiency. The significance of this observation is clearly illustrated by the marked hypoglycemia that developed when the basal glucagon level was restored and exogenous glucose was not infused to compensate for the altered hepatic response to the hormone. In the above experiment, despite the fact that the plasma glucagon level was returned to 119% of its control value and that rapidly developing hypoglycemia probably triggered compensatory glucose production,[13] glucose output was still 24% less than its initial value. Additional evidence supporting desensitization comes from our observation that the response to an increase in glucagon was also blunted following a period of glucagon deficiency (data not shown). Glucagon was infused at a rate four times the basal rate into three of the animals used in the present study (from 255 to 300 min). This maneuver raised glucose production to 5.8 mg/kg · min, a deficient response when compared with the rate of 8.5 mg/kg · min attained in animals not previously subjected to glucagon deficiency.[4]

The reduced glucose production which occurred coincident with glucagon restoration is not likely to have been the result of a downward drift in the basal rate of hepatic glucose output for several reasons. In control experiments reported elsewhere, replacement of both insulin and glucagon at fixed basal rates was not associated with significant time-dependent changes in the production rate or level of plasma glucose.[8,11,13] Thus, in those experiments, glucagon retained its effectiveness in stimulating glucose production over a 4.5-h period. In saline infusion experiments of similar duration, glucose production fell by approximately 12% (0.3 mg/kg · min), but even a fall of this magnitude cannot explain the deficiency observed during the glucagon restoration period in the present study (1.0 mg/kg · min).

With regard to the use of somatostatin in these studies, we have previously shown that this hormone has no effects on the parameters being measured other than those mediated through changes in insulin and glucagon secretion.[13] In control experiments glucose turnover was the same in somatostatin-infused animals receiving basal amounts of exogenous insulin and glucagon as in animals infused with saline.[13] It has been reported that somatostatin can sensitize the liver to glucagon in vivo.[14] Our data, however, indicate that the effect of hyperglucagonemia on glucose production is unaffected by somatostatin (Cherrington et al., manuscript in preparation) and it now seems that the sensitization observed by Sacca et al.[14] was the result of under-insulinization rather than the presence of somatostatin.[15] In vitro data supporting the assumption that somatostatin has no direct effects on glucose metabolism include our observation that it failed to significantly alter basal or hormone-stimulated glucose output by hepatocytes[16] and our finding that basal and insulin-stimulated glucose uptake by fat and muscle were unaffected by the peptide.[16] The lack of an effect of somatostatin on the liver in vitro is consistent with some[17,18] but not all other data.[19,20] It should be noted, however, that even when direct effects of somatostatin on glucose production have been observed, the concentration of the peptide required to elicit the effect was substantially greater than the levels which result from the infusion of the peptide into whole animals at rates similar to those used in the present experiments.[21] With respect to the present study, the alterations observed in glucose production in the pancreatectomized hormone-replaced dogs (Figure 2) were almost identical to those observed in the hormone-replaced animals infused with somatostatin, confirming that the observed hepatic desensitization was not due to the presence of somatostatin.

There are several possible mechanisms by which a decrease in the sensitivity of the liver to glucagon might occur. These include modification of glucagon receptor number or affinity, inhibition of adenylate cyclase, activation of phosphodiesterase, interference with cAMP activation of protein kinase, or other effects on events subsequent to protein kinase activation. The most likely way to explain an inhibition brought about by any of these mechanisms is to hypothesize that by lowering the plasma glucagon level, one allows the unapposed action of insulin to manifest itself and that the consequences of this unapposed action remain evident for sometime following restoration of the glucagon level. It is well documented that insulin can substantially inhibit the accumulation of cAMP in response to submaximal concentrations of glucagon[22] and that it can block the action of exogenously added cAMP in perfused livers and isolated hepatocytes.[23] It has been suggested that such an effect is

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brought about by an inhibition of adenylate cyclase[24,25] and/or an activation of phosphodiesterase.[26–28] In addition, experiments using perfused rat liver have demonstrated an insulin-induced reduction in protein kinase activity in the absence of any change in the concentration of cAMP.[29] Finally, with regard to post-protein kinase effects, insulin has been shown to cause an immediate inactivation of phosphorylase and subsequent activation of glycogen synthase in the livers of monkeys,[30] rats,[31] and pancreatectomized dogs.[32] Converting these enzymes back to the forms that favor glycogenolysis may be a time-dependent process that would not immediately reflect a change in hormonal balance from relative hyperinsulinemia to normal. Such a lag might account for the attenuated rate of glucose production upon restoration of basal glucagon in our protocol.

One cannot conclude on the basis of the present study that glucagon deficiency does not sensitize any of the components of the response system. It is possible, for instance, that an increased synthesis or affinity of the glucagon receptor is being masked by inhibitory changes taking place at a subsequent step (i.e., phosphodiesterase activation). The present study indicates only that the overall process of glucose production is not sensitized. The fact that sensitization did not occur may be taken to support the concept that glycogen depletion and hyperglycemia are the primary reasons for the acute fall in glucose production which is seen in response to a selective increase in glucagon.[4] If such is the case, one would not predict a sensitization in the present study, since neither the hepatic glycogen level nor the plasma glucose level changed significantly during the 3 h of glucagon lack. On the other hand, it is still possible that an internally regulated enzymatic change is involved in downregulation providing it only functions in response to an increase in the plasma glucagon level. That is to say the desensitization-resensitization cycle only occurs when the glucagon concentration rises and subsequently falls. Such a possibility is supported by the fact that sensitization did not occur in response to an absolute deficiency of the hormone in the present study, and that the effect of a basal amount glucagon on glucose production is not evanescent.[13] When Rizza and Gerich[33] administered glucagon at a rate of 3 ng/kg · min to normal man, glucose production increased rapidly but transiently, and a second increment in glucagon brought about 1 h after cessation of the original infusion induced a normal response, suggesting that resensitization did occur. Interpretation of the above study is difficult, however, since the insulin level was not controlled. Cherrington et al.,[34] on the other hand, did control insulin and observed a tachyphylaxis in the hepatic response to successive infusions of glucagon. Once again, however, interpretation is difficult since the recovery period was brief. Thus the question of resensitization following a period of glucagon excess remains to be clarified.

One important implication of the present study is that a preexisting hormonal milieu can acutely condition the response of the liver to a given insulin-glucagon molar ratio. Some years ago, it was suggested by Unger et al.[35] that the molar ratio of insulin to glucagon (I/G ratio) might most appropriately express the net metabolic effect to be expected of these two antagonistic hormones in a given situation.[36] Further studies indicated, however, that a given I/G ratio can be associated with quite different hepatic effects. Cherrington et al.[34] examined the relevance of the I/G ratio in vivo in studies in depancreatized dogs maintained on constant basal intraportal insulin infusions. In these dogs, which could not mobilize extra insulin, glucagon administration caused glucose production to rise and then fall despite continuing hyperglucagonemia (i.e., despite a constant I/G ratio). In other similar studies, glucose production could be maintained during glucagon infusion only when the glucagon infusion rate was increased progressively.[37] The present data suggest further that the effectiveness of a given I/G ratio is also influenced by the preexisting ratio of the two hormones, thereby further complicating use of this index as an indicator of metabolic effectiveness.

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical assistance of Ray Lamb and Barbara Santoro. We are most grateful for the excellent secretarial skills of Patsy Barrett, Lissa Hancock, Carolyn Sielbeck, and Christi Riley. John J. Deri is an M.D. candidate at Columbia University, New York, New York. Alan D. Cherrington was a recipient of the Solomon A. Berson Research and Career Development Award of the American Diabetes Association.

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