Interaction of Somatostatin, Glucagon, and Insulin on Hepatic Glucose Output in the Normal Dog

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SUMMARY

The interaction of insulin and glucagon during infusion of somatostatin (SRIF), which suppresses secretion of these hormones, was investigated in normal, postabsorptive, conscious dogs. Hepatic glucose output (production) and over-all glucose uptake by the tissues was measured with 3-^{3}H-glucose, administered by a priming injection along with a constant infusion. Infusion of SRIF (1.5-5.0 μg./min.) for 90 minutes resulted in a moderate hypoglycemia associated with a decrease in glucose production. In some animals glucose production and plasma glucose levels returned to normal before the end of SRIF infusion. Glucose uptake tended to follow plasma glucose levels. Upon termination of SRIF infusion, glucose production and uptake and plasma glucose increased sharply.

Infusion of glucagon (1 μg./kg./hr.) along with SRIF (3.3 μg./min.) caused an exaggerated increase in glucose production and hyperglycemia over that of glucagon infusion alone. Infusion of a smaller dose of glucagon (0.2 μg./kg./hr.) for two hours produced only small increases in plasma glucose and glucose production; addition of SRIF during the third hour caused a significant increase in glucose production and plasma glucose. Addition of insulin (0.03 U./kg./hr.) to the glucagon infusion had little effect. However the further addition of SRIF failed to produce the marked increase in glucose production and hyperglycemia seen with glucagon-SRIF infusion.

It is concluded that acute insulin deficiency produced during SRIF infusion makes the liver more sensitive to the effects of glucagon and that the response of the liver to glucagon and other hyperglycemic agents may be modulated by insulin. DIABETES *25*:116-21, February, 1976.

The hypothalamic polypeptide somatostatin (SRIF) has been shown to decrease the secretion of glucagon and insulin in man,[1-4] baboon,[5] dog,[6] and rat.[7] In contrast to the hyperglycemia observed following administration of alloxan or streptozotocin or pancreatectomy, administration of SRIF results in a mild hypoglycemia.[1-6] The latter response is believed to reflect suppression of glucagon secretion.

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Glucagon and insulin have a number of opposing effects on carbohydrate metabolism. The availability of SRIF, the first agent capable of suppressing the secretion of both glucagon and insulin, affords an opportunity to study the interrelationship of these hormones on specific metabolic events. In the present study SRIF is used to explore the interaction of glucagon and insulin on hepatic glucose output and plasma glucose concentration. The findings reveal that insulin modulates the sensitivity of the liver to the hyperglycemic effect of glucagon.

METHODS

All experiments were carried out on trained, unanesthetized, normal dogs, weighing 16-20 kg., at about 18 hours after their daily meal. The animals

were maintained on a modified Lusk diet with 38 per cent of the calories derived from carbohydrates, 39 per cent from fat, and 23 per cent from protein.[8] Glucose production and uptake were measured with 3-^{3}H-glucose, administered as a priming injection along with a constant infusion into the saphenous vein.[9] Serial blood samples were collected in heparinized syringes from the jugular vein through an indwelling polyethylene catheter inserted percutaneously, by needle, shortly prior to each experiment. The blood was promptly centrifuged and the plasma deproteinized with $Ba(OH)_2$ and $ZnSO_4$ according to the method of Somogyi.[10] Aliquots of plasma were frozen to await analysis for insulin. For glucagon analysis 4 ml. of blood was placed in chilled tubes containing 0.2 ml. of Trasylol and 0.2 ml. of 2.4 per cent Na_2EDTA (1.2 mg./ml. blood). After centrifugation the plasma was separated and frozen.

Plasma glucose was determined by the glucose oxidase method[11] on a Beckman Glucose Analyzer. Plasma insulin was determined by the radioimmunoassay method of Hales and Randle[12] with a kit from Schwarz-Mann, Orangeburg, N.Y. Glucagon was determined by the radioimmunoassay method of Faloona and Unger[13] with antiserum 30K obtained from Dr. Roger Unger and ^{125}I-glucagon obtained from Nuclear Medical Laboratories, Dallas. Cyclic somatostatin was obtained from Bachem, Inc., Marina Del Rey, California. Glucagon and crystalline insulin were kindly donated by Dr. Mary Root, Eli Lilly and Co., Indianapolis.

The method for determination of plasma glucose specific activity was described in detail elsewhere.[9] Rates of hepatic glucose output (production) and over-all glucose uptake by the tissues were calculated for the steady state as described before;[9,14] during periods of changing plasma glucose concentrations, 0.7 of the initial glucose-pool size was used as the rapidly mixing compartment of the glucose pool.[15-17]

RESULTS

The effects of infused somatostatin (1.5-5.0 μg./min., 0.08-0.25 μg./kg./min.) in 12 experiments on six dogs are shown in figure 1. Somatostatin produced a moderate decrease in plasma glucose concentration that was statistically significant ($P < 0.001$) between 30 and 75 minutes. In a third of the experiments plasma glucose returned to normal or above by 90 minutes despite continued infusion of SRIF. In all instances a significant hyperglycemia followed termination of SRIF infusion.

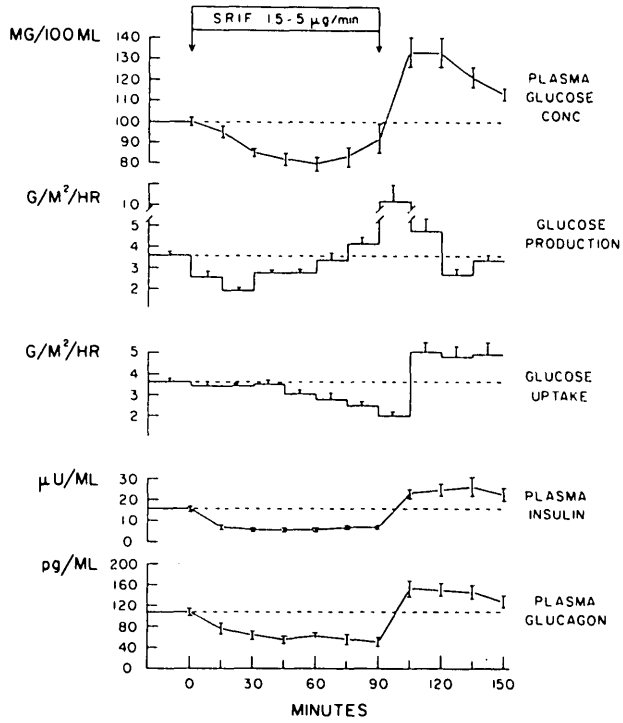

FIG. 1. Effect of infusion of somatostatin (SRIF) for 90 minutes at 1.5-5.0 μg./min. (0.08-0.25 μg./kg./min.) in 12 experiments on six normal dogs. The priming injection and constant infusion of 3-^{3}H-glucose was started 180 minutes prior to zero time. The control values were obtained in the period immediately preceding zero time, and the dashed time is an extension of the control value for reference. Plasma glucose is significantly lower ($P < 0.001$) between 30 and 75 minutes and significantly higher ($P < 0.001$) beyond 90 minutes. Glucose production is decreased significantly ($P < 0.01$) only during the first 60 minutes and is markedly increased after termination of the infusion. Glucose uptake is decreased significantly ($P > 0.01$) only at 90 and 105 minutes. Plasma insulin and glucagon concentrations are significantly ($P < 0.01$) depressed throughout infusion.

Glucose production decreased significantly ($P < 0.01$) during the first 60 minutes, but again in at least a third of the experiments returned to control values prior to the end of SRIF infusion. A marked increase in glucose production occurred following termination of the SRIF infusion, and this was largely responsible for the observed concomitant hyperglycemia. Over-all glucose uptake by the tissues decreased significantly only at 90 and 105 minutes ($P < 0.01$). For the most part, glucose uptake declined in correspondence with decreases in plasma glucose concentration; therefore the metabolic clearance of glucose (glucose uptake ÷ prevailing plasma glucose concentration, multiplied by 100) showed little change except for a decrease at 90 and 105 minutes. Plasma insulin and glucagon

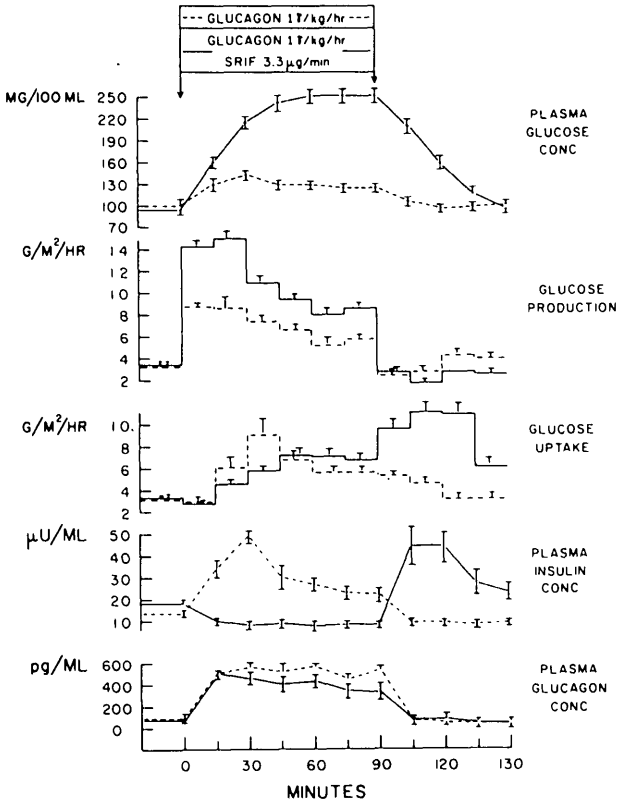

FIG. 2. Infusion of glucagon (1 μg./kg./hr.) (- - -) for 90 minutes into four normal dogs produced a significant hyperglycemia and increases in glucose production and uptake and in plasma insulin and glucagon concentrations. When SRIF (3.3 μg./min.) was infused along with the glucagon (——) there was an exaggerated increase in plasma glucose concentration and in glucose production ($P < 0.05$-0.02 at 0-30 minutes, compared with glucagon alone). Glucose uptake was not significantly different from that with glucagon alone, except after termination of infusions. Plasma insulin levels did not rise and indeed remained depressed because of the SRIF infusion, except after termination of the infusion.

concentrations fell as expected, and some rebound occurred on termination of the infusion.

In order to determine whether or not SRIF infusion impaired hepatic glucose output per se, the effectiveness of infused glucagon on glucose output was examined. As shown in figure 2, infusion of a small dose of glucagon (1 μg./kg./hr.) in the normal animal produced a moderate hyperglycemia that was accompanied by an increased glucose production and glucose uptake by the tissues. There was also the expected rise in plasma insulin concentration in response to the hyperglycemia. When SRIF (3.3 μg./min.) was infused along with glucagon there was a striking potentiation of the hyperglycemic effect of glucagon. It should be noted that this response was associated with a potentiation of the glucagon-induced increase in hepatic glucose output.

Glucose uptake during glucagon infusion in the normal dog showed a moderate, transient increase, as observed previously. The combined infusion of SRIF and glucagon resulted in similar increases in glucose uptake, except for a larger increase on termination of the combined infusions, presumably due to the marked hyperglycemia and hyperinsulinemia.

The role of insulin deficiency in the exaggerated response to glucagon was studied next. Glucagon was infused at a dose (0.2 μg./kg./hr.) that had barely perceptible effects in the normal dog. After a two-hour infusion to establish control values, an infusion of SRIF (3.3 μg./min.) was added and the combined infusions continued during the third hour. As shown in figure 3, plasma glucagon levels increased moderately (about 100-150 per cent), resulting in a small (under 10 per cent) rise in plasma glucose during the first two hours. Glucose production, glucose uptake, and plasma insulin concentration showed small and transient changes that were qualitatively similar to changes obtained with larger doses of glucagon. The addition of SRIF resulted in a marked rise in plasma

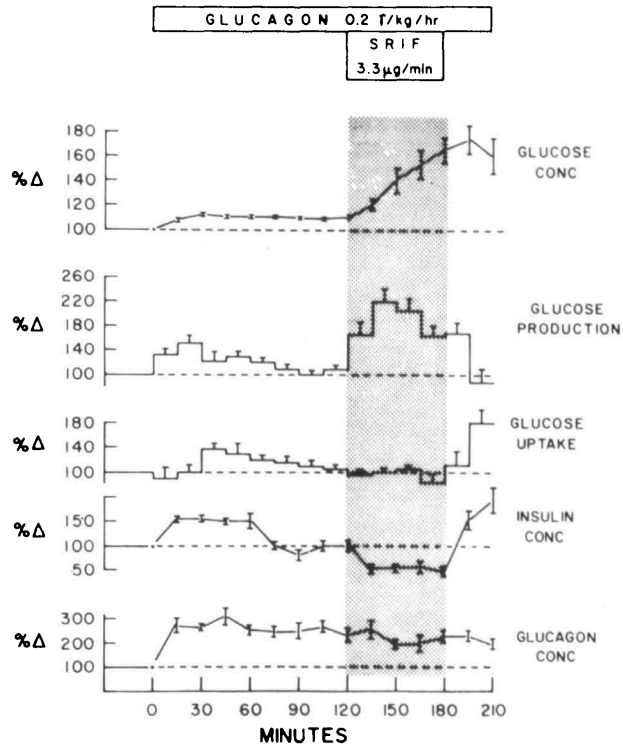

FIG. 3. Infusion of glucagon (0.2 μg./kg./hr.) for two hours caused insignificant changes in plasma glucose (under 10 per cent), glucose production, and glucose uptake. Addition of SRIF (3.3 μg./min.) at two hours caused a significant increase in plasma glucose and glucose production. Plasma insulin levels were depressed.

glucose concentration accompanied by an increase in glucose production.

The effect of addition of insulin on the above parameters is shown in figure 4. The small dose of insulin (0.03 U./kg./hr.) had little effect on the glucagon-induced changes in the control period. However, the addition of SRIF to the combined infusion of glucagon and insulin failed to produce the marked hyperglycemia and the increase in glucose production observed during infusion of SRIF and glucagon only (figure 3).

DISCUSSION

The present study, while supporting the contention that glucagon is important in maintaining plasma glucose concentration, adds a significant qualification to this role. It is shown here that insulin has an important influence in modulating the response of the liver to glucagon. This is apparent from the following observations: First, when the secretion of insulin and glucagon was suppressed by somatostatin, the replacement of glucagon alone resulted in an exaggerated increase in hepatic glucose output. More convincing, however, is the finding that this exaggerated response to glucagon was removed by infusion of very small amounts of insulin (figure 4). Thus, acute deficiency of insulin makes the liver more sensitive to glucagon, and this is attenuated by small amounts of insulin.

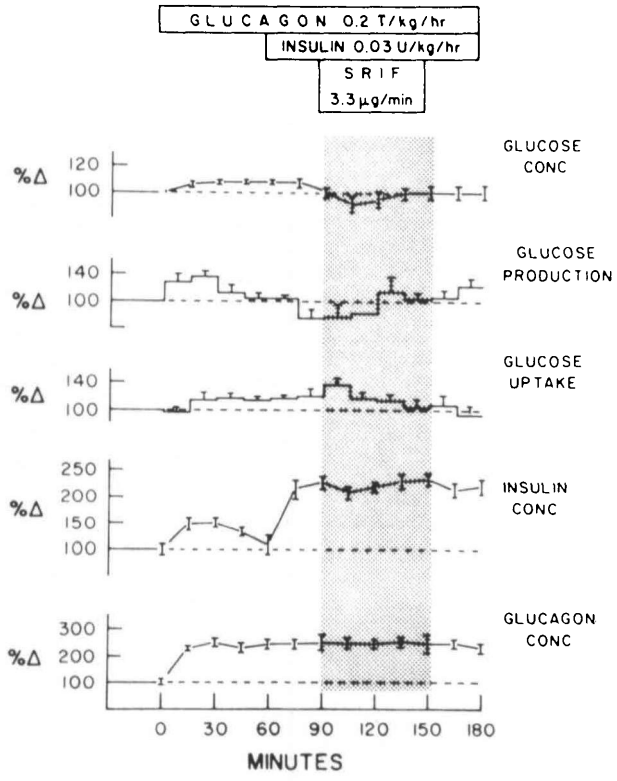

FIG. 4. Glucagon infusion during first hour produced small changes similar to those in figure 3. Addition of insulin (0.03 U./kg./hr.), which doubled the circulating plasma insulin concentration, had only minimal effects; however, upon addition of SRIF (3.3 μg./min.) there was no increase in plasma glucose and glucose production, as noted in figure 3.

The suggestion that insulin exerts a restraining effect on hepatic glucose output was made in earlier studies in 1959.[18] In normal dogs a constant infusion of insulin produced a sustained hypoglycemia that evoked an increased hepatic glucose output during the infusion period. On termination of the insulin infusion, and without additional lowering of plasma glucose levels, there was a prompt, further increase in hepatic glucose output. It was concluded that the infused insulin had restrained the hepatic glucose output even in the face of hypoglycemia. It was further demonstrated that even in the basal, postabsorptive state the circulating endogenous insulin was restraining hepatic glucose output. This was evident from the finding that injection of anti-insulin serum in the normal dog resulted in a prompt increase in hepatic glucose output and hyperglycemia.[19] The acute response to anti-insulin serum occurs without a rise in plasma glucagon levels[20] and in the absence of the adrenal gland.[19]

The individual findings reported here are in harmony with reports by others. The administration of glucagon, along with infusion of somatostatin, has been shown to produce an excessive hyperglycemic response in man[21] and dog.[22] The ability of small amounts of insulin to inhibit glucagon-stimulated hepatic glucose output by perfused normal rat liver has been well-documented by numerous studies[23-25] and reemphasized in the recent report by Parrilla et al.[26]

The role of glucagon in the regulation of blood glucose concentration has been the subject of much interest and debate. Infusion of glucagon into normal animals, including man, stimulates many metabolic events, including glycogenolysis, gluconeogenesis, and glucose production. Whether these effects are influenced by the changes in glucagon occurring under physiologic conditions is less clear. In this regard the availability of a radioimmunoassay for measuring glucagon and the discovery of somatostatin have facilitated exploration of this question.

Plasma glucagon concentration has been shown to increase in a number of circumstances that presumably call for an increased availability of glucose.[20,27,28] It has also been reported that glucagon secretion is not suppressed by glucose in diabetics as effectively as in normal subjects, suggesting that an inappropriate glucagon secretion may be a contributing factor to diabetes hyperglycemia.[22,29]

The discovery and availability of somatostatin evoked renewed interest and gave emphasis to the role of glucagon in glucose homeostasis. Attention was directed to glucagon by the observation that infusion of somatostatin in the baboon, while lowering plasma insulin, produced a hypoglycemia rather than the expected hyperglycemia.[5] This was attributed to a suppression of glucagon secretion, which was subsequently shown to be depressed by somatostatin. Infusion of glucagon along with somatostatin increased plasma glucose concentrations. In these reported studies[21,22] insulin was not replaced, and the main emphasis was placed on the role of glucagon in maintaining plasma glucose homeostasis.[34]

Recognition of the participation of insulin in the regulation of plasma glucose concentration has led to the proposal that the glucagon:insulin (G:I) ratio may effectively regulate the minute-to-minute control of blood glucose levels.[30] A molar ratio of glucagon to insulin of about 1:3 was noted in normal subjects after an overnight fast.[31] Similar ratios were found to be effective in maintaining prolonged glucose production in the acutely depancreatized dog.[32] Using the perfused rat liver, Parrilla et al.[26] found that the effect on glucose output was unchanged when the G:I ratio remained unchanged despite marked variations in the absolute amounts of infused insulin and glucagon.

Conceptually the G:I ratio has been very useful, but there may be some reservations about its general applicability. Thus, despite the demonstration that an increased G:I ratio stimulated gluconeogenesis, ureogenesis, ketogenesis, and proteolysis in the perfused rat liver, doubts were raised that such high G:I ratios occur in vivo, and therefore it has been questioned whether the aforementioned metabolic events are affected by fluctuations of the G:I ratio under physiologic conditions.[26] Likewise, Vranic and Cherrington[33] found that in acutely depancreatized dogs a sustained increase in hepatic glucose output by glucagon infusion required a continuously decreasing ratio of insulin to glucagon. It is clear that a given change in the G:I ratio can be brought about by either increasing the amount of one hormone or decreasing the amount of the other hormone. Since these hormones differ in their specific effects on metabolism and in sensitivity of tissue responses to them, it would appear that the character of the change in the ratio may be more important than the ratio as such.

The present findings do not detract from the major role of glucagon in plasma glucose homeostasis. They do point to a direct involvement of insulin in modulating the liver responses to glucagon. This phenomenon is not limited to glucagon, in that insulin deficiency produced by somatostatin sensitizes the liver to the hyperglycemic effects of dibutyryl cyclic AMP and isoproterenol (unpublished observations). The underlying biochemical nature of this sensitization by insulin deficiency remains to be explained.

ACKNOWLEDGMENTS

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