Studies on the Mechanism Underlying the Influence of Alanine Infusion on Glucose Dynamics in the Dog

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SUMMARY

These experiments have been designed to study the influence of alanine infusion on glucose dynamics in the dog and to further elucidate the role of pancreatic hormones in the interaction of alanine with glucose homeostasis. The primed constant infusion of glucose-2-t was used in order to quantitate the rates of glucose production by the liver (Ra) and glucose utilization (Rd). In a first group of experiments, the intravenous infusion of alanine at the rate of 2 mg./kg./min. produced a moderate enhancement of plasma insulin (IRI), while pancreatic glucagon (IRG) increased more consistently. This different pattern of IRI and IRG response caused the insulin/glucagon molar ratio to decline progressively throughout the experiment. Both rates of glucose turnover increased significantly during alanine infusion. Since Ra rose more rapidly than Rd did initially, hyperglycemia developed. Later, glucose production slowly decreased and, in spite of the sustained hyperglucagonemia, reached levels very close to the baseline in the second part of the experiment. A significant direct correlation between Ra and IRG was found, while the changes in Ra correlated inversely with those in I/G molar ratio.

In a second group of experiments, alanine was infused at the same dose together with 0.4 μg./kg./min. of cyclic somatostatin. In the first part of the infusion, IRG fell more than IRI did, so that I/G ratio increased. Later, IRI levels maintained at low values while IRG returned slowly to the baseline and consequently I/G ratio significantly decreased. Glucose production fell rapidly soon after the beginning of the infusion, and therefore hypoglycemia developed. Later, Ra increased progressively to levels above baseline and plasma glucose returned to the preinfusion levels. As in the first group of experiments, a significant direct correlation between Ra and IRG and an inverse correlation between the changes in Ra and I/G ratio were observed.

These experiments demonstrate that alanine infusion produces an acceleration of glucose turnover and that a clear interrelationship between the release of glucose by the liver and the mobilization of pancreatic hormones exists. Finally, the experiments with somatostatin indicate that hyperglucagonemia is one of the mechanisms underlying the stimulatory effect of alanine on glucose production. DIABETES *26*:262-70, April, 1977.

Many recent studies have demonstrated the peculiar role of the amino acid alanine as a major protein-derived gluconeogenic precursor.[1] Alanine is released by muscle and taken up by the liver in greater amounts than any other amino acid in the postabsorptive state as well as after a prolonged fast[2,3] and may be readily converted by the liver to glucose both in vitro and in vivo.[4-7] A decreased availability of alanine has been implicated in the development of hypoglycemia in pregnancy,[8] after ethanol ingestion,[9] and in ketotic hypoglycemia of childhood.[10] Although these studies have elucidated the central role of alanine as key intermediate between protein catabolism and glucose generation, the metabolic con-

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sequences of the infusion of exogenous alanine have not been completely investigated. While it is well documented that alanine administration stimulates the release of insulin and glucagon,[11-14] not unequivocal results have been presented as to the glycemic response to this amino acid, and no attempt as yet has been made to quantitate this response in kinetic terms. Some authors, in fact, have reported a hyperglycemic effect of alanine,[8,11,12] whereas others have not observed any appreciable modification of glycemia, and even a decrease of plasma glucose has been reported in certain experiments.[13-15] The different pattern of glycemic response to alanine can be theoretically ascribed to the different responsiveness of pancreatic alpha and beta cells in the various experimental situations. On the other hand, it must be stressed that the maintenance of normoglycemia or the occurrence of minor changes in plasma glucose after alanine administration does not necessarily exclude the possibility of relevant modifications in glucose turnover, especially if one takes into account that the release of both insulin and glucagon is activated by alanine. In this regard, it was found that insulin and glucagon, when concurrently infused into depancreatized dogs in titrated amounts, do not neutralize each other but rather act synergistically to increase glucose turnover with minimal changes in plasma glucose concentration.[16,17]

With these considerations in mind, we have undertaken the present work in order to (1) obtain quantitative information on the effects of intravenous infusion of alanine on the rates of glucose production and utilization in the normal dog, (2) correlate the changes in glucose production by the liver with those of plasma glucagon and insulin/glucagon molar ratio in an attempt to get more insight into the mechanism of alanine interaction with glucose turnover, and (3) establish whether the mobilization of pancreatic hormones is entirely responsible for the changes in glucose dynamics induced by alanine. In order to answer this last question, alanine was infused in dogs that were receiving somatostatin, which acted to inhibit the release of both pancreatic hormones in response to alanine stimulus.

MATERIALS AND METHODS

Experimental procedures. The experiments were carried out on 16 mongrel dogs of both sexes weighing 10-20 kg. After overnight fasting, the animals were anesthetized with an intravenous injection of sodium pentobarbital (30 mg./kg.). During the study, additional doses of 10 mg./kg. were given as required. Body temperature was periodically checked with a rectal thermometer and kept constant by a heating pad. The glucose turnover was estimated by the primed constant-infusion technique. At the beginning of each experiment (t=0), a priming dose of glucose-2-^{3}H was administered through a catheter placed in the inferior vena cava via the saphenous vein, followed by the constant infusion, which was continued throughout the experimental period. The ratio of the priming dose to the constant infusion was approximately 80:1. For the determination of glucose-specific activity, arterial blood samples were collected every 10 minutes from t=60 to t=180 through a second catheter inserted in the abdominal aorta via the circumflex femoral artery. The data obtained from t=60 to t=90 were used for the calculation of the baseline values of the rates of glucose turnover. At t=90, in a first group of experiments including nine dogs, alanine was administered by intravenous constant infusion at the rate of 2 mg./kg./min. for 60 minutes. L-alanine (Serva Feinbiochemica, Heidelberg) was dissolved in distilled water, and the solution was buffered to pH 7.4 with NaOH. In a second group of experiments, seven dogs received the same dose of alanine for 60 minutes together with 0.4 μg./kg./min. of cyclic somatostatin (Bachem, Inc., Marina Del Rey, Calif.).

Analytic procedures. In order to determine the specific activity of glucose, plasma samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$. An aliquot of the supernatant was used for glucose assay by the oxidase method (Boehringer, Mannheim). Another aliquot was evaporated by drying at 70° C. to remove tritiated water. This is the only form in which tritium is irreversibly lost from position 2 in the isomerization of the hexose-6-phosphates and is present in plasma in detectable amounts.[18,19] The dry residue was dissolved with 1 ml. of water and then mixed with 10 ml. of Aquasol (New England Nuclear). Radioactivity assay was done with a Nuclear-Chicago Liquid Scintillation Spectrometer, and the specific activity of glucose was expressed as nCi./mg. Radioimmunoassay of insulin was performed by the double-antibody procedure, with dog insulin used as standard.[20] For the determination of plasma glucagon concentration, blood samples were collected into chilled tubes containing 1.2 mg. of EDTA and 500 U. of Trasylol per milliliter of blood. Pancreatic glucagon was determined by radioimmunoassay[21] using antiserum 30K (Diabetes Research Fund, Dallas), glucagon-^{125}I

(Nuclear Medical Laboratories, Inc., Dallas), and pork glucagon standard (Novo Research Institute). Finally, plasma alanine levels were determined by an enzymatic method[22] using alanine dehydrogenase (Boehringer).

Calculations. In the basal state when a dynamic equilibrium prevailed (t=60 to 90 minutes), the glucose turnover rate (mg./kg./min.) was calculated by the isotopic dilution equation: Rt=Ra=Rd=F/SA, where Rt=the rate of glucose turnover, Ra=the rate of endogenous glucose production, Rd=the rate of over-all glucose utilization, F=the rate of infusion of the tracer (nCi./kg./min.), and SA=the specific activity of plasma glucose at equilibrium (nCi./mg.). Since in the postabsorptive state the liver is essentially the only source of glucose, Ra can be assumed as the rate of hepatic glucose production. In nonsteady states, Ra and Rd were calculated by Steele's equations[23] in their derivative form, which permit the evaluation of the continuous changes in the rates:

$$Ra_t = \frac{F - p\,V\,g_t\,dSA/dt}{SA_t}\,;\quad Rd_t = Ra_t - p\,V\,dg/dt$$

where g is the plasma glucose concentration (mg./ml.), V represents the glucose distribution volume, and p is the pool fraction, which was assumed to be 0.65 according to Cowan and Hetenyi.[24] The values of g_t, SA_t, dg/dt, and dSA/dt were calculated from their respective polynomials fitted by the method of least squares. In any case, a fourth- or a fifth-degree polynominal was able to describe satisfactorily the course of glucose and specific activity. The assay of glucose turnover by the primed constant-infusion and the pool-fraction technique has been recently validated for both steady and nonsteady states.[25] All calculations were performed with a program written in BASIC using a WANG 2200 system. The results are presented as mean ± S.E., and the statistical significance of the difference among two means was estimated by the Student *t*-test.

RESULTS

The modifications of plasma insulin and glucagon concentrations induced by alanine infusion are depicted in figure 1. A moderate but significant increase in plasma levels of insulin were observed. IRI rose from the mean basal level of 16 ± 1.5 μU./ml. to the maximum value of 22 ± 3 μU./ml. after 40 minutes from the start of the infusion and then very slowly

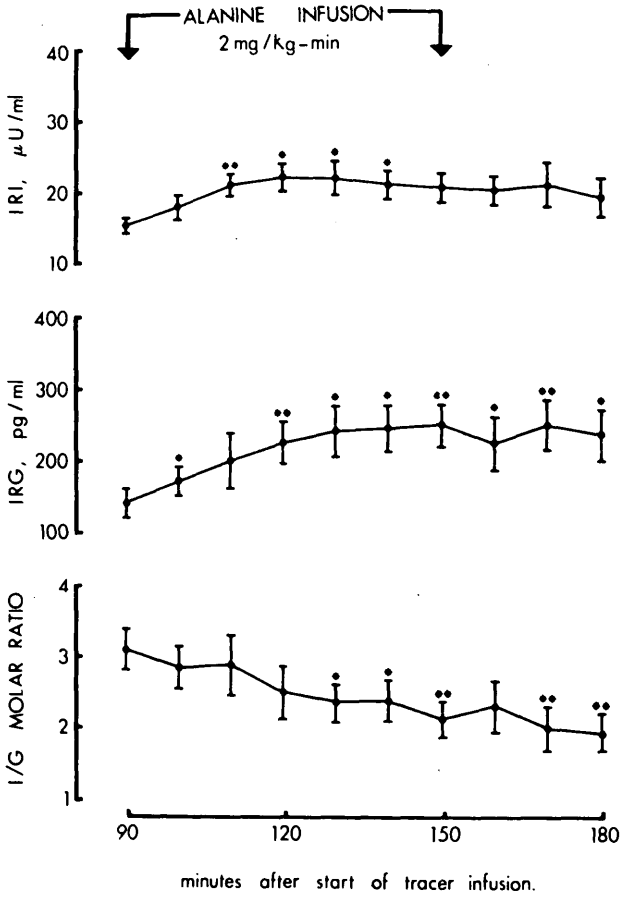

FIG. 1. Effect of the intravenous infusion of alanine at the rate of 2 mg./kg./min. on plasma concentrations of insulin (IRI) and glucagon (IRG) and on insulin:glucagon molar ratio in nine normal dogs. One or two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. P values refer to the significance of the changes from the basal state (paired *t*-test).

declined toward the preinfusion levels. IRG increased more consistently than IRI did and at the end of the infusion reached the maximum value of 250 ± 32 pg./ml., representing a 77 per cent increase above the baseline (141 ± 18 pg./ml.). Interestingly, a sustained and significant hyperglucagonemia was present also in the postinfusion period. The different pattern of IRI and IRG response to alanine accounts for the progressive and statistically significant decline of I/G molar ratio, which persisted throughout the experimental period.

The changes in plasma glucose and in the rates of glucose production and utilization are illustrated in figure 2. Plasma glucose increased significantly after alanine and stabilized at a plateau in the second half of the infusion at a level of about 134 mg./100 ml., which represents a 19 per cent increase above the basal

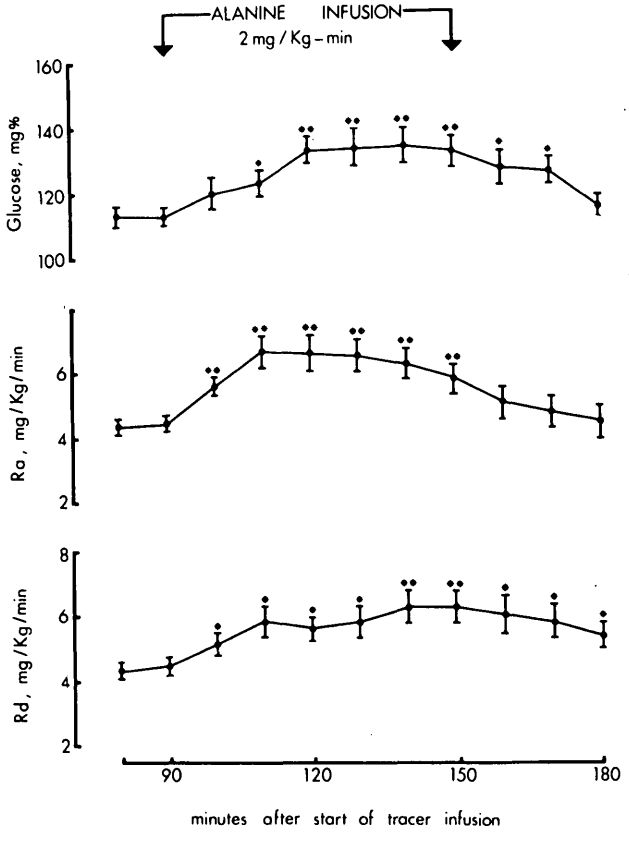

FIG. 2. Effect of the intravenous infusion of alanine at the rate of 2 mg./kg./min. on plasma glucose levels and on the rates of hepatic glucose production and over-all uptake by tissues in nine normal dogs. One or two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. P values refer to the significance of the changes from the basal state (paired *t*-test).

value of 114 ± 3 mg./100 ml. Hyperglycemia was supported by a rapid increase in glucose production, which exceeded that of glucose uptake in the initial part of the infusion. Later, Ra showed a progressive decline and returned to values very close to the baseline at the end of the experiment, when hyperglucagonemia was still present. The enhancement of Rd in these experiments was less pronounced than that of Ra, and the maximum increase was observed at the end of alanine infusion.

In figure 3A the values of Ra are plotted against the plasma levels of pancreatic glucagon. A significant direct correlation between these two parameters was found. In order to establish whether a relation existed also between Ra and I/G molar ratio, the absolute differences of Ra from the baseline were plotted against similar differences in I/G ratio. In figure 3B the existence of a significant inverse correlation between the changes in Ra and I/G ratio is demonstrated.

The modifications of plasma insulin and glucagon following the combined infusion of alanine and somatostatin are presented in figure 4. IRI levels promptly fell after the start of the infusion and then stabilized at a plateau, which persisted throughout the infusion period. The pattern of glucagon changes was somewhat different from that of IRI. In fact, IRG fell more consistently in the initial phase, and this caused the I/G molar ratio to increase (the lack of statistical significance may depend on the very large variability). Later, IRG returned progressively to the basal levels—without, however, exceeding them, and therefore I/G ratio fell significantly. Soon after the discontinuation of the infusion, an immediate increase of all the parameters was recorded.

The modifications of plasma glucose and glucose dynamics during the infusion of alanine plus somatostatin are illustrated in figure 5. Plasma glucose levels fell immediately after the beginning of the infusion, reaching the nadir of 87 ± 4 mg./100 ml. at 20

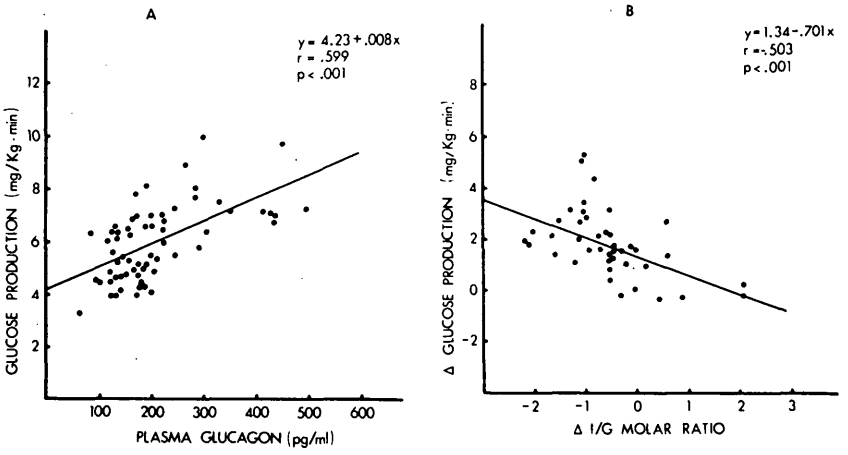

FIGURE 3

(A) Relation between hepatic glucose production and plasma glucagon concentration during alanine infusion in nine normal dogs. (B) Relation between the changes in hepatic glucose production and in insulin:glucagon molar ratio during alanine infusion.

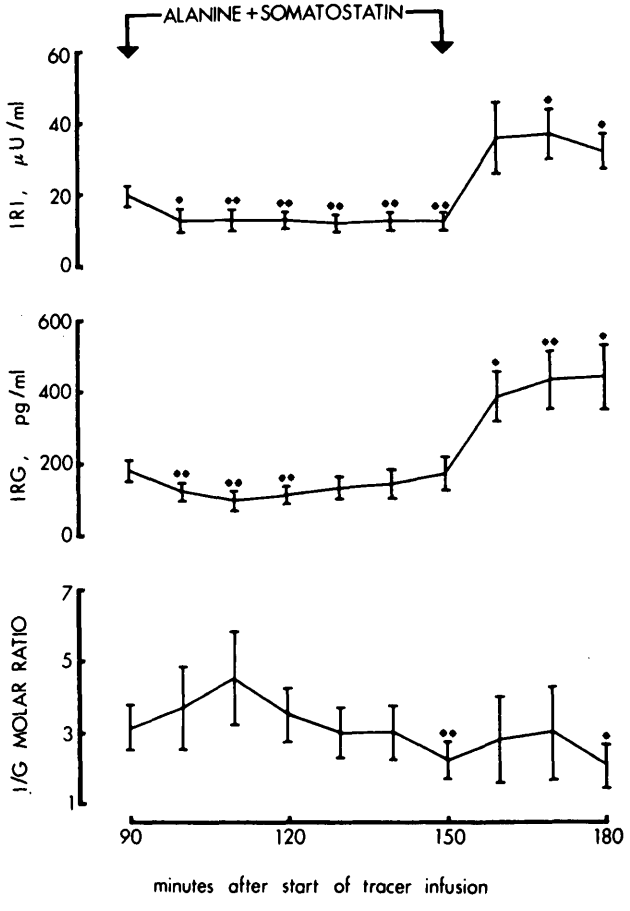

FIG. 4. Effect of the combined infusion of alanine and somatostatin (2 mg./kg./min. + 0.4 μg./kg./min.) on plasma concentrations of insulin (IRI) and glucagon (IRG) and on insulin:glucagon molar ratio in seven normal dogs. One or two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. P values refer to the significance of the changes from the basal state (paired *t*-test).

minutes, which represents a 19 per cent decrease from the basal level of 109 ± 2 mg./100 ml. However, in the second part of the experiment, glucose levels slowly recovered and at the end of the infusion were not statistically differentiated from the baseline. The changes in Ra were grossly parallel to those in glycemia, except in the late phase of the infusion, when Ra recovered more consistently and exceeded significantly the base values. Rd displayed opposite modifications to those of Ra. However, the deviations from the baseline were quite small and never reached a statistical significance. The discontinuation of the infusion resulted in a prompt and significant enhancement of glycemia and both rates of glucose turnover. As in the experiments in which only alanine was administered, a significant direct correlation was found between Ra and IRG (figure 6A), while the changes in Ra correlated inversely with those in I/G ratio (figure 6B).

The changes in plasma alanine concentration are illustrated in figure 7. In the first group of experiments, the infusion of alanine increased the endogenous levels of this amino acid from a mean basal value of 358 ± 54 μmoles per liter to 1,084 ± 161 μmoles per liter at 60 minutes. In the dogs infused with alanine plus somatostatin, plasma alanine concentration rose more markedly, reaching the level of 1,879 ± 388 μmoles per liter at the end of the infusion period.

DISCUSSION

It is well known that protein ingestion or infusion of certain amino acids stimulates the function of endocrine pancreas. In this regard, the most extensively

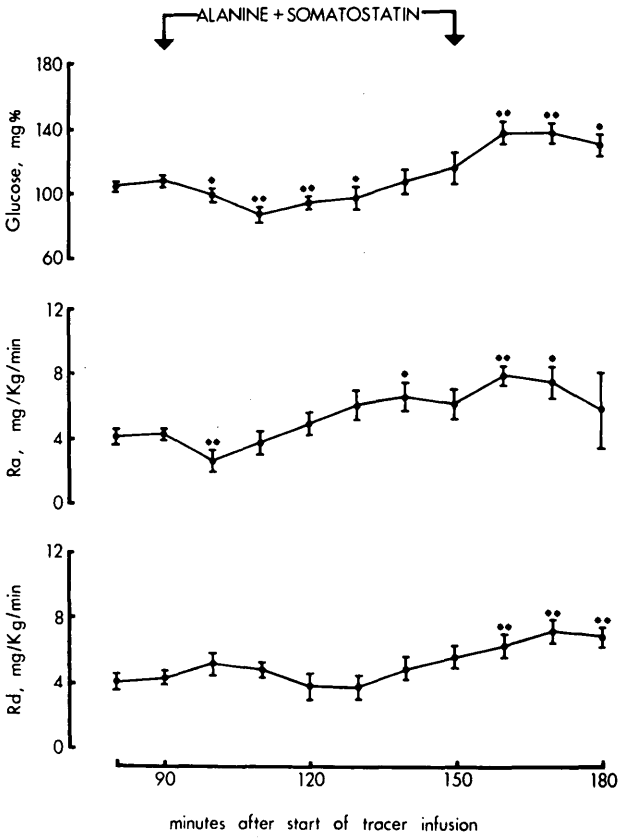

FIG. 5. Effect of the combined infusion of alanine and somatostatin (2 mg./kg./min. + 0.4 μg./kg.-min.) on plasma glucose levels and on the rates of hepatic glucose production and over-all uptake by tissues in seven normal dogs. One or two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. P values refer to the significance of the changes from the basal state (paired *t*-test).

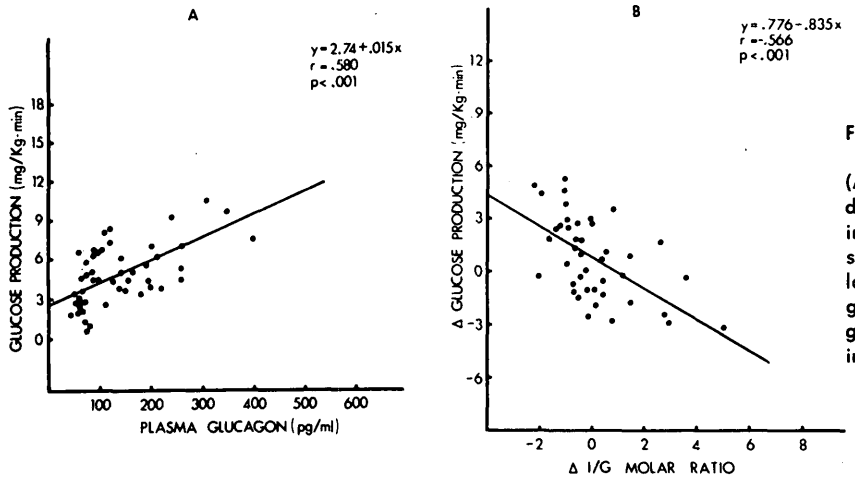

FIGURE 6

(A) Relation between hepatic glucose production and plasma glucagon levels during the combined infusion of alanine and somatostatin in seven normal dogs. (B) Relation between the changes in hepatic glucose production and in insulin: glucagon molar ratio during the combined infusion of alanine and somatostatin.

studied amino acid is arginine, whose administration often is associated with the maintenance of normoglycemia or exerts only a minor hyperglycemic effect[26-30] in spite of its demonstrated effectiveness as insulin- and glucagon-releasing agent.[26-33] In recent tracer studies, Cherrington et al. have demonstrated that the maintenance of normoglycemia during arginine infusion is due to the synchronous increase in the rates of glucose production and utilization and that the mobilization of pancreatic hormones is solely responsible for the increase in glucose turnover.[33,34] Regarding alanine, although the peculiar role of this amino acid in many physiologic and pathophysiologic conditions has been clarified by many recent investigations, the metabolic effects of exogenously administered alanine are not yet completely elucidated. The ability of alanine to stimulate glucagon and to a lesser extent insulin release has been demonstrated,[11-14] and the results of the present study confirm this finding. Furthermore, it has been reported that alanine infusion moderately raises the levels of plasma glucose[8,11,12] and that alanine administration to children with ketotic hypoglycemia or to therapeutically starved obese subjects causes reversal of hypoglycemia.[10,13] However, it must also be recalled that alanine is incapable of consistently increasing plasma glucose levels in the postabsorptive state and in obese subjects.[12-15] Inasmuch as in these conditions glucagon response to alanine is reduced while that of insulin is enhanced,[12,35] it may be inferred that the different responsiveness of alpha and beta cells plays a primary role in conditioning the type of glycemic response to alanine. In our experiments, alanine infusion constantly exerted a hyperglycemic effect, supported by the increase of hepatic glucose release, which exceeded that of glucose utilization. Of particular interest is the existence of a direct correlation between the absolute values of plasma glucagon and Ra. Insulin response to alanine, although present in all the experiments and statistically significant, was quite weak. This minor responsiveness of pancreatic beta cells in comparison to alpha cells accounts for the progressive reduction of I/G molar ratio —which, in turn, is responsible for the enhancement of hepatic glucose release and, consequently, of plasma glucose. Also supporting this view is the existence of a signifi-

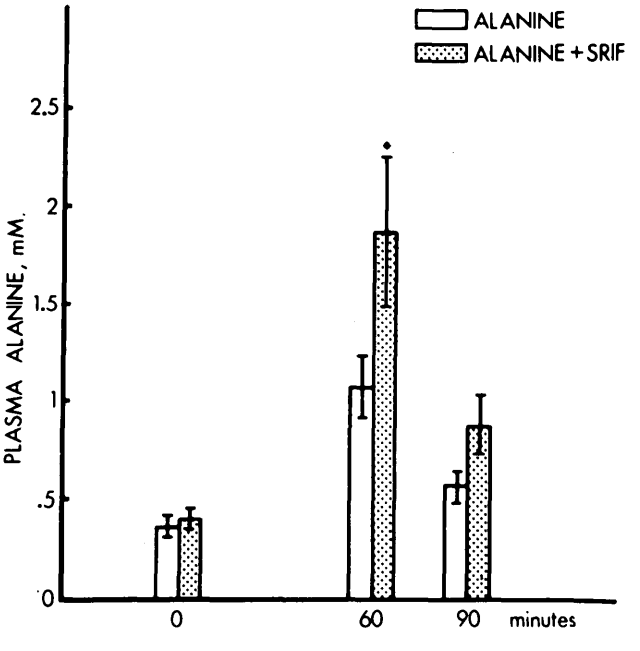

FIG. 7. Changes in arterial alanine concentration induced by the infusion (from t=0 to t=60) of alanine or alanine plus somatostatin (SRIF). Asterisk indicates p=0.05.

cant inverse correlation between the changes in Ra and I/G ratio. These results suggest that the activation of the hepatic production of glucose during alanine infusion, in the presence of a concomitant release of both pancreatic hormones, is dependent on the particular pattern of insulin and glucagon response. In this regard, it is pertinent to recall that Genuth observed a hyperglycemic effect of alanine in insulin-dependent diabetic patients who are characterized by increased basal glucagon levels[12,36] and by elevated glucagon response to protein ingestion[25] but not in normal subjects.[15] Moreover, Wise et al. have reported that fasting enhanced glucagon and glycemic responses to alanine and that obesity depressed the intensity of both responses, while opposite changes in plasma insulin occurred.[12] In these experiments, in addition, alanine failed to increase plasma glucagon and glucose in diabetic patients with chronic pancreatitis. The results of our study substantiate these previous observations that the glycemic response to alanine is related to the particular pattern of pancreatic hormonal response and extend this knowledge with quantitative information on glucose dynamics. Of particular interest in our experiments was the observation that glucose production achieved the maximum level at 20 minutes after the start of alanine infusion and then returned slowly to levels very close to the baseline in spite of the sustained hyperglucagonemia. This transient effect of glucagon on glucose production has been observed also in depancreatized dogs,[17] in diabetic patients after protein ingestion,[37] and in normal man.[38] Our results demonstrate that the same phenomenon occurs in normal dogs when endogenous glucagon secretion is activated by alanine. Moreover, they provide evidence that the availability of gluconeogenic substrate is not a causal factor, since glucose production was clearly decreasing at the end of the infusion when the arterial concentration of alanine was three times the basal level.

The experiments with somatostatin have been performed in order to answer the question of whether the activation of pancreatic hormone secretion is the only mechanism responsible for the acceleration of glucose turnover evoked by alanine infusion. In these experiments, we observed a prompt fall in insulin concentration, which persisted throughout the combined infusion of alanine and somatostatin. In contrast to insulin, plasma glucagon decreased initially but later gradually recovered, without, however, exceeding the preinfusion levels. After the discontinuation of the infusion, a prompt rebound of insulin and glucagon was observed, and this phenomenon very likely depends on the different kinetics of the two substances. In fact, elevated levels of alanine persisted also in the postinfusion period at a time when somatostatin, because of its short half-life,[39] presumably had completely disappeared from the blood stream. In this group of experiments, plasma glucose levels did not increase in response to alanine infusion, but rather initially decreased and later recovered slowly, showing, on the whole, a parallel pattern to that of glucagon. The most interesting changes in glucose dynamics were observed in Ra, which displayed opposite modifications to those in I/G molar ratio. In fact, Ra fell immediately after the beginning of the infusion when I/G ratio increased and then recovered gradually, exceeding the basal levels in coincidence with the late diminution of I/G ratio. Also in these experiments a direct correlation between Ra and IRG and an inverse relation between Ra and I/G ratio were found. These results reinforce the concept that alanine's ability to modify glucose production and glycemia is related to the pattern of insulin and glucagon response. However, the possibility that factors other than the mobilization of pancreatic hormones may be also operative in the action of alanine on glucose dynamics cannot be completely excluded. We have, in fact, observed an increase of Ra above the baseline at the end of the combined infusion of alanine and somatostatin, in spite of the fact that plasma glucagon has returned only to the basal level. Two possible explanations for this phenomenon can be put forth: (1) The liver is extremely sensitive to small relative increments of plasma glucagon in the presence of somatostatin and hypoinsulinemia; and (2) alanine can partially increase glucose production by acting as substrate in the absence of absolute hyperglucagonemia, provided insulin levels are reduced.

The modifications of plasma alanine concentrations during the infusion of this amino acid deserve some considerations. In the dogs infused with alanine and somatostatin, plasma levels of alanine increased more markedly than in the experiments with alanine alone. Since somatostatin completely abolished glucagon's response to alanine, this result indirectly confirms previous observations that glucagon lowers plasma alanine levels.[40-42] The mechanism underlying this phenomenon has not been clarified as yet. The ability of glucagon to increase the uptake of alanine by the perfused liver and to stimulate gluconeogenesis from alanine in vivo has been demonstrated in both dogs and humans.[6,40,41] Furthermore, the impaired hyper-

glycemic response and the lack of hypoalaninemic effect of glucagon in patients with acute viral hepatitis have suggested that glucagon indeed acts by enhancing hepatic alanine uptake and its incorporation into glucose.[42] In view of this knowledge, one might be tempted to explain simply the higher levels of plasma alanine found in somatostatin-treated dogs as the consequence of a reduced incorporation of alanine into glucose because of the blunted glucagon response. On the other hand, it has been recently reported that glucagon infusion in man, while increasing the intrahepatic conversion of alanine to glucose, does not modify the splanchnic extraction of this amino acid.[41] Therefore, the possibility that changes in alanine metabolism at some extrahepatic level may have produced hyperalaninemia in our experiments with somatostatin should also be taken into consideration.

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