

Splanchnic Metabolism of Alanine in Intact Man

Effects of Somatostatin and Somatostatin Plus Insulin

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SUMMARY

We examined splanchnic metabolism of alanine in 15 normal males under three sets of conditions: infusion of saline (control studies); infusion of somatostatin (SRIF) (bihormonal deficiency of insulin and glucagon); and infusion of somatostatin plus insulin (selective glucagon deficiency). Net splanchnic alanine uptake (NSAU) remained stable over 2 h during infusion of saline. Infusion of SRIF was associated with a fall in estimated hepatic plasma flow (EHPF) whether or not insulin was infused concomitantly. With SRIF only, arterio-hepatic venous alanine differences increased such that NSAU remained stable over 2 h, despite the fall in EHPF. In contrast, with selective glucagon deficiency, NSAU fell significantly after 2 h, an effect consequent on a fall in EHPF and a delayed fall in arterio-hepatic venous (A-HV) alanine differences. Our studies are compatible with a role for basal glucagon in maintenance of splanchnic extraction of alanine in normal man. However, the SRIF-initiated fall in EHPF may exert an influence on A-HV alanine differences independent of changes in pancreatic hormone secretion. **DIABETES** 28:486-490, May 1979.

The role of glucagon in regulating hepatic alanine metabolism has remained controversial. Glucagon has been shown to stimulate hepatic alanine uptake in the isolated perfused rat liver.¹ However, in previous studies in man from our laboratory, infusion of glucagon at physiologic concentrations over a 60-min period was not associated with an increase in net splanchnic alanine uptake (NSAU).² Also, when somatostatin (SRIF) plus glucagon were infused to intact dogs, arterial alanine levels and hepatic alanine uptake were unchanged over a 60-min period.³ Furthermore, the induc-

tion of selective glucagon deficiency for 1 h in the dog was not associated with a fall in NSAU.³ On the other hand, studies of longer duration have demonstrated a delayed but progressive rise in plasma alanine during prolonged glucagon deficiency, suggesting diminished hepatic alanine extraction.⁴

To investigate the role of glucagon in maintaining hepatic alanine uptake, SRIF was administered to normal man in the presence and absence of an insulin infusion designed to maintain basal levels of insulin. SRIF inhibits release of both insulin and glucagon⁵ and may provide a useful tool for dissecting the individual effects of these hormones on NSAU. Wahren et al.⁶ administered SRIF for 1 h in males fasted 12-14 h and noted a 25% fall in NSAU. They failed, however, to observe SRIF-induced changes in NSAU among females studied after 60-64 h of starvation.⁶ The interpretation of the effects of glucagon and insulin withdrawal in these studies is complicated by the associated hypoglycemia induced by SRIF infusion. Therefore, the present study was undertaken to withdraw glucagon, in the presence and absence of basal insulin, but with euglycemia maintained, in order to evaluate the role of glucagon in the regulation of splanchnic alanine uptake.

MATERIALS AND METHODS

Fifteen healthy nonobese men between the ages of 20 and 35 yr were studied. The mean body wt was 72.9 ± 2.0 kg (range 56.3-86.0 kg). Studies began between 1200 and 1400 h after a 12-14-h fast. The protocols for this study were reviewed and approved by the Vanderbilt University Committee for the Protection of Human Subjects. The nature, purpose, and possible risks of the procedures were fully explained to each subject before obtaining his voluntary consent.

Hepatic venous and brachial artery catheterization were performed as previously described.⁷ After a 20-30-min basal period, saline, SRIF (0.9 mg/h), or SRIF (0.9 mg/h) plus insulin (0.15 mU/kg/min) were infused intravenously for 2 h. In the latter two groups, euglycemia was maintained during SRIF infusion by intravenous glucose ad-

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TABLE 1
Arterial glucose, insulin, and glucagon levels in three series of studies*

	Time (min)										
	-20	-10	0	15	30	45	60	75	90	105	120
Glucose (mg/dl)											
Saline											
Mean	93.8	95.2	94.5	94.8	95.2	95.5	96.5	95.0	95.8	97.5	97.8
SEM	4.0	4.1	3.9	4.1	4.2	3.9	4.3	4.6	4.6	3.6	3.9
SRIF only											
Mean	100.0	98.9	99.9	98.7	99.0	103.4	106.9	109.3	112.4	118.3	123.3
SEM	1.7	1.9	2.0	1.9	2.2	2.5	3.7	4.7	5.0	7.2	8.3
SRIF + insulin											
Mean	96.8	94.8	94.8	95.5	96.2	96.5	98	96.2	96.8	98.2	98
SEM	1.6	1.8	1.7	2.8	3.2	2.8	3.1	2.8	2.9	3.6	3.5
Insulin (μ U/ml)											
Saline											
Mean	7.5	8.0	7.0	5.2	7.5	8.0	8.2	9.0	10.2	9.0	5.2
SEM	1.5	1.7	1.6	1.3	2.4	3.4	2.3	3.4	4.9	2.5	1.3
SRIF only											
Mean	10.3	10.2	9.0	5.6	4.2	3.9	2.7	2.3	3.0	2.7	1.5
SEM	3.1	4.1	3.1	2.5	2.8	1.9	1.1	1.1	1.6	0.8	1.0
SRIF + insulin											
Mean	9.9	7.9	9.5	12.2	13.0	13.9	12.8	13.0	11.0	10.2	13.2
SEM	1.5	1.7	1.5	1.6	2.4	2.2	2.4	2.1	2.7	0.8	1.9
Glucagon (pg/ml)											
Saline											
Mean	123.5	147.2	128	142	128.2	147.8	133	133.2	127.5	134.2	125.8
SEM	18.0	33.3	19.8	25.5	36.9	43.9	50.5	26.9	30.9	28.0	32.4
SRIF only											
Mean	157	154.2	170.1	71.9	58.6	67.1	64.4	49.9	69.3	61.9	52.4
SEM	18.0	25.4	24.2	7.0	4.0	8.4	5.6	4.7	4.1	7.8	5.9
SRIF + insulin											
Mean	183	182	189	116	88	82	83	91	83	90	95
SEM	48	47	44	17	18	23	22	18	22	20	12

* Course of glucose, insulin, and glucagon levels during infusions of saline only, SRIF only, and SRIF plus insulin. SRIF, somatostatin.

ministered at a variable rate according to the glucose clamp technique of Andres et al.⁸ We hoped by this means to minimize contraregulatory mechanisms attendant on hypoglycemia. To determine hepatic plasma flow, indocyanine green was infused throughout the study via a peripheral vein.⁹ Cyclic SRIF was kindly provided by Dr. Jean Rivier, Salk Institute, La Jolla, California.⁵ Methods of preparation of hormones for infusion have been described elsewhere.⁷ Alanine concentrations were determined by a

rapid short-column chromatography technique.² Insulin, glucagon, and glucose determinations were performed by methods described elsewhere.⁷

RESULTS

Results are shown in Tables 1, 2, and 3 and in Figures 1 and 2.

Control study. When saline only was infused, arterial glucose, insulin, and glucagon concentrations (Table 1) and

TABLE 2
Estimated hepatic plasma flow (L/min) in three series of studies*

	Time (min)										
	-20	-10	0	15	30	45	60	75	90	105	120
Saline											
Mean	0.819	0.846	0.806	0.818	0.851	0.880	0.846	0.947	0.955	0.964	0.946
SEM	0.120	0.128	0.129	0.120	0.143	0.112	0.098	0.128	0.140	0.158	0.153
SRIF											
Mean	0.864	0.852	0.827	0.661	0.7	0.678	0.717	0.747	0.77	0.77	0.77
SEM	0.05	0.03	0.06	0.05	0.05	0.05	0.03	0.04	0.05	0.04	0.04
SRIF + insulin											
Mean	0.93	0.96	0.93	0.592	0.602	0.601	0.581	0.62	0.645	0.657	0.627
SEM	0.07	0.07	0.135	0.13	0.08	0.08	0.09	0.101	0.12	0.09	0.16

* Course of ENPF during infusion of saline, SRIF only, and SRIF plus insulin. ENPF, estimated hepatic plasma flow; SRIF, somatostatin.

TABLE 3
Arterial alanine concentrations ($\mu\text{mol/L}$) in three series of studies*

	Time (min)											
	-20	-10	0	15	30	45	60	75	90	105	120	
Saline												
Mean	239	239	238	239	231	228	232	228	221	216	216	
SEM	7.2	14.2	9.7	11.7	11.9	14.3	14.9	15.1	16.5	18.6	19.8	
SRIF only												
Mean	283	274	276	293	295	279	288	279	286	284	296	
SEM	14.5	17.2	16.6	16.2	15.5	13.3	13.5	10.8	11.6	9.7	11.6	
SRIF + insulin												
Mean	263	262	254	263	266	266	282	286	292	293	307	
SEM	30.7	27.7	30.9	30.0	26.7	27.6	27.0	27.4	26.7	28.5	29.3	

* Course of arterial alanine concentrations during infusion of saline only, SRIF only, and SRIF plus insulin. SRIF, somatostatin.

estimated hepatic plasma flow (EHPF) (Table 2) remained constant. Arterial alanine levels fell modestly and not significantly from a mean of 239 ± 7.2 to $216 \pm 19.8 \mu\text{M/L}$ over the 2-h period of the study (Table 3). Arterio-hepatic venous (A-HV) alanine differences (Figure 1) and NSAU remained stable throughout the study (Figure 2).

Infusion of somatostatin. The administration of somatostatin only resulted in a marked suppression of circulating plasma insulin and glucagon concentrations (Table 1). Plasma glucose concentrations were supported by the infusion of glucose at a mean rate of 0.73 mg/kg/min until the splanchnic glucose production rate, which fell initially, had returned to normal. A modest rise to $123.3 \pm 8.3 \text{ mg/dl}$ in arterial glucose concentration occurred at the end of the infusion period. The infusion of SRIF resulted in an immediate fall in EHPF (Table 2) that persisted for the duration of the study, the mean fall varying between 9 and 22% of basal flow. An increase in A-HV alanine difference was observed that was significant by 15 min ($P < 0.05$) and persisted for the 2 h of the SRIF infusion (Figure 1). Arterial alanine concentrations and NSAU did not change significantly over the 2 h of the SRIF infusion (Figure 2). The constancy of NSAU was consequent on the divergent changes in A-HV alanine and in EHPF: an increase in the former (Figure 1) and a fall in the latter (Table 3).

Infusion of SRIF plus insulin. The infusion of SRIF plus insulin resulted in a sharp fall in arterial glucagon levels, whereas plasma insulin levels were maintained between $10\text{--}14 \mu\text{U/ml}$ (Table 1). Plasma concentrations of glucose were supported by the infusion of glucose at mean rate of 1.26 mg/kg/min for the entire study period (Table 1). Esti-

mated hepatic plasma flow fell sharply by about 25% (Table 2) and remained suppressed for the 2-h period of infusion of SRIF plus insulin.

A-HV alanine differences increased during infusion of SRIF plus insulin from a mean basal value of 108 to $132 \mu\text{mol/L}$ after 30 min. The rise in A-HV alanine differences was not sufficient to compensate for the fall in EHPF, and NSAU (Figure 2) fell significantly throughout the infusion of SRIF plus insulin ($P < 0.05$ or less for all eight time periods between 15 and 120 min). Furthermore, during the second hour, there was a significant fall in A-HV alanine differences to $81 \mu\text{mol/L}$. Thus by the end of the second hour, NSAU had fallen about 50% as a result of a 30% fall in EHPF and a 25% fall in A-V alanine differences. Mean arterial alanine concentrations rose from a mean basal value of 263 ± 30.7 to $282 \pm 27 \mu\text{mol/L}$ after the first hour and reached $307 \mu\text{mol/L}$ at 2 h (Table 3, $P < 0.05$ by paired *t* test).

DISCUSSION

In the present studies, we examined net splanchnic alanine exchange in intact man under conditions of glucagon deficiency with and without maintenance of basal insulin levels over a 2-h period. To this end, we first infused SRIF to a group of seven normal subjects. In the face of impressive insulinopenia and glucagonopenia, no change was observed in NSAU over 2 h: whereas SRIF infusion was associated with a significant fall in EHPF, A-HV alanine differences increased by about 20%, which compensated for the reduced plasma flow.

A different picture, however, emerged under conditions

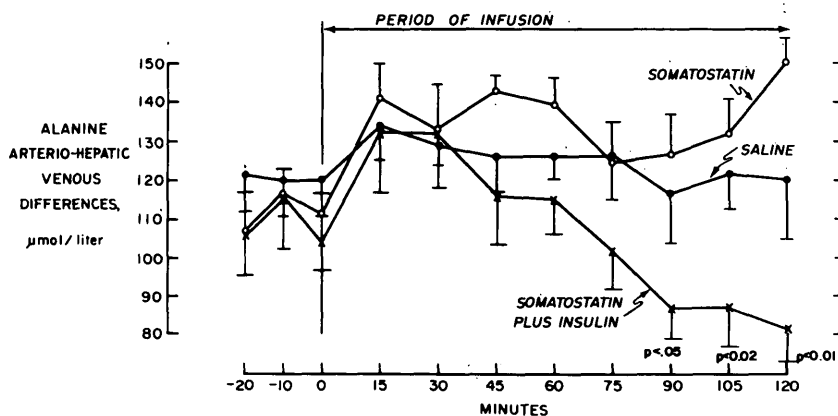


FIGURE 1. Arterio-hepatic venous differences of alanine in normal men before and during the infusion of saline, SRIF, and SRIF plus insulin. Values are means \pm SE of means.

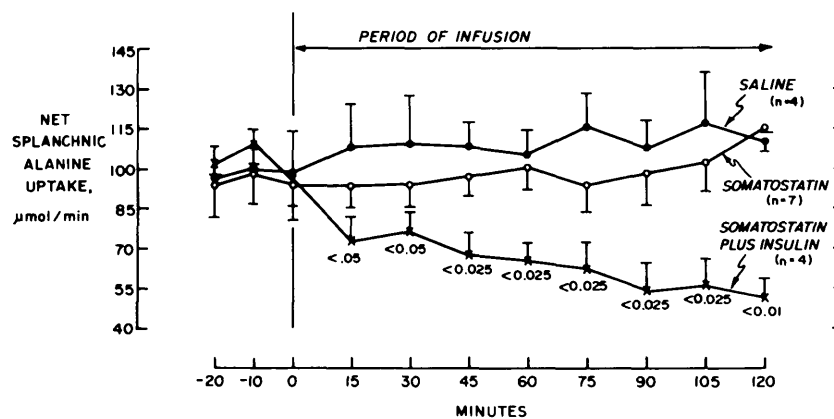


FIGURE 2. Net splanchnic alanine uptake in normal men before and during infusion of saline, SRIF, and SRIF plus insulin. Values are means \pm SE of means.

of selective glucagon lack (that is, during infusion of SRIF plus insulin): NSAU fell from 101 to 65 $\mu\text{mol}/\text{min}$ after the first hour and to 50 $\mu\text{mol}/\text{min}$ after the second hour. The fall in NSAU during the first hour was entirely consequent on a fall in EHPF, but during the second hour there was also a significant decrease in A-HV alanine differences.

Our results may appear at variance with earlier studies by Jennings et al.³ from this laboratory. When SRIF plus insulin were infused into intact dogs, arterial alanine levels remained stable and hepatic alanine uptake was unchanged over 60 min of observation.³

In the present studies, when selective glucagon deficiency was produced, a fall in A-HV alanine differences was observed only during the second hour of the experiment. These results are in keeping with studies in the dog and in man from several laboratories. Cherrington et al.⁴ infused SRIF plus insulin into conscious dogs for a 4-h period. After a delay of about 2 h, a rise in arterial alanine levels was observed that reached 100 $\mu\text{mol}/\text{L}$ above basal by the end of the study. Infusion of radiolabeled alanine was accompanied by a progressive rise in arterial ¹⁴C-alanine levels, suggesting that there was impaired removal of alanine under conditions of glucagon deficiency. Sacca et al.¹⁰ infused alanine into anesthetized dogs with and without SRIF. Alanine levels rose to significantly higher plasma concentrations in the presence of SRIF, results compatible with reduced alanine clearance in the near-absence of glucagon. Gerich et al.¹¹ observed that venous alanine levels rose sharply in a group of diabetic subjects at 4–6 h after the start of SRIF infusion. The hyperalaninemia was attributed to glucagon lack and was significantly less in the face of the endogenous hyperglucagonemia of the diabetic state. It appears therefore that the changes in alanine extraction associated with glucagon lack are not immediate but develop after 1–2 h of glucagonopenia.

It is of interest that a fall in NSAU was observed over a 2-h observation period under conditions of "isolated" glucagon deficiency (basal insulin levels present), whereas NSAU remained unchanged during combined deficiency of glucagon and insulin. The role of insulin in the regulation of hepatic alanine extraction remains unresolved. Chiasson et al.¹² infused insulin into dogs at either 1 or 5 mU/kg/min while euglycemia was maintained by variable infusion of exogenous glucose. Despite circulating insulin levels of 72 and 213 $\mu\text{U}/\text{ml}$, respectively, in these studies, NSAU remained unchanged. In addition, in unpublished studies in normal men fasted for 48 h, Chiasson et al. ob-

served that insulin was without effect on NSAU, again under conditions in which euglycemia was maintained by variable glucose infusion. Similarly, when Felig and Wahren¹³ infused glucose (2 mg/kg/min for 45 min) into normal men fasted overnight, thereby raising endogenous insulin from 8 to 16 $\mu\text{U}/\text{ml}$, net splanchnic alanine uptake remained unchanged. On the other hand, when these workers infused glucose at 25 mg/kg/min, plasma glucose levels rose to 292 mg/dl, arterial insulin levels rose to 50 $\mu\text{U}/\text{ml}$, and the A-HV alanine differences fell from 117 to 79 $\mu\text{mol}/\text{L}$.¹³ The same workers also reported a fall in NSAU after administering an oral glucose load to normal subjects, and they have concluded that insulin exerts an inhibitory effect on NSAU.¹⁴ These studies do not distinguish, however, between effects of insulin and possible independent effects of hyperglycemia. Although the potential role of insulin in the control of NSAU thus remains unclear, it appears from our studies that the attenuation in NSAU in the first 2 h of glucagon deficiency is demonstrable only in circumstances when basal insulin is present.

Although our data thus provide evidence that monotropic glucagon deficiency produces a fall in net splanchnic alanine extraction, there are certain reservations in the interpretation of these studies. Somatostatin is a useful tool for inhibiting endogenous insulin and glucagon release, but its infusion is associated with a consistent but variable fall in EHPF. In our studies, we in fact observed a consistent early increase in A-HV alanine differences during the first hour of infusion of SRIF alone or SRIF plus insulin (Figure 1). The increase of A-HV alanine may be triggered by a fall in EHPF and may provide a compensatory mechanism for the maintenance of NSAU. Therefore, it is possible that the fall in hepatic plasma flow may influence A-HV alanine concentrations' differences and, hence, NSAU independently of any change in pancreatic hormone secretion. These disadvantages of SRIF do not obtain in the dog where the agent appears to have only a minimal influence on EHPF. There is concordance between the results we obtained in man and those obtained by Sacca et al.¹⁰ and Cherrington et al.⁴ in the dog, and, taken together, the data are compatible with a role for glucagon in the maintenance of splanchnic extraction of alanine.

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REFERENCES

- ¹ Mallette, L. R., Exton, J. H., and Park, C. R.: Effects of glucagon on amino acid transport and utilization in the perfused rat liver. *J. Biol. Chem.* 244:5724-28, 1969.
- ² Chiasson, J-L., Liljenquist, J. E., Sinclair-Smith, B. C., and Lacy, W. W.: Gluconeogenesis from alanine in normal postabsorptive man. Intrahepatic stimulatory effect of glucagon. *Diabetes* 24:574-84, 1975.
- ³ Jennings, A. S., Cherrington, A. D., Liljenquist, J. E., Keller, U., Lacy, W. W., and Chiasson, J-L.: The roles of insulin and glucagon in the regulation of gluconeogenesis in the postabsorptive dog. *Diabetes* 26:847-56, 1977.
- ⁴ Cherrington, A. D., Lacy, W. W., and Chiasson, J-L.: Effect of glucagon on glucose production during insulin deficiency in the dog. *J. Clin. Invest.* 57:664-77, 1978.
- ⁵ Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R.: Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science (Wash., D.C.)* 179:77-79, 1973.
- ⁶ Wahren, J., Efendic, S., Luft, R., Hagenfeldt, L., Bjorkman, O., and Felig, P.: Influence of somatostatin on splanchnic glucose metabolism in postabsorptive and 60-hour fasted humans. *J. Clin. Invest.* 59:299-307, 1977.
- ⁷ Liljenquist, J. E., Mueller, G. L., Cherrington, A. D., Keller, U., Chiasson, J-L., Perry, J. M., Lacy, W. W., and Rabinowitz, D.: Evidence for an important role of glucagon in the regulation of hepatic glucose production in man. *J. Clin. Invest.* 59:369-74, 1977.
- ⁸ Andres, R., Swerdloff, R. S., Pozefsky, T., and Coleman, D.: Manual feedback technique for the control of blood glucose concentration. *In Automation in Analytical Chemistry*, Skeggs, L. J., Ed. New York, Mediad, 1977, pp. 486-91.
- ⁹ Leevy, C. M., Mendenhall, C. L., Lesko, W., and Howard, M. M.: Estimation of hepatic blood flow with indocyanine green. *J. Clin. Invest.* 41:1169-79, 1962.
- ¹⁰ Sacca, L., Trimarco, B., Perez, G., and Rengo, F.: Studies on the mechanism underlying the influence of alanine infusion on glucose dynamics in the dog. *Diabetes* 26:262-70, 1977.
- ¹¹ Gerich, J. E., Lorenzi, M., Bier, D. M., Schneider, V., Tsalikion, E., Karam, J. H., and Forsham, P. H.: Prevention of human diabetic ketoacidosis by somatostatin: evidence for an essential role for glucagon. *N. Engl. J. Med.* 292:985-89, 1975.
- ¹² Chiasson, J-L., Liljenquist, J. E., Finger, F. E., and Lacy, W. W.: Differential sensitivity of glycogenolysis and gluconeogenesis to insulin infusions in dogs. *Diabetes* 25:283-91, 1976.
- ¹³ Felig, P., and Wahren, J.: Influence of endogenous insulin secretion on splanchnic glucose and amino acid metabolism in man. *J. Clin. Invest.* 58:1702-11, 1971.
- ¹⁴ Felig, P., Wahren, J., and Hendler, R.: Influence of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. *Diabetes* 24:468-75, 1975.