Regulation of Serine Dehydratase Induction in Rat Liver[1] (36938)

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Previous studies have shown that the rat liver enzyme serine dehydratase (L-serine hydrolyase (E C 4.2.1.13)) can be induced by force feeding of enzymatically hydrolyzed casein (1, 2) and by administration of glucagon (1–3) to rats previously maintained on a protein-free diet. The level of enzyme is high in the alloxan diabetic rat (4, 5). Enzyme activity is low in pancreatectomized rats and is not increased by fasting, force feeding of amino acid (AA) or hydrocortisone (HC) administration (7, 8). Serine dehydratase (SDH) can be induced in the adrenalectomized rat by hydrocortisone (2, 4) glucagon (4) or amino acid intubation (2, 6). Hydrocortisone may potentiate the stimulation by glucagon or cAMP in the pancreatectomized rat (7, 9). Serine dehydratase induction can be prevented by glucose intubation by a mechanism independent of insulin (2, 4) and cAMP (10). Pituitary hormones seem to have no role in regulating serine dehydratase levels (9).

Data obtained utilizing inhibitors of RNA and protein synthesis (5) and also immunochemical isolation (2) indicate that the induction results from increased enzyme synthesis.

Our studies indicate that an intermediate factor(s) is responsible for the induction of SDH following AA intubation. Additional data suggests that some factor other than insulin is released in response to AA infusion which inhibits the glucagon but not the HC induction of the enzyme.

Materials and Methods. Male rats, 180–200 g, were obtained from Holtzman Co., Madison, WI. Animals were housed in light–dark regulated rooms but were fed *ad libitum* on a protein-free diet for 5 days before the experiment. Adrenalectomies were performed 20 hr before initiation of the experiment during which interval the animals were maintained on 0.9% NaCl in the drinking H_2O. All experiments were begun at the end of the light period (8:00 AM) at which time the food was removed. Glucagon (1 mg) and hydrocortisone (10 mg) were administered subcutaneously while intravascular infusion was via the corpus cavernosum of the penis. All animals not receiving amino acids (enzymatically hydrolyzed casein) were infused with a volume of 0.9% NaCl equal to the volume received by experimental animals. All animals except diabetic rats were adrenalectomized.

Enzyme analysis. After 8 hr, the animals were decapitated, the livers were removed and frozen. The livers were homogenized in 3 vol of buffer (0.1 *M* Tris, 0.2 *M* KCl, 0.001 *M* EDTA, 0.001 *M* dithiothreitol and 0.001 *M* pyridoxal phosphate) with a Polytron homogenizer. Enzyme assays were performed upon the supernatant after centrifugation at 105,000*g* for 60 min as previously described (11). Enzyme activity is expressed as micromoles of product formed per minute per gram of liver.

Isotope distribution. Liver samples were

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TABLE I. Inhibition of Glucagon Induction of SDH by Intravascular Infusion of Amino Acids.

Treatment	NAD (μmole/min/g liver ± SE)			
	No. rats	Expt I	No. rats	Expt II
Control	(3)	0.7 ± 0.2	(4)	2.4 ± 0.6
Glucagon (1 mg)	(3)	9.5 ± 0.8	(4)	13.1 ± 2.5
Glucagon + amino acids	(3)	3.7 ± 0.3[a]	(5)	3.5 ± 0.9[b]
Amino acids	(4)	1.0 ± 0.4[a]		—
Glucagon + histidine		—	(4)	2.7 ± 0.4[c]
Glucagon + NaCl		—	(4)	2.4 ± 0.5[c]

[a] Amino acids (50 mg) at 0 time and 2 hr, intravascularly.
[b] Amino acids (125 mg) at 0 time, intravascularly.
[c] Histidine and NaCl (125 mg) at 0 time, intravascularly.

homogenized in 10 vol of distilled H_2O and then placed in a boiling H_2O bath for 7 min. Aliquots of the supernatant were taken for radioactivity determinations. Serum and liver supernatants were counted by liquid scintillation in Scintisol.

Glucagon was purchased from Eli Lilly Co. and hydrocortisone acetate from Pfizer. Lactic dehydrogenase was from Calbiochem and NADH was from Sigma. α-methyl D-glucoside-methyl-^{14}C (12.3 mCi/mmole) was from Calatomic and *l*-amino cyclopentane-1-carboxylic acid-2-5^{3}H (cycloleucine) (81.3 mCi/mmole) was from New England Nuclear.

Results. Although previous experiments indicated that the oral intubation of AA induced SDH (1, 2), the administration of AA by intravascular infusion did not induce SDH. When the AA were infused in conjunction with glucagon the normal glucagon induction was inhibited by 60–80% (Table I). The HC induction of SDH was unaffected by AA infusion (Table II). The AA infusion was only antagonistic to the glucagon-mediated induction, thus the inhibition may be at the level of cAMP as glucagon but not HC acts via stimulation of cAMP (12, 13).

It has been reported that insulin inhibits the adenyl cyclase system (14, 15) and most of the AA except histidine are known to stimulate insulin release (16, 17), thus the possibility that insulin was the inhibitor was examined. Both histidine and NaCl (administered at concentrations similar to that of the AA mixtures) inhibited the glucagon induction (Table I) but had no effect on the HC induction (Table II) indicating that insulin was not the specific factor involved in inhibiting the glucagon induction. Infusion of physiological saline did not inhibit the induction.

In order to further test a possible inhibitory role for insulin, the effect of infusion was assayed in the diabetic rat. The normally high level of SDH in the diabetic rat was inhibited 50% within 20 hr of an initial infusion of AA. The blood glucose levels exceeded

TABLE II. Effect of Amino Acid Infusion on the Hydrocortisone Induction of SDH.

Treatment	NAD (μmole/min/g liver ± SE)			
	No. rats	Expt I	No. rats	Expt II
Control	(4)	0.7 ± 0.2	(3)	1.3 ± 0.2
Hydrocortisone (10 mg)	(4)	3.9 ± 0.5	(3)	3.5 ± 0.6
Hydrocortisone + amino acids[a]	(4)	3.8 ± 0.8	(3)	5.3 ± 1.0
Hydrocortisone + histidine[a]		—	(4)	3.8 ± 0.8
Hydrocortisone + NaCl[a]		—	(4)	4.6 ± 0.7

[a] Amino acids, histidine and NaCl = 125 mg at 0 time, intravascularly.

TABLE III. Effect of Amino Acid Infusion on SDH Levels in the Diabetic Rat.[a]

Treatment	No. rats	(μmole/min/g liver $\pm$ SE) Serine dehydratase	Glucokinase
Control	(5)	181 $\pm$ 14	0.6 $\pm$ 0.1
Amino acids[b]	(5)	99 $\pm$ 22	0.7 $\pm$ 0.1

[a] Rats were made diabetic by the intravenous administration of 10 mg of alloxan 7 days before sacrifice.

[b] Amino acids infused at 20 hr (60 mg) and 10 hr (100 mg) before sacrifice.

400 mg % in all rats and the glucokinase levels were extremely low, indicating that the animals were diabetic (Table III).

In addition, the role of insulin was further examined by measuring ^{3}H-cycloleucine and ^{14}C-methyl glucoside transport. As shown in Table IV the distribution of ^{3}H-cycloleucine between serum and liver is the same in both the glucagon- and glucagon plus AA-treated animals. The serum levels of ^{3}H-cycloleucine at 30 min and 8 hr after injection of glucagon were similar between the 2 groups. Hydrocortisone also enhanced the transport of ^{3}H-cycloleucine to the liver but again the AA infusion had no effect on the distribution.

Both glucagon and glucagon plus AA increased the rate of uptake of ^{14}C-methyl glucoside by the liver (Table V). The methyl glucoside was injected at the beginning of the experiment, thus the ratio between liver and serum is a measure of uptake by the liver rather than a change in the distribution equilibrium as was measured with the cycloleucine which had been allowed to equilibrate for 20 hr before the start of the experiment. The enhanced uptake of ^{14}C-methyl glucoside would suggest that insulin is being released at similar rates after both the glucagon and glucagon plus amino acid treatment. Similar differences were observed when the uptake of ^{14}C-methyl-*o*-glucose was measured after 3 hr. Preliminary evidence indicates that the level of immunoreactive insulin was not different between the glucagon and glucagon plus AA treatment (data not shown).

TABLE IV. Effect of Glucagon, Hydrocortisone and Amino Acids on ^{3}H-Cycloleucine Distribution.[a]

Treatment	No. rats	dpm/g liver / dpm/ml serum $\pm$ SE
Control	(4)	0.48 $\pm$ 0.08
Glucagon (1 mg)	(4)	1.63 $\pm$ 0.3
Glucagon + amino acids[b]	(4)	1.46 $\pm$ 0.2
Hydrocortisone (10 mg)	(4)	0.64 $\pm$ 0.08
Hydrocortisone + amino acids[b]	(4)	0.75 $\pm$ 0.06

[a] Animals sacrificed at 3 hr.

[b] 150 mg of amino acids (0.75 ml). Other animals received 0.75 ml of 0.9% NaCl, intravascularly.

Discussion. Although insulin inhibits the accumulation of cAMP (14, 15) and as such might be expected to inhibit the induction of SDH by glucagon, the available evidence suggests that this is not the case (4, 10). In the current study the evidence also weighs against insulin being implicated in the blocking of the glucagon induction of SDH in the liver of rats administered AA intravascularly. This conclusion follows from our observation that the glucagon induction of SDH was inhibited by (a) intravascular administration of 125 mg of either NaCl or the amino acid histidine, which reportedly does not stimulate insulin release (16, 17) and (b) the intravascular injection of AA to diabetic rats. Also the distribution of radioactively labeled, nonmetabolizable glucose and amino acid was the same in rats given only glucagon as in glucagon-treated rats injected intravascularly with AA. Glucagon alone has been reported to stimulate insulin release as effectively as either glucose or amino acids (18, 19), therefore, high serum levels of insulin would already be in circulation. It is of interest that intravascular administration of AA does not block the induction of SDH by HC. At present the factor(s) responsible for inhibiting glucagon but not HC induction of SDH in these studies is unknown, however, under similar experimental conditions, epinephrine was found to inhibit glucagon but not HC

TABLE V. Effect of Glucagon and Amino Acids on ^{14}C-Methyl Glucoside Uptake.

Treatment	dpm/g liver / dpm/ml serum			
	No. rats	30 min[a]	No. rats	60 min[a]
Control	(3)	0.53 ± 0.14	(3)	0.54 ± 0.15
Glucagon	(3)	0.89 ± 0.27	(3)	0.89 ± 0.25
Glucagon + amino acids[b]	(3)	0.82 ± 0.05	(3)	0.80 ± 0.10

[a] Animals sacrificed at 30 and 60 min.

[b] 150 mg of amino acids (0.75 ml). Other animals received 0.75 ml of 0.9% NaCl, intravascularly.

induction of SDH (20). Experiments are currently in progress which are aimed at clarifying what role, if any, epinephrine has in the inhibitory action of intravascularly injected amino acids on SDH induction in glucagon-treated rats.

Although the cycloleucine distribution between serum and liver was not affected by intravascular injection of AA when compared to glucagon plus 0.9% NaCl, the liver levels were significantly increased compared to controls which were injected intravascularly with an equal volume of 0.9% NaCl. The enhanced liver uptake by both glucagon- and HC-treated rats is comparable to that previously reported (21–23). It should be noted that the dose of amino acids infused is 10–20 times the total amino acid content normally circulating in blood. Thus the uptake of cycloleucine by the liver of animals receiving amino acids actually represents larger amounts of amino acids than animals receiving glucagon alone. Therefore, supplying large amounts of amino acids to the liver is not a sufficient stimulus to induce serine dehydratase and the induction observed after amino acid intubation must be mediated via an intermediate factor.

Intubation of AA in intact rats results in increased serum glucagon levels (16, 17, 24) and increased levels of cAMP (3) and SDH (1–3) in liver. In contrast, intubation of AA in pancreatectomized rats does not induce liver SDH (7, 8). When the above is considered along with the observation that protein ingestion stimulates release of pancreozymin (25) which in turn stimulates glucagon release (26), it appears unlikely that AA act directly to stimulate SDH induction. Although it is currently under investigation, the nature and the mechanism of action of the factor responsible for antagonizing glucagon induction is unknown at this time.

Summary. When amino acids are infused intravascularly, the enzyme, serine dehydratase, is not induced in liver of adrenalectomized rats as it is when amino acids are given orally. The amino acids appear not to act directly on the liver to induce the enzyme. In fact when injected intravascularly, they inhibit the normal glucagon induction of serine dehydratase but not that induced by hydrocortisone. Intravascular administration of either histidine or concentrated NaCl is inhibitory as are amino acids administered intravascularly to the diabetic rat. The uptake of amino acids and glucose by the liver of glucagon-treated rats after amino acid infusion as measured by distribution of ^{3}H-cycloleucine and ^{14}C-methyl glucoside is the same as in rats treated with glucagon only. Thus it appears that insulin is not the factor responsible for the inhibition of enzyme induction.

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