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Plasma Insulin, Glucagon, and Gut Glucagon-like Immunoreactivity during Experimentally Induced Hyperammonemia in Rats (40735)

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A number of reports have demonstrated alterations in intermediary metabolism during ammonia intoxication. Hyperammonemia from administration of ammonium salts to pigs (1) and rats (2), injection of crystalline jackbean urease in rats, or administration of urea to horses was accompanied by hyperglycemia (3–5). Schlienger *et al.* (6) reported that cirrhotic humans suffering from hepatoencephalopathy had increased arterial blood ammonia and decreased glucose tolerance. It has been postulated that alterations in carbohydrate metabolism seen during ammonia intoxication may be due to altered release of pancreatic and/or gut hormones. In this communication we are reporting circulating concentrations of insulin, glucagon, and gut glucagon-like immunoreactivity (GLI) in rats during acute hyperammonemia.

Materials and methods. Male Sprague–Dawley rats (270–370 g) individually housed under conditions of controlled lighting (12 hr light:dark cycle) and temperature (22 ± 1°C) were allowed free access to the American Institute of Nutrition reference diet (7) (Bio-Serv Inc., Frenchtown, N.J.) and tap water for at least 7 days prior to being used in an experiment.

Plasma sampling. Each animal was surgically fitted (8) with an indwelling jugular cannula 1–2 days prior to an experiment. One to two hours prior to experimental treatment each rat was placed into an individual cage covered by wire mesh. A piece of polyethelene tubing was passed through the wire mesh and connected to each jugular cannula. This allowed for remote collection of blood samples with minimal disturbance to the animals. At the time of sampling a 1.0-ml blood sample was withdrawn through the cannula into a heparinized syringe and transferred into a small test tube immersed in ice and containing 500 units of Trasylol (FBA Pharmaceuticals, New York, N.Y.). The plasma was separated by centrifugation and stored at −20°C until thawed for analyses.

Determination of plasma ammonia, glucose, insulin, glucagon, and GLI. Plasma ammonia was assayed according to Chaney and Marbach (9) and glucose using hexokinase and glucose 6-phosphate according to Bondar and Mead (10). Immunoreactive insulin, glucagon and GLI in plasma were quantified by radioimmunoassay. For the insulin assay, I^{125}-labeled porcine insulin (New England Nuclear, Boston, Mass.) served as radioactive tracer and guinea pig anti-porcine insulin (Research Products International, Elk Grove, Ill.) as the primary antiserum. The standard used was purified rat insulin (Lot No. 615-063-12), generously supplied by Dr. Mary Root, Eli Lilly and Company, Indianapolis, Indiana. Bound and free insulin were separated using goat anti-guinea pig IgG (Research Products International).

Glucagon was assayed according to the procedure of Faloona and Unger (11). I^{125}-Porcine glugacon was purchased from Nuclear Medical Supply (Dallas, Tex.). Purified porcine glucagon (Lot No. 258-V016-235; Dr. Mary Root, Eli Lilly and Co.) was used as the standard.

Gut glucagon-like immunoreactivity (GLI) was quantified in an assay done in parallel with the glucagon assay, with anti-glucagon serum k4023 (Dr. Lise G. Heding, Novo, Denmark) substituted for the 30K antiserum. Since antiserum 30K is specific for pancreatic glucagon (11) and antiserum k4023 binds both pancreatic glucagon and

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GLI (12), the GLI concentration was calculated from the difference in 30K glucagon and k4023 glucagon immunoreactivity in each sample (12, 13).

Experimental protocol. Hyperammonemia was produced in animals by a single intraperitoneal injection of 25 units /kg body wt of purified jackbean urease (Sigma, St. Louis, Mo.) dissolved in saline (0.9% NaCl). Each rat was injected within 2 (fed) or at 20 (fasted) hr following removal of feed. Control animals were injected with an equivalent volume of saline alone. Blood samples were collected from each animal at 0, .5, 1, 2, 3, and 4 hr following injection. All of the data were analyzed by Student's *t* test.

Results. The data in Fig. 1 show that fed and fasted rats had developed a sustained hyperammonemia within 30 min following urease injection. Plasma glucose was significantly ($P < 0.025$) elevated by 1 hr in fed rats and 2 hr in fasted rats (Fig. 1). In fasted rats plasma insulin concentrations in urease-treated rats were elevated at 30 min where they remained throughout the experiment (Fig. 2A). In the fed rats the increase in plasma insulin in response to urease was not as dramatic as in fasted rats, but there was a tendency for the insulin levels to remain elevated as compared to controls from 30 min to 3 hr following urease injection (Fig. 3A). There were no consistent changes in plasma glucagon or GLI in response to urease treatment in

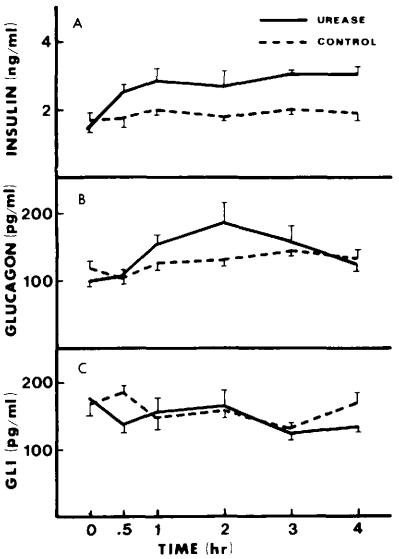

FIG. 2. Concentrations of insulin, glucagon, and GLI in plasma of fasted rats following an injection of urease or saline.

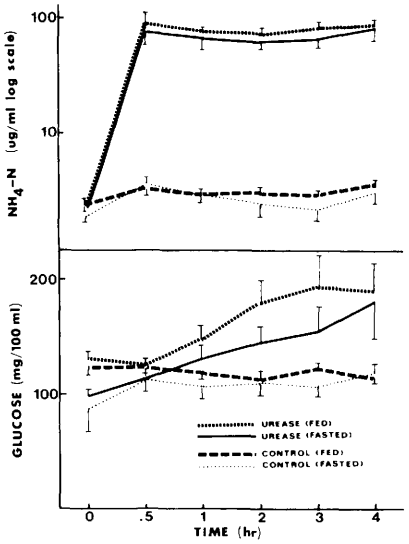

FIG. 1. Plasma concentrations of ammonia N and glucose in fed and fasted rats following an injection of urease or saline.

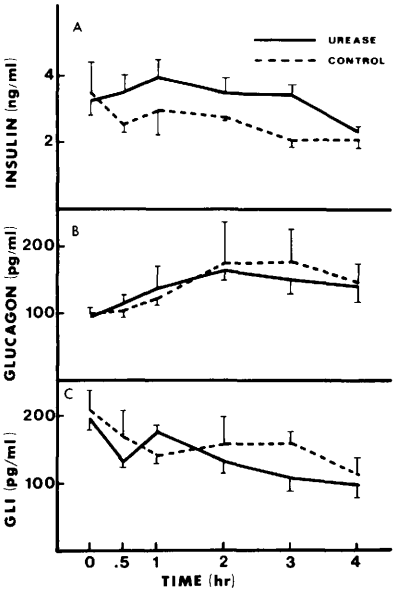

FIG. 3. Concentration of insulin, glucagon, and GLI in plasma of fed rats following an injection of urease or saline.

TABLE I. AVERAGE PLASMA CONCENTRATION (±SEM) OF AMMONIA, GLUCOSE, INSULIN, AND GLUCAGON AND GLI IN FED RATS FOLLOWING TREATMENT WITH UREASE OR SALINE[a]

	Treatment		Significance of treatment effect
	Saline	Urease	
Ammonia (μg/ml)	3.3 ± 0.1	83.4 ± 7.2	$P < 0.005$
Glucose (mg/100 ml)	118 ± 2	167 ± 17	$P < 0.025$
Insulin (ng/ml)	2.5 ± 0.1	3.3 ± 0.2	$P < 0.005$
Glucagon (pg/ml)	140 ± 23	143 ± 15	N.S.
GLI (pg/ml)	147 ± 14	124 ± 14	N.S.

[a] There were five animals in each treatment group.

either fed or fasted rats (Figs. 2B, 2C, 3B, and 3C). However, plasma glucagon levels were significantly ($P < 0.05$) elevated compared to controls at 2 hr following urease injection in fasted rats (Fig. 2).

When all of the data were averaged across all sampling times following urease or saline injection, urease-injected rats had significantly higher plasma ammonia, glucose, and insulin concentrations compared to controls in both the fed and fasted groups. However, urease injection caused no significant change in the average concentrations of plasma glucagon or GLI in either fed or fasted rats (Tables I and II).

Discussion. The present results in fed and fasted rats agree with earlier reports (3, 4) that urease injection produces hyperammonemia accompanied by hyperglycemia. Elevated blood glucose also followed hyperammonemia induced by injection of ammonium salts (1), portal stricture (14), and hepatic cirrhosis (15). Recently Eisenstein and Strack (16) observed that isolated rat islets released a single spike of glucagon in response to ammonia exposure. It has also been reported that ammonia infusions into intact or pancreatectomized dogs increased plasma glucagon (17). However, in the present study urease induced hyperammonemia did not consistently alter plasma concentrations of glucagon or gut GLI. Similarly, oral dosing of humans with ammonia increased plasma ammonia levels but failed to elevate circulating glucagon concentration (18). The reasons for the apparent lack of agreement in the results of these studies are unclear.

The elevated plasma insulin in both fed and fasted rats (Tables I and II) during prolonged hyperammonemia is similar to that observed following ammonia infusion in dogs (17). Interestingly it has been observed that insulin release in response to a variety of stimuli is inhibited by ammonia. Exposure of pancreatic tissue from golden hamsters (19) and isolated rat islets (20) to ammonia inhibited glucose stimulated insulin secretion. Similarly, ammonium acetate infusions suppressed glucose stimulated insulin release in humans (21) and rats (22). We have also observed that the ability of arginine to stimulate insulin release is decreased in hyperammonemic rats (23). *In vitro* insulin secretion stimulated by glyceraldehyde, Ba^{2+} and sulfonylurea was also inhibited by ammonia (20). Based upon such evidence it is tempting to speculate

TABLE II. AVERAGE PLASMA CONCENTRATIONS (±SEM) OF AMMONIA, GLUCOSE, INSULIN, GLUCAGON, AND GLI IN FASTED RATS FOLLOWING TREATMENT WITH UREASE OR SALINE[a]

	Treatment		Significance of treatment effect
	Saline	Urease	
Ammonia (μg/ml)	2.8 ± 0.2	71.0 ± 16.2	$P < 0.005$
Glucose (mg/100 ml)	110 ± 8	144 ± 17	$P < 0.05$
Insulin (ng/ml)	1.9 ± 0.1	2.8 ± 0.1	$P < 0.005$
Glucagon (pg/ml)	127 ± 6	146 ± 13	N.S.
GLI (pg/ml)	155 ± 6	144 ± 3	N.S.

[a] There were five animals in each treatment group.

that the elevated plasma insulin concentrations that we and others (17) observed during prolonged ammonia intoxication may be due not to stimulated release but rather a decrease in the metabolic clearance rate of the circulating hormone. Further research is needed to test this possibility.

It appears from the present study that the hyperglycemia accompanying ammonia intoxication is not a consequence of increased glucagon and GLI or decreased insulin release and is related to other factors. Insulin resistance has been noted in rats during hyperammonemia produced by urease treatment (3), ammonia infusion, and portal stricture (14). Similarly, in the present study hyperglycemia during ammonia intoxication occurred in spite of elevated plasma insulin.

Ammonia may also elevate blood glucose concentrations by inhibiting tricarboxylic acid cycle activity (24) or stimulating gluconeogenesis from lactate (25). Moreover, Prior *et al.* (3) observed that hyperammonemic rats had depleted stores of glycogen in brain, liver, and muscle tissue. Liver slices from normal rats incubated with ammonia showed an increased rate of glycogenolysis and glucose production (3). Adrenal and pituitary hormones may also contribute to elevated blood glucose during hyperammonemia. Adrenalectomy substantially reduced the hyperglycemic response to ammonia in rats (3) and the addition of ammonia to rat pituitary glands *in vitro* stimulated growth hormone release (26).

Summary. Fed and fasted rats injected with urease showed a dramatic and sustained elevation in plasma ammonia concentrations. Plasma glucose concentrations were also elevated following urease treatment in both fed and fasted rats. The increase in plasma ammonia following urease treatment was accompanied by a significant increase in the average plasma insulin concentrations in both groups. There was no consistent effect of ammonia intoxication on plasma glucagon or gut glucagon-like immunoreactivity.

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