structures that are more abundant in the prefrontal cortex and olfactory bulbs than in the striatum (compare the passive uptake values). Third, the properties of DA uptake receptor sites may be different in the limbic system than in the extrapyramidal system. This is consistent with the differential effects of certain neuroleptics on limbic and extrapyramidal DA activity as reported by others [2]. It is also consistent with the fact that cocaine inhibited DA uptake less effectively in the limbic system than did chilling (Fig. 1, panels B and C). If this latter mechanism contributed, even in part, to the present findings, then there may be important differences in DA neurotransmission in the various DA systems (i.e. a configurational difference in uptake sites in the respective carrier molecules). A search for such differences may lead us to better understand and treat "functional" and organic disorders related to DA activity.

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Hypoglycin toxicity: studies of ammonia metabolism

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Hypoglycin A, α-amino (methylenecyclopropyl) propionic acid, is an amino acid found in unripe ackee fruit and in the seeds of several varieties of maple trees [1, 2]. Ingestion in man causes Jamaican vomiting sickness, a disorder characterized by hypoglycemia [3], encephalopathy, small vacuole fatty degeneration of the viscera [4], and a distinctive organic aciduria dominated by acidic metabolites of hypoglycin and substrates of FAD-dependent dehydrogenases, e.g. glutarate, ethylmalonate and β-hydroxyisovalerate [5]. The precise biochemical mechanisms responsible for the *in vivo* blocks in gluconeogenesis and fatty acid metabolism are not clear. Hypoglycin A is metabolized by transamination and oxidative decarboxylation to (methylenecyclopropyl) acetyl-CoA (MCPA-CoA) [6]. The toxicity is due to simultaneous inhibition of several acyl-CoA dehydrogenases by MCPA-CoA [7, 8]. In addition, mitochondrial sequestration of carnitine and/or coenzyme A by MCPA and other fatty acids may contribute to the toxicity [9].

Pent-4-enoate is structurally related to MCPA, and administration to the rat causes hypoglycemia, hyperammonemia, encephalopathy and fatty degeneration of the liver [10], presumably due in part to inhibition of fatty acid oxidation by a metabolite, pent-2,4-dienoyl-CoA [11].

Jamaican vomiting sickness is also similar in many respects to Reye's syndrome, a human disorder of obscure etiology, in which hyperammonemia is common.

Similarities between Jamaican vomiting sickness, intoxication due to pent-4-enoate, and Reye's syndrome have prompted speculation of possible hyperammonemia in hypoglycin intoxication [12, 13]; however, direct measurement of blood ammonia in hypoglycin intoxication has not been reported.

Hypoglycin A was prepared from Jamaican ackee by the method of Kean [14]. It eluted from UR-30 anion exchange resin on a Beckman model 120 amino acid analyzer as a single peak which co-chromatographed with leucine and had a molecular weight of 141 by mass spectrometry. (Methylenecyclopropyl) acetic acid (MCPA) was prepared from the hypoglycin [6]; the trimethylsilyl (TMS)-ester chromatographed as a single peak (M.U. 11.72) by GC on OV-22 and had the appropriate molecular weight (184) and spectrum by combined gas chromatography-mass spectrometry.

Sprague–Dawley rats (approx. 150 g) were maintained on commercial rat chow. They were fasted but allowed free access to water beginning 15 hr prior to i.p. injection of

Table 1. Effect of hypoglycin i.p. on plasma ammonia concentration*

	Plasma ammonia (μg/100 ml)		
	2 hr	4 hr	24 hr
Control	75 ± 10 (3)	58 ± 15 (3)	67 ± 10 (8)
Hypoglycin	139 ± 23† (3)	118 ± 19† (3)	297 ± 62‡ (7)

* Values are expressed as means ± one standard deviation. The number of observations is indicated in parentheses.
† Significantly different from control ($P < 0.05$).
‡ Significantly different from control ($P < 0.01$).

Table 2. Urinary nitrogen, urea and orotic acid*

	Total nitrogen (mmoles)	Urea (mmoles N)	Orotic acid (μg/mg creatinine)
Control	10.5 ± 0.9 (4)	8.7 ± 0.3 (4)	13.4 ± 4.2 (5)
Hypoglycin	16.8 ± 3.7† (3)	12.8 ± 2.8† (3)	4.3 ± 1.4‡ (4)

* Urine was collected over the 24 hr following hypoglycin injection (100 mg/kg, i.p.). Values are expressed as means ± one standard deviation. The number of observations is indicated in parentheses.
† Significantly different from control ($P < 0.05$).
‡ Significantly different from control ($P < 0.01$).

Table 3. Citrulline synthesis by rat liver mitochondria*

	Citrulline synthesis (% control)		
Substrate (10^{-3} M)	Hypoglycin A (5×10^{-3} M)	MCPA (1×10^{-3} M)	MCPA (4×10^{-3} M)
Glutamate	116	30	5
Succinate		68	13

* Results are the mean of duplicate determinations expressed as the percentage of citrulline synthesis in mitochondria without added inhibitor. The reproducibility of the assay is shown in Ref. 17.

hypoglycin (100 mg/kg) [4] in 0.9% saline. Control animals were injected with saline. In each of three treated and three control rats, blood was taken from the tail under ether anesthesia 2, 4 and 24 hr after injection. In four other hypoglycin-treated rats and five controls, urine was collected for 24 hr over dry ice and blood was taken at 24 hr.

Plasma ammonia was measured in duplicate by a resin absorption method adapted to 0.1 ml [10]. The blood urea nitrogen (BUN) was measured using standard clinical chemical procedures. Urine total nitrogen and urea were measured as previously described [15]. Urine orotic acid was measured as described elsewhere [16].

Citrulline synthesis in rat liver mitochondria was measured as described previously [17]. The difference between the groups was analyzed by Student's *t*-test.

Hypoglycin-treated animals had elevated plasma ammonia levels at 2, 4 and 24 hr (Table 1); the hyperammonemia was greatest at 24 hr. Serum urea nitrogen was high in the hypoglycin-injected animals at 24 hr [72 ± 18 (S.D.) (seven rats)] compared to controls [19 ± 7 (eight rats)], $P < 0.01$.

Urine total nitrogen and urea were elevated significantly in hypoglycin-treated rats, and urinary orotic acid was reduced significantly (Table 2).

MCPA (1×10^{-3} M), but not hypoglycin, inhibited citrulline synthesis by rat liver mitochondria by about 70%. Inhibition was similar with both glutamate and succinate as oxidizable substrate (Table 3).

We have demonstrated the presence of hyperammonemia after hypoglycin injection. A similar abnormality may occur in patients with Jamaican vomiting sickness.

The cause of the observed hyperammonemia is not clear. Nitrogen turnover was increased but it seems unlikely that a 50% increase in nitrogen turnover would have led to hyperammonemia if the urea cycle had been intact. The increased nitrogen excretion may have been due to increased protein breakdown because of the inhibition of fatty acid oxidation. Hypoglycin is known to lead to a complex organic acidemia [18]. Hyperammonemia is found in patients with propionic, methylmalonic, isovaleric, glutaric and other organic acidemias [13]. Injection of propionic acid causes hyperammonemia, in amino acid loaded rats, that parallels a decrease in hepatic *N*-acetyl glutamate [13]. Pent-4-enoate, a structural analog of MCPA, also decreases *N*-acetyl glutamate in isolated rat hepatocytes [19]. However, the inhibition of citrulline synthesis in rat liver mitochondria by pent-4-enoate is qualitatively and quantitatively different from the effects of other organic acids [17]. The inhibition of citrulline synthesis in rat liver mitochondria by pent-4-enoate, but not that by propionate, is reversed when succinate is the oxidizable substrate [17]. MCPA is similar to propionate in this regard. Hypoglycin intoxication causes modest increases in plasma citrulline, suggesting some degree of impairment at the argininosuccinate synthetase step [12]; however, the lack of orotic aciduria suggests a major block at the carbamyl phosphate

synthetase step. This would be consistent with the proposal that many organic acids may inhibit ureagenesis by decreasing acetyl-CoA, thus decreasing *N*-acetyl glutamate synthesis. It is also possible MCPA or MCPA-CoA directly inhibits carbamyl phosphate synthetase.

In summary, the effects of hypoglycin A (100 mg/kg) on nitrogen metabolism were studied in rats. Hypoglycin administration caused hyperammonemia. Urine total nitrogen and urea were increased in the hypoglycin-treated rats. Urine orotic acid was significantly lower. Citrulline synthesis in rat liver mitochondria was inhibited by (methylenecyclopropyl) acetic acid, a metabolite of hypoglycin.

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