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Letter to the Editor: Amino Acid Metabolism in Dysmature Newborn Rats—Possible Explanation for the Antihypoglycemic Effect of Prenatal Glucocorticoids

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The animal model of the uteroplacental insufficiency state described originally by Wigglesworth (4) has enabled investigators to study small for gestational age or dysmature progeny with specific emphasis on glucose homeostasis. We have previously shown that 91% of dysmature rat pups develop hypoglycemia 2-4 hr after birth and that prenatal glucocorticoid treatment significantly reduces this tendency (2). Our subsequent investigations have included an examination of the possible relationship between the protective effect of steroid hormones and the availability or utilization of gluconeogenic substrates.

Pregnant Sprague-Dawley rats with accurately timed gestations (within 12 hr) were utilized for this study. Under ether anesthesia, a low midline laparotomy was performed on the 17th-19th day after mating, and the appropriate surgical procedure was carried out as described in detail earlier (2); thus, vascular supply to one uterine horn was compromised with a ligature of silk suture (dysmature group), while the opposite horn was left intact, these animals serving as controls. At term (22 days of gestation), delivery by cesarian section was performed, and the rat pups were weighed immediately on a Mettler PT 1200 electronic balance. A reduction in weight of more than 15% compared to the mean of control littermates was considered evidence of intrauterine growth retardation (IUGR) on the ligated side. The experimental groups of rat pups with IUGR included the following. *Group 1* (newborns at birth) was comprised of fetally malnourished pups which were killed as soon as possible (within 1-5 min) after birth; blood from severed neck vessels for determination of plasma amino acid patterns was sampled before death. *Group 2* (nutritionally deprived newborns) was comprised of dysmature pups which were maintained without nutritional support for 2-4 hr at an environmental temperature of 32°, with sampling of blood carried out during this interval. The weight of these animals was noted at birth and at the time of death. Experimental conditions for *group 3* (glucocorticoid-treated animals) were as follows. The dams of these dysmature progeny received an intramuscular injection of 9-α-fluoroprenisolone acetate (1 mg/kg) 24 and 48 hr before delivery; these small for gestational age animals of *group 3* were then divided into two subgroups—one killed at birth, with sampling of blood as noted previously, and the other maintained under conditions of starvation similar to *group 2*, with blood sampling performed and the pups killed at 2-4 hr of age. Blood samples were collected in heparin-lithium fluoride tubes and were rapidly centrifuged. The plasma was then utilized for glucose and aminogram determinations using a Beckman ERA 2001 glucose analyzer and a Beckman 120M amino acid analyzer, respectively.

Table 1. *Plasma aminograms of control and intrauterine growth-retarded rat pups*[1]

Amino acid	Controls at birth (n = 15)	IUGR at birth (n = 9)	IUGR at 24 hr (n = 4)	IUGR + steroid at birth (n = 6)	IUGR + steroid at 24 hr (n = 10)
Alanine	0.51 ± 0.03	0.77 ± 0.10	0.34 ± 0.14	2.31 ± 0.34[2]	1.07 ± 0.26[2]
Valine	0.35 ± 0.01	0.37 ± 0.07	0.07 ± 0.02	0.47 ± 0.05	0.24 ± 0.05
Glycine	0.24 ± 0.09	0.38 ± 0.03	0.25 ± 0.03	0.43 ± 0.032	0.41 ± 0.07
Isoleucine	0.13 ± 0.004	0.16 ± 0.009	0.04 ± 0.005	0.13 ± 0.02	0.09 ± 0.02
Leucine	0.27 ± 0.007	0.34 ± 0.02	0.06 ± 0.01	0.34 ± 0.03	0.19 ± 0.05
Proline	0.21 ± 0.01	0.43 ± 0.04	0.07 ± 0.04	0.40 ± 0.05	0.38 ± 0.10
Threonine	0.33 ± 0.009	0.49 ± 0.03	0.11 ± 0.02	0.36 ± 0.03	0.16 ± 0.03
Serine	0.25 ± 0.08	0.35 ± 0.02	0.11 ± 0.01	0.41 ± 0.05	0.18 ± 0.03
Aspartic acid	0.023 ± 0.003	0.006 ± 0.002	0.022 ± 0.003	0.002 ± 0.006	0.019 ± 0.003
Methionine	0.10 ± 0.003	0.13 ± 0.01	0.06 ± 0.009	0.12 ± 0.01	0.08 ± 0.03
Phenylalanine	0.15 ± 0.008	0.24 ± 0.02	0.06 ± 0.004	0.36 ± 0.001	0.13 ± 0.03
Glutamic acid	0.14 ± 0.01	0.19 ± 0.01	0.10 ± 0.02	0.25 ± 0.03	0.13 ± 0.02
Tyrosine	0.16 ± 0.04	0.25 ± 0.04	0.12 ± 0.07	0.24 ± 0.03	0.25 ± 0.04
Lysine	0.93 ± 0.06	1.21 ± 0.11	0.24 ± 0.09	1.37 ± 0.11	0.58 ± 0.15
Histidine	0.05 ± 0.07	0.04 ± 0.05	0.07 ± 0.06	0.08 ± 0.09	0.03 ± 0.006
Total amino acid	4.06 ± 0.15	5.69 ± 0.28	1.81 ± 0.25	7.46 ± 0.58[2]	4.30 ± 0.86[2]
GNG amino acid	1.33 ± 0.04	1.95 ± 0.12	0.74 ± 0.22	3.62 ± 0.43[2]	2.26 ± 0.47[2]

[1] Mean values ± SE are shown in micromoles per ml. IUGR = intrauterine growth retardation; GNG = gluconeogenic.
[2] $P < 0.01$, as compared to the untreated IUGR group of corresponding age.

Plasma amino acid profiles determined at birth and at 2-4 hr of age in untreated and treated dysmature pups are shown in Table 1. As reported by Roux and Jahcan (3), both groups of

IUGR pups showed significantly higher total plasma amino acid levels at birth, compared to control pups of normal body size, with particular increases in gluconeogenic amino acids (alanine, glycine, proline, and valine). A fall to low levels of circulating amino acids was observed at 2–4 hr in the untreated progeny, coincident with the development of hypoglycemia. The group administered corticosteroids prenatally had more pronounced increases of plasma gluconeogenic amino acids than untreated animals, with alanine especially elevated. Despite 3-fold greater apparent utilization in animals administered glucocorticoid prenatally, high alanine levels were maintained in the treated dysmature group; a similar pattern was noted for glutamine-glutamate. In addition, an inverse correlation between plasma glucose at birth and plasma alanine levels was noted for all animals ($r = -0.55, P = 0.01$), as well as an inverse correlation between plasma glucose and the four gluconeogenic amino acids ($r = -0.62, P = 0.001$).

Thus, the reduced tendency to develop hypoglycemia in corticosteroid-treated dysmature rat pups seems to be associated with both greater availability and increased utilization of glucogeneic amino acids. We therefore postulate that the hormone may act both to increase the supply of gluconeogenic substrate and to stimulate enzymes essential for gluconeogenesis.

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Arginine Esterase and Lysosomal Hydrolases in Liver from Cystic Fibrosis Subjects*

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Summary

The total activity and isoelectric focusing patterns of arginine esterase, cathepsin B_1, and several lysosomal hydrolases were normal in liver from two patients with cystic fibrosis. No abnormalities were observed in values for pH optimum, K_m, and V_{max} for arginine esterase and cathepsin B_1 in liver from cystic fibrosis patients compared to those values for liver from the control subject. Soybean trypsin inhibitor at concentrations up to 100 μg/ml had no effect on liver arginine esterase or cathepsin B_1.

Speculation

The data suggest that none of the enzymes studied in this work is directly related to the genetic defect in cystic fibrosis. Liver arginine esterase is different from the major arginine esterase found in plasma and saliva but may be identical to the minor component. The reported deficiency (12–15) of the major plasma arginine esterase may be a secondary phenomenon resulting from inadequate exocrine (chiefly pancreatic) secretion.

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