Pediat. Res. 7: 184–191 (1973)

Alanine
amino acids
diabetes mellitus
glucagon
gluconeogenesis
low birth weight
newborn

The Effect of Intravenous Glucagon on Plasma Amino Acids in the Newborn

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Extract

The relationship between glucagon stimulation at a pharmacologic dose level (300 μg/kg) and amino acids in systemic venous plasma was examined in four groups of newborn infants. Full term infants (FT), (mean age, 12.5 hr), infants of diabetic mothers (IDM), (mean age, 3.33 hr), infants who were small for gestational age (SGA), (mean age, 7.5 hr), were studied on the day of birth either before, or 4 hr after the first feeding; FT infants were studied on the 3rd day of life (mean age, 80 hr), 4 hr after feeding.

The mean total amino acid concentration in venous plasma on the day of birth is 2,999 ± 147 μmol/liter in FT infants, 2,952 ± 536 μmol/liter in IDM infants, and 1,867 ± 320 μmol/liter in the SGA group ($P < 0.005$ compared with FT group). Since plasma amino acid levels normally fall rapidly, the level in the IDM group is lower than expected for the postnatal age of this group on the day of birth. Several amino acids, notably, glutamine plus asparagine, and glycine contribute to the very low total amino acid level in SGA infants. Total amino acid concentration in FT infants falls to 2,444 ± 395 μmol/liter by the 3rd day of life. Two important glucogenic amino acids, glycine and alanine, account for half of the net decline, whereas the branched chain amino acids show a net rise in plasma by the 3rd day.

Glucagon infusion produces a hypoaminoacidemic response at 30 min on the 3rd day of life, a reaction which resembles that of the adult. Glutamine plus asparagine (absolute change (Δ), −165 μmol/liter), glycine (Δ, −55 μmol/liter), alanine (Δ, −45 μmol/liter), and proline (Δ, −40 μmol/liter), the amino acids with the most important gluconeogenic functions in liver and kidney, account for about 60% of the total net decline in the total plasma amino acid level.

The response to glucagon is attenuated on the day of birth in the FT infant. The IDM group is insensitive to glucagon, whereas the response in the SGA group mimics FT infants.

Blood glucose on the day of birth is depressed in IDM (20.2 ± 4.4 mg/100 ml) and SGA (28.6 ± 3.5 mg/100 ml) infants compared with full term infants (45 ± 4.7 mg/100 ml). Basal serum insulin on the day of birth is elevated fivefold above FT values (2.0 ± 0.77 μU/ml) in IDM and SGA groups. All groups have an insulinemic response to glucagon (Δ, > + 23 μU/ml) and the peak insulin response is highest in the SGA group. The basal level of serum growth hormone is elevated in the SGA group on the day of birth (51.9 ± 15.6 ng/ml) compared with full term infants (15.1 ± 4.1 ng/ml). All groups show a growth hormone (HGH) response to glucagon.

Speculation

On the day of birth, mechanisms which protect the fetus from hypoglycemia *in utero* (maternal glucose supply and high growth hormone levels) are withdrawn, and splanchnic glucogenic mechanisms should become activated. The apparent inability of the neonate to extract the important gluconeogenic amino acids, alanine and proline, from plasma after a glucagon stimulus on the 1st day of life may account for the susceptibility to hypoglycemia after birth when added stresses exist, as in the infant who is small for gestational age or born to a diabetic mother.

Introduction

The factors contributing to the instability of blood glucose levels in the postnatal period are not fully understood. Whereas the availability of liver glycogen stores to support euglycemia at this time of life has been extensively investigated, the ability of the neonate to utilize amino acids as substrates for hepatic gluconeogenesis has received relatively little attention. The glucogenic potential of amino acids in the adult has become a topic of major interest [14, 16], and we chose to investigate this area of metabolic interlock in the newborn infant. The concentration of individual amino acids in the plasma was examined on the day of birth, when hypoglycemia is more common, and on the 3rd day of life when control of blood glucose has usually been achieved. The hypoaminoacidemic response to a single injection of glucagon on the day of birth was studied in full term infants, infants born to mothers with diabetes mellitus, and infants who were small for gestational age. The response was compared with that observed in full term infants in the 3rd day after birth. The amino acid response to glucagon is blunted on the day of birth, particularly in infants prone to hypoglycemia.

Methods

The nature of the investigation was explained to each mother, and babies were studied only after informed consent was obtained. The study was approved by the ethics committees of the hospital.

The subjects included 13 full term infants of normal birth weight between 4 and 7 hr of age (day of birth group); 7 full term infants of normal birth weight between 75 and 86 hr of age (3rd day group); 6 infants between 2 and 6 hr of age who were referred immediately after birth because their mothers had diabetes mellitus and hyperglycemia during pregnancy (3 insulin dependent, 3 gestational) (IDM group); and 6 infants whose birth weights were less than 1,950 g at birth after more than 36 weeks gestation, so that their weight was at least 20% below the mean value predicted from the Lubchenko growth charts. The infants in this group were between 3 and 19 hr old at the time of investigation (SGA group). Pertinent biographic data on the four groups of infants are summarized in Table I.

The feeding regimes for the four groups were as follows. Full term infants on the 1st day of life were offered 5% glucose and water for the first feeding at not later than 12 hr of age. Full term infants on the 3rd day were fed on evaporated milk formula or breast milk every 4 hr. The IDM group were all studied before the first feeding as were six of the seven SGA infants. All studies were performed either before the first feeding or 4 hr after a feeding.

Six of the full term, normal infants and all of the other infants received a single dose of glucagon (300 μg/kg) administered intravenously on the day of birth at the time of the first amino acid analysis. Blood was drawn at zero time and at 2, 5, 15, 30, 45, and 60 min after the injection for determination of blood glucose, insulin, and growth hormone. One milliliter or less of heparinized systemic venous blood was obtained at 30 min after glucagon injection for amino acid analysis.

Suitable precautions were taken to prevent artifacts which can change amino acid concentration during preparation and analysis of the sample [29]. The plasma was immediately separated from the cells by centrifugation and deproteinized with 5 volumes of cold, sulfosalicylic acid (3% w/v). The supernatant was recovered by centrifugation at 10,000 $\times$ *g*, for 10 min at 4°. The deproteinized plasma was applied to the resin columns of a Beckman/Spinco amino acid analyzer [33], modified for simultaneous analysis of small volume samples on "acidic" and "basic" columns [30]. The error of analysis by this method is not more than $\pm$3% for each amino acid. No attempt was made to determine glutamine and asparagine separately. As-

Table I. Subject groups, relevant data

Infants	Range of gestational age, wk	Range of birth weights, g	Mean age at time of investigation, hr[1]	Plasma levels (mean ± SEM)				
				Basal glucose, mg/100 ml	Insulin, μM/ml		Growth hormone, ng/ml	
					Basal	Peak	Basal	Peak
Full-term, *day 1*	38–42	2,950–3,650	12.5 (4.5–16.5)	45 ± 4.7	2.0 ± 0.77	30.8 ± 14.6	15.1 ± 4.1	151 ± 19.4
Full term, *day 3*	38–42	2,720–3,990	80 (75–86)	47 ± 3.5	1.6 ± 0.9	25.1 ± 2.2	7.4 ± 1.0	23.7 ± 6.3
IDM[2]	33–38	2,065–3,400	3.33 (2–5)	20.2 ± 4.4	45 ± 19.6	83.8 ± 15.8	11.3 ± 3.17	85 ± 21.9
Small for gestational age	35–40	1,190–1,956	7.5 (3–19)	28.6 ± 3.5	10.8 ± 3.9	167 ± 71.1	51.9 ± 15.6	175 ± 35.5

[1] The range of ages is shown within parentheses.
[2] Infants of diabetic mothers.

paragine contributes approximately 10% of the ninhydrin-reacting material in the combined peak [4]. "Asparagine plus glutamine" in the text refers to the integrated glutamine-asparagine peak on the elution chromatogram.

Immunoreactive insulin (IRI) and growth hormone were assayed in plasma by methods described previously [18]. Sera from infants of insulin-dependent mothers were assayed following precipitation of maternal anti-insulin antibodies with alcohol by the method of Heding [17]; the insulin values in these patients represent the "free" insulin. Glucose was determined by the glucose oxidase method on whole blood.

Results

Plasma Amino Acid Concentration on Day of Birth

The mean total concentration of amino acids in plasma of normal full term infants and of infants born to diabetic mothers was not significantly different in the two groups (2,999 ± 147 μmol/liter, and 2,953 ± 536 μmol/liter, respectively). The corresponding value for the SGA group was significantly lower (1,867 ± 320 μmol/liter, $P < 0.005$).

There were few significant differences in the concentration of the individual plasma amino acids of the IDM and full term infant groups; taurine and threonine were elevated, and glycine was low in the former (Table II). On the other hand, the concentration of many amino acids was significantly lower in the SGA group when compared with the typical normal, full term infant; significant differences at the 0.05 level or lower were found for serine, asparagine plus glutamine, glycine, methionine, isoleucine, and histidine (Table II).

Plasma Amino Acid Concentrations in Full Term Infants on Day of Birth Compared with 3rd Day of Life

The total amino acid concentration in plasma falls about 18% between the day of birth (2,999 ± 147 μmol/liter, mean ± SD) and the third day of life (2,444 ± 395 μmol/liter). There is a statistically significant difference in the concentration of several individual amino acids at the two different ages after birth (Fig. 1). Glycine and alanine together account for about half of the net decline in total amino acid concentration; these two substances are important for gluconeogenesis. There is also a slight or modest increase in the concentration of several amino acids in plasma (Fig. 1); the increase of the branched chain amino acid, leucine, is statistically significant.

Effect of Glucagon Administration on Plasma Amino Acids in Full Term Normal Infants under 24 Hr of Age

Glucagon significantly lowers the plasma concentration of many amino acids in normal full term infants on the day of birth (Fig. 2). The quantitative response is greatest for glutamine plus asparagine and glycine. Alanine and proline are quite unresponsive to glucagon administration on the 1st day of life in this group of infants. The diminution in the plasma concentration of the branched chain amino acids is small by comparison, but statistically significant ($P < 0.01$). Because insulin normally modulates the level of branched chain amino acids in plasma, this response in the neonate could reflect an insulin effect, secondary to the glucagon infusion.

Effect of Glucagon Administration on Plasma Amino Acids in SGA Infants on Day of Birth

Although the initial plasma amino acid concentration of the SGA infant is generally lower than in the normal full term infant (Table I), the SGA group can still respond to a pharmacologic dose of glucagon. The plasma level of many amino acids falls after glucagon infusion (Fig. 2); the pattern of the response resembles that of the full term normal infant; the quantitative changes are less, however, than in the control group.

Table II. Plasma amino acid concentrations on day of birth, μmol/liter[1]

	Full term (n = 13)[2] mean ± SD	SGA (n = 6)[3] mean ± SD	P[5]	IDM (n = 6)[4] mean ± SD	P[5]
Taurine	124 ± 42	135 ± 38	NS	238 ± 65	<0.001
Threonine	171 ± 63	135 ± 65	NS	299 ± 113	<0.01
Serine	177 ± 65	107 ± 49	<0.05	192 ± 71	NS
Glutamine/asparagine	710 ± 154	421 ± 120	<0.001	571 ± 137	NS
Proline	181 ± 46	155 ± 35	NS	200 ± 39	NS
Glutamic	81 ± 34	97 ± 32	NS	72 ± 28	NS
Glycine	340 ± 94	205 ± 56	<0.005	262 ± 32	<0.01
Alanine	355 ± 75	296 ± 92	NS	292 ± 49	NS
Valine	116 ± 35	85 ± 46	NS	131 ± 31	NS
Cystine	115 ± 23	96 ± 34	NS	97 ± 32	NS
Methionine	30 ± 8	19 ± 7	<0.02	27 ± 5	NS
Isoleucine	33 ± 11	21 ± 7	<0.05	35 ± 14	NS
Leucine	62 ± 19	57 ± 28	NS	64 ± 16	NS
Tyrosine	55 ± 14	51 ± 24	NS	76 ± 24	NS
Phenylalanine	65 ± 12	53 ± 26	NS	66 ± 7	NS
Ornithine	64 ± 18	53 ± 36	NS	69 ± 32	NS
Lysine	193 ± 58	155 ± 81	NS	243 ± 15	NS
Histidine	85 ± 26	57 ± 25	<0.05	102 ± 25	NS
Arginine	51 ± 14	41 ± 21	NS	40 ± 19	NS
Total	2,999 ± 147	1,867 ± 320	<0.005	2,952 ± 536	NS

[1] SGA: small for gestational age; IDM: infants of diabetic mothers; NS: not significant.
[2] Full term normal infants, 4–17 hr after birth (mean age, 12.5 hr).
[3] Infants who are small for gestational age, 3–19 hr after birth (mean age, 7.5 hr).
[4] Infants born to diabetic mothers, 2–6 hr after birth (mean age, 3.33 hr).
[5] P values calculated by Student's t test, using the full term infant group as control subjects.

Effect of Glucagon on Plasma Amino Acids in IDM Patient Group on Day of Birth

The plasma concentration of most amino acids was not significantly altered by glucagon infusion in the infants born to diabetic mothers, in contrast to the response from the other two groups of infants (Fig. 2).

Effect of Glucagon Administration on Plasma Amino Acids of Normal, Full Term Infants on 3rd Day of Life

Glucagon infusion provoked a greater fall of plasma amino acids on the 3rd day of life than on the day of birth (Fig. 2). The drop in the plasma level of alanine and proline is significant at this age and it resembles the adult response to glucagon [23].

Hormone Response to Glucagon Infusion

The preinfusion plasma levels of insulin and growth hormone were higher on the day of birth in the IDM and SGA infant groups when compared with the full term normal group (Table I). Glucagon infusion stimulates an abrupt insulin release on the day of birth in all infants; the peak occurred between 2 and 5 min after glucagon administration in all subjects. The peak was lowest in the normal full term group, in whom the hypoaminoacidemic response for some of the glucogenic amino acids was blunted, and in whom the initial plasma IRI level was lowest; plasma levels of HGH rose after glucagon infusion in all groups. The greatest absolute rise (Δ) of HGH was observed 45 min after glucagon and occurred in the full term normal group on the day of birth; the smallest rise was found in the same type of infant on the 3rd day of life. Blood glucose levels were significantly depressed before glucagon, in IDM and SGA groups. The absolute rise after glucagon was not significantly different in the four groups, but the peak glucose level, 30–45 min after glucagon, was significantly lower in IDM and SGA groups [27].

Discussion

The mechanism causing postnatal blood glucose instability holds continuing interest. Long standing observations in the newborn reveal that capillary blood glucose falls in the initial hours after birth [8, 11], and that the rise in blood glucose after glucagon, on the first day of life, is less than in the older neonate

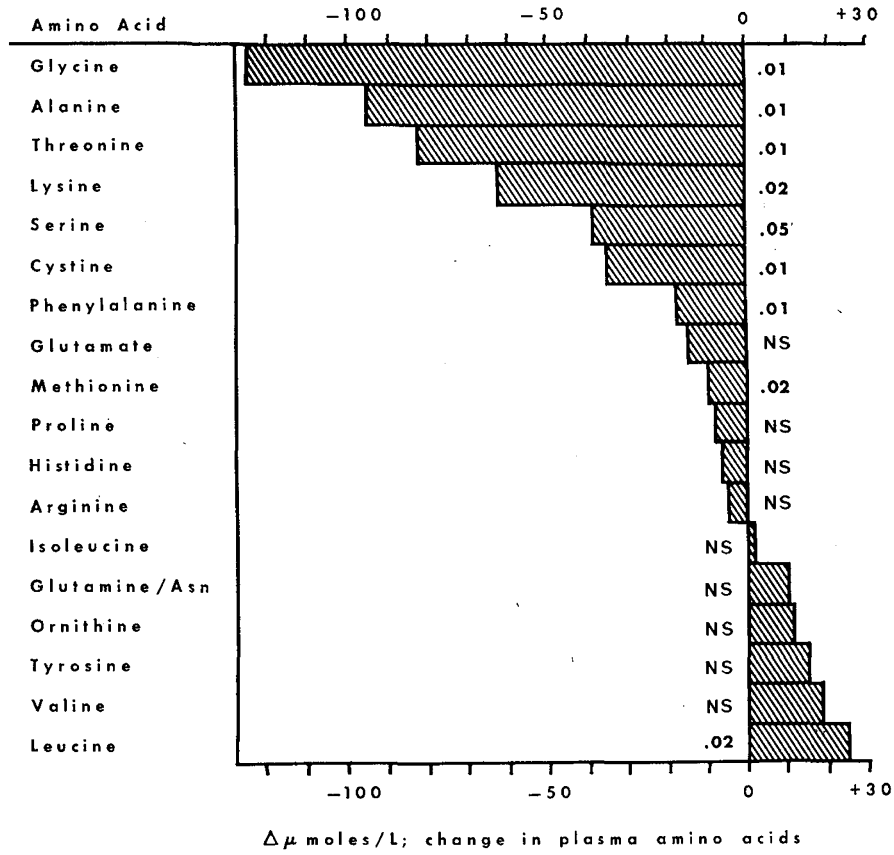

Fig. 1. Change in concentration of individual amino acids in systemic venous plasma of full term normal infants between the day of birth (13 infants) and the 3rd day of life (a separate group of 7 infants). Hatched horizontal bars represent the difference (absolute change (Δ) in micromoles per liter of venous plasma) between the mean values in the two groups. A negative value indicates a fall in concentration; a positive value means a rise. The statistical significance of the change assessed by Student's *t* test is indicated by the *P* value shown against the bar.

subject [25]. Our own studies [27] show that endogenous circulating levels of venous plasma insulin are similar in full term infants under 24 hr of age and on the 3rd day of life (Table I). After pharmacologic doses of glucagon, the rise in venous plasma glucose, free fatty acids, and insulin is not significantly different on the 3rd day of life and on the day of birth, but basal and peak levels of glucose are significantly lower in the IDM and SGA groups. On the other hand, growth hormone levels in venous plasma are initially higher and rise more after glucagon stimulation in the under-24-hr age group. These observations indicate that the human infant has numerous protective mechanisms to facilitate release of glycogen stores and glucose utilization in the first 24 hr of life. Nevertheless, the newborn infant often manifests hypoglycemia. For this reason, we thought it pertinent to examine amino acids in venous plasma to determine whether these substrates for glucogenesis are used differently in the initial hours after birth.

Our observations on systemic venous plasma amino acids in full term normal infants on the day of birth are similar to those published in the literature [9, 19].

The decline in the plasma content of total and many individual plasma amino acids, between the day of birth and the 3rd day of life, corresponds to the findings of others [10, 19]. The natural trend toward a transient, relative hypoaminoacidemia immediately after birth probably reflects a number of physiologic factors including utilization of the augmented plasma amino levels [7] with which the neonate is normally endowed by virtue of the fetal-maternal transplacental gradient [6, 20], the low intake of protein and total calories which is characteristic of the first days of life, and an effect of acute starvation [2, 15].

There is no difference in the total plasma amino

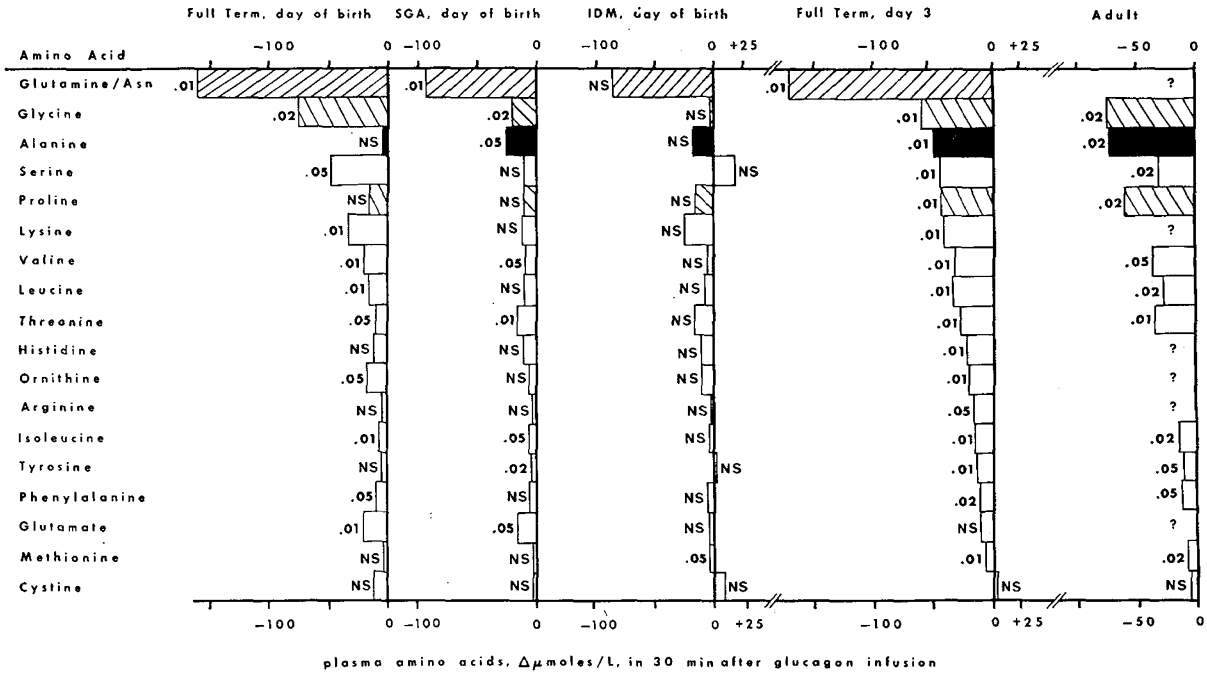

Fig. 2. Change in concentration of individual amino acids, in systemic venous plasma of infants before and 30 min after intravenous glucagon infusion (300 μg/kg). A negative value indicates a fall in amino acid concentration (absolute change (Δ) in micromoles per liter of plasma) after glucagon administration. Bar graphs show the mean values for six full term infants between 4 and 17 hr of age on the day of birth; seven full term infants on the 3rd day of life; six infants in the infants of diabetic mothers (*IDM*) group between 2 and 6 hr of age on the day of birth, and six infants in the small-for-gestational-age (*SGA*) group, between 3 and 19 hr of age on the day of birth. The comparable values for adults are taken from the data of Felig *et al.* [12]; amino acids indicated by a question mark were not reported in the latter study. Statistical significance of response is indicated. The four amino acids indicated by shading account for 75% of the hypoaminoacidemic response to glucagon in the adult. It is believed that glucagon promotes extraction of glycine and proline mainly by kidney, alanine mainly by liver, and glutamine by both organs.

acid concentration, and little difference except for taurine and threonine, in the IDM group when compared with the full term normal infants who were studied at an older mean age. However, because plasma amino acid levels are falling rapidly on the day of birth [19], the levels in the IDM group are actually lower than anticipated for infants of early postnatal age. When SGA infants are compared with normal infants it is apparent that SGA infants have depressed total amino acid concentration in plasma, and this finding is even more marked if the age factor is considered; the hypoaminoacidemia is accounted for by markedly low levels of several individual amino acids including glutamine plus asparagine.

Four amino acids are of particular interest in the present study. Alanine and glutamine are extracted by the splanchnic tissues of the resting adult in amounts that average about 120 μmol/liter of circulating plasma for the two amino acids [16, 22], and they account for two-thirds of the total amino acid extraction by splanchnic tissues; the balance is accounted for by glycine and proline which are extracted from the renal circulation in particular, in amounts averaging about 60 μmol/liter circulating plasma in the adult [15]. However, the actual amount extracted cannot be calculated without knowing the plasma flow per unit of time through the tissues.

Rapid glucagon administration is accompanied by transient hypoaminoacidemia in the adult [5, 12]. The fall in alanine and glycine alone comprises about a third of the decline in total α-amino nitrogen under this stimulus. Glucagon apparently achieves its depressive effect on plasma amino acids partly through a direct effect on increased hepatic uptake of alanine and other amino acids [23], and probably also through an indirect effect mediated by insulin, which causes increased storage in muscle of certain amino acids, notably the branched chain group, phenylalanine and tyrosine [13]. The hepatic effect is probably more important in the fasted state, and the 30-min plasma re-

sponse has been used in mature subjects to evaluate the effect of glucagon on amino acids [12]. We have not yet examined whether a difference in timing of the response on the day of birth accounts for any of our observations in this age group.

If euglycemia in the human neonate is supported to any extent by glucogenic amino acids, then a failure to extract alanine and proline from plasma may contribute to the observed instability of blood glucose in the initial hours after birth. It has already been suggested that a primary deficiency of plasma alanine may invoke the syndrome known as ketotic hypoglycemia in later infancy [25]. We are proposing that the problem in the normal neonate is not in the availability of alanine, but in its utilization. In addition to the indirect evidence for the role of glucogenic amino acids in man, there is good direct evidence from animal studies that certain amino acids are important glucose precursors in splanchnic tissues after glucagon stimulation [21, 28]. Diminished ability of the human neonate to extract proline from plasma after glucagon stimulation might be analogous to the well documented reduced ability of nonhuman kidney to take up and to oxidize proline in the postnatal period [3]. Impaired extraction of alanine after glucagon administration in the human neonate may reflect diminished specific activity of L-alanine-2-oxoglutarate aminotransferase during late fetal and early postnatal life, analogous to that of other mammalian species [31, 32]. The inability of amino acids to participate in these initial steps of glucogenesis in the neonate would appear to be more likely than a defect at later steps, inasmuch as pyruvate carboxylase and phosphoenolpyruvate carboxykinase are apparently present in human liver at birth [1].

It is known that glucagon secretion in the dog is provoked by hyperalaninemia [24], and it is believed that the modest physiologic hyperalaninemia of early starvation in man [15] is sufficient to initiate glucagon release under these conditions. There is relative hyperalaninemia in the initial hours after birth and this might stimulate endogenous glucagon release, in which case the response to exogenous glucagon might be attenuated. Direct measurement of plasma glucagon in the neonate would clarify this point of interest.

The infants of diabetic mothers show little hypoaminoacemic response to glucagon, which suggests that their adaptation to a relatively rich caloric environment during the late weeks of gestation may contribute to the obtunded amino acid response to glucagon. It is possible that effects of established hyperinsulinemia on amino acid distribution and the normal physiologic unresponsiveness to glucagon both contribute to the relative inertness of plasma amino acids on the day of birth in this group. The infants of low birth weight were able to respond to glucagon with a small but significant decrease of three important gluconeogenic amino acid (alanine, glutamine-plus-asparagine and glycine), whereas proline was typically unresponsive. However, several of the insulin-sensitive amino acids dropped significantly after glucagon infusion, perhaps reflecting the obvious hyperinsulinemia produced in this group by the glucagon infusion. The fact that a rise in blood glucose followed a pharmacologic dose of glucagon in all four groups of infants in the present study does not rule out the likelihood that the observed impairment of the amino acid response plays a role in postnatal hypoglycemia in the endogenous or physiologic state.

Summary

Amino acids were measured in systemic venous plasma on the day of birth and on the 3rd day of life in normal full term infants, and on the day of birth in infants of diabetic mothers and in infants who were small for their gestational age. The effect of age and intravenous glucagon administration (300 μg/kg) was examined.

Total amino acid concentration in plasma falls significantly between the day of birth and the 3rd day of life in normal full term infants. The level of glucogenic amino acids is more likely to fall than the level of ketogenic amino acids, and the latter may actually rise in plasma. Plasma amino acid levels were lower than expected during the early hours after birth in infants of diabetic mothers and in infants who were small for gestational age.

Glucagon produces a hypoaminoacidemic response in the newborn infant on the 3rd day of life, which resembles the response in the adult. Full term normal infants under 24 hr of age have a blunted amino acid response, particularly with regard to alanine and proline. Infants of diabetic mothers are almost unresponsive to a glucagon stimulus on the day of birth. The response in the small-for-gestational-age group on the day of birth was also less than in control infants, although the glucogenic amino acids, glutamine, glycine, and alanine, fell significantly. The basal and peak blood glucose levels before and after glucagon respectively are depressed in the latter two groups, compared with full term infants.

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34. We are very grateful to Mr. Rudi Oppliger and Mr. Jaromir Vajgent for the many amino acid analyses which they performed for this study.
35. This work was supported by Grants nos. MT-1085, MA-2952, and MA-3037, Medical Research Council of Canada, and by Grant no. R-232-70, United Cerebral Palsy Association's Research and Educational Foundation.

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37. Accepted for publication December 11, 1972.

Printed in U.S.A.