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The metabolic effects of glucagon infusion in normoglycaemic and hypoglycaemic small-for-gestational-age infants

I. Changes in blood glucose, blood lactate and plasma free fatty acids

By

SCHULTZ, K., MESTYÁN, J., SOLTÉSZ, GY. and HORVÁTH, M.

Department of Paediatrics, University Medical School, Pécs

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The response to glucagon infusion of blood glucose, blood lactate and plasma FFA was studied in 7 normoglycaemic and 7 hypoglycaemic SGA infants. The infants received glucagon at a rate of 0.2 μg/kg/min into a cephalic vein for a period of four hours.

Glucagon caused a marked and significant rise in blood glucose in both groups: from 66 ± 12 mg/100 ml to 136 ± 16 mg/100 ml in the normoglycaemic, and from 22 ± 2 mg/100 ml to 69 ± 13 mg/100 ml in the hypoglycaemic infants. The time course of the response was different: while in the former group blood glucose declined after the second hour of glucagon infusion, in the hypoglycaemic group the response persisted throughout the test period.

In the normoglycaemic SGA infants FFA fell rapidly in response to glucagon, whereas no significant change occurred in hypoglycaemic infants. The different baseline lactate concentrations were not affected significantly.

Current concepts concerning hypoglycaemia in intrauterine malnourished newborn infants have been focused upon the role of gluconeogenesis, which appears to be impaired in comparison to well-nourished neonates [3, 6, 10, 11, 18]. The postnatal accumulation of gluconeogenic amino acids, lactate and pyruvate in the plasma strongly suggests a reduced hepatic uptake of these glucose precursors and hence a decreased glucose synthesis [6, 11]. The possible pathogenic importance of diminished gluconeogenesis is further supported by the observation that hyperaminoacidaemia associated with hypoglycaemia is largely due to increased levels of prominent glucogenic amino acids (alanine, glycine, proline), and the extent of their accumulation in the plasma is very much dependent on the severity of glucose deficiency [6, 11].

On the basis of these recent findings it seemed logical to explore whether glucagon, an important regulator of glucogenolysis and gluconeogenesis, is capable under such metabolic conditions to stimulate endogenous glucose production by increasing the utilization of glucogenic amino acids and thus to prevent their accumulation in the plasma of severely underweight newborn infants. In view of its physiological role in gluconeogenesis it appeared reasonable to assume that glucagon might be used as a therapeutic tool in neonatal hypoglycaemia to stimulate

hepatic glucose synthesis by the gluconeogenic pathway. Such an attempt based primarily on the glucogenolytic effect of exogenous glucagon has already been made [9], but because of the variability of the results it was not recommended for therapy of neonatal hypoglycaemia in small-for-gestational-age (SGA) infants.

Material and Methods

Two groups of SGA newborns were studied: seven normoglycaemic and seven hypoglycaemic infants, who were admitted to our referral neonatal units for observation and treatment. Assesment of body size in relation to gestational age was based on the position of the infants on our local intrauterine growth chart: birth weight was lower than the 10th percentile, and all infants appeared clinically wasted. The average percentage weight deficit amounted to 31.6% in the normoglycaemic and 43.2% in the hypoglycaemic babies.

Data for the two groups of infants are summarized in Table I. The normoglycaemic group included 4 full-term (gestational age >37 weeks) and 3 preterm (gestational age <37 weeks) infants; the hypoglycaemic group comprised of 3 full-term and 4 preterm infants. From Table I it can be seen that in the hypoglycaemic group mean birth weight and postnatal age at which the infants were examined, were lower than in the normoglycaemic SGA infants. Two normoglycaemic infants were born in asphyxia and four hypoglycaemic infants after a toxaemic pregnancy.

All studies were performed either before the first feeding, or four hour after a feeding of evaporated milk formula, or breast milk. Hypoglycaemia was diagnosed shortly after admission between 3 and 18 hours postnatally. In full-term infants a blood glucose level below 30 mg/100 ml, in preterm infants one below 20 mg/100 ml was accepted as the criterion of hypoglycaemia. Neither on admission nor during glucagon infusion were symptoms attributable to hypoglycaemia observed.

All infants received an infusion of glucagon at a rate of 0.2 μg/kg/min into a cephalic vein for a period of four hours. Blood was drawn from the cubital vein at zero time and at 1, 2, 3 ,4 hours for blood glucose, blood lactate, and plasma free fatty acid determination. Blood samples for amino acid analysis were obtained at zero time, and in the 2nd and 4th hour of the test period.

Glucose and lactate concentration was determined by the orthotoluidine method

Table I

Gestational age, birth weight and postnatal age in the two groups of infants

	Normoglycaemic SGA infants	Hypoglycaemic SGA infants
Gestational age, wk	36,6 (32–38)	36,0 (31–43)
Birth weight, g	2066 (1300–2790)	1500 (1070–1860)
Postnatal age at time of investigation, hr	13 (3–31)	8 (3–18)
No. of infants	7	7

Ranges for age, weight and postnatal age are in parentheses.

described by Price [15] and by the method reported by Huckabee [7], respectively. Plasma FFA was analysed according to Dalton and Kowalsky [4].

Differences between group averages were compared by the standard t test.

Results

Mean ± SE of glucose, lactate and FFA, obtained prior to and at hourly intervals during glucagon infusion in the two groups of intrauterine malnourished infants are summarized in Tables II, III and IV.

Glucagon infusion caused a significant rise in blood glucose from 66 ± 12 mg/100 ml to a maximum level of 136 ± 16 by 120 minutes in the normoglycaemic, and from 22±2 mg/100 ml to 69±13 by 180 minutes

Table II

Blood glucose level* before and during glucagon infusion in normoglycaemic and hypoglycaemic SGA infants

	Before glucagon infusion	During glucagon infusion			
		1 hr	2 hr	3 hr	4 hr
Normoglycaemic SGA infants	66 ±12	129‡ ±13	135‡ ±16	103 ±19	71 ±13
Hypoglycaemic SGA infants	22 ±2	53‡ ±7	64‡ ±12	69‡ ±13	63‡ ±15

* Concentrations are given in mg/100 ml (mean ± SE)

\+ $p < 0.05$

‡ $p < 0.001$

Table III

Blood lactate level* before and during glucagon infusion in normoglycaemic and hypoglycaemic SGA infants

	Before glucagon infusion	During glucagon infusion			
		1 hr	2 hr	3 hr	4 hr
Normoglycaemic SGA infants	20 ±5	18 ±3	21 ±4	25 ±6	28 ±10
Hypoglycaemic SGA infants	32 ±6	33 ±5	29 ±5	29 ±6	29 ±6

* Concentrations are given in mg/100 ml (mean ± SE)

TABLE IV

Plasma FFA* level before and during glucagon infusion in normoglycaemic and hypoglycaemic SGA infants

	Before glucagon infusion	During glucagon infusion			
		1 hr	2 hr	3 hr	4 hr
			**	**	**
Normoglycaemic SGA infants	1418 ±210	1022 ±220	490 ±79	459 ±154	565 ±163
Hypoglycaemic SGA infants	529 ±141	677 ±209	563 ±223	292 ±73	357 ±129

* Concentrations are given in μ E/l (mean ± SE).
** $p < 0.01$.

in the hypoglycaemic SGA infants. These peak concentrations represented increments of 134% and 309% respectively. The absolute and relative rises in blood glucose undoubtedly show that hypoglycaemic underweight infants are capable to mobilize endogenous glucose in response to glucagon infusion. Not only the magnitude but also the time course of the blood glucose changes during glucagon administration was different in the two study groups (Fig. 1). While in the normoglycaemic SGA neonates blood glucose declined rapidly after the second hour of glucagon infusion, in the hypoglycaemic group the elevated level persisted throughout the test period. This striking difference in the type of response is best shown by the glucose concentration obtained at the end of infusion; in the normoglycaemic infants it almost returned to the starting value, while in the hypoglycaemic ones it still exceeded the baseline by 248%.

In SGA newborns with normal blood glucose concentrations, the lactate level showed a slight tendency

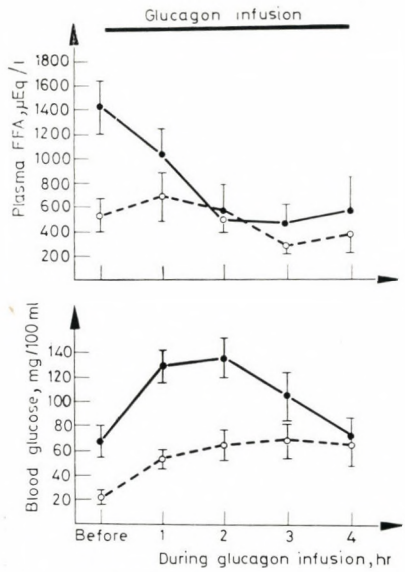

FIG. 1. Changes in blood glucose and plasma FFA in response to glucagon infusion in normoglycaemic (●) and hypoglycaemic (o) SGA infants. The upper part shows the mean response ± SE in plasma FFA, and the lower part the mean response ± SE in blood glucose

to rise towards the end of glucagon infusion (Table III). In contrast, in the hypoglycaemic infants the somewhat, but not significantly, higher baseline level remained practically unchanged.

The significantly different plasma FFA concentrations were differently affected by glucagon infusion (Table IV and Fig. 1): while the high FFA declined rapidly and significantly in normoglycaemic SGA infants, first a transient rise and later a small reduction of the much lower FF Alevel occurred in hypoglycaemic SGA infants. These changes were not significant.

As regards the mean blood urea level, while in the normoglycaemic group a gradual rise was observed, no change occurred in the hypoglycaemic SGA infants (Table V). This difference, although statistically insignificant, points to a glucagon stimulated glucose production from amino acid precursors in normoglycaemic SGA babies.

TABLE V

Blood urea nitrogen level* before and during glucagon infusion in normoglycaemic and hypoglycaemic SGA infants

	Before glucagon infusion	During glucagon infusion	
		2 hr	4 hr
Normoglycaemic SGA infants	18.4 ±4.3	22.4 ±4.9	33.3 ±11.6
Hypoglycaemic SGA infants	17.2 ±3.5	15.7 ±2.4	17.7 ±2.1

* Concentrations are given in mg/100 ml (mean ± SE)

Discussion

The results outlined undoubtedly show, that not only the normoglycaemic but also the hypoglycaemic SGA infants responded to glucagon infusion by a considerable increase in blood glucose. Hepatic glucose release, which resulted in a threefold rise from the hypoglycaemic baseline values, raised the blood glucose value to normal neonatal levels which persisted until the end of the 4 hour infusion period. The course of the response was different in the normoglycaemic SGA infants; in these the doubled blood glucose level declined during the second half of the test period.

The glucagon-induced increase in blood glucose concentration supported earlier [1, 14] and recent [5] results obtained by single intramuscular or intravenous injection of glucagon, and showed that intrauterine malnutrition is not necessarily associated with an inability to increase glucose production. Even the liver of hypoglycaemic SGA infants was capable to respond to glucagon stimulation. In contrast, Le Dune [9] in her report on the effect of 30 μg/kg glucagon in normal and hypoglycaemic SGA neonates concluded that the latter infant category showed a significantly lower response than the former in terms of maximum glucose levels attained throughout a 90 minute test period. Evaluation of a difference in glucagon stimulation in terms of maximum glucose levels may, however, be misleading when infant-groups with considerably different

baseline values are compared. It is the increment which should be used as an index of hepatic glucose release. Using this indicator, Le Dune's results are in fact not inconsistent with the present observation. Still, a comparison with our findings is somewhat hampered by the difference in approach to the problem and in the mode of glucagon administration. The amount of glucagon did not differ much between the two series of examinations: while we infused glucagon at a constant rate of 0.2 μg/kg/min representing a total amount of 48 μg/kg for the 4 hour infusion period, Le Dune gave 30 μg/kg in a single intramuscular injection.

In the normoglycaemic SGA infants the rapid decline of blood glucose following the second hour of glucagon infusion indicated hormonal or metabolic events opposing the hyperglycaemic effect of glucagon. On the contrary, in the hypoglycaemic group the marked glucose response persisted throughout the entire 4 hour observation period. This unopposed action of glucagon suggests an essential difference between the two groups of SGA infants as far as the influence and the interactions of hormones in the control of glucose homeostasis are concerned.

The most likely cause of the suppression of the effect of glucagon is an increased insulin secretion. It is well-known that glucagon stimulates insulin secretion not only by causing hyperglycaemia but also by a direct stimulating effect upon the cells of the pancreas [2, 12, 17]. It is tempting to suggest that the persistently elevated blood glucose concentration in the hypoglycaemic SGA infants throughout the test period may be due to a weak or lacking insulin response to exogenous glucagon.

Although the present study failed to provide direct evidence favouring this interpretation, the changes in plasma FFA and amino acids associated with glucagon infusion also point toward the possibility that an altered glucagon-insulin relationship lies at the root of the different metabolic behaviour of the two groups of intrauterine malnourished infants. Le Dune [9] and Reisner et al. [16] studied the effect of glucagon on plasma insulin concentration in SGA infants, and concluded that the fasting and the maximum insulin levels were higher in the hypoglycaemic babies. In SGA infants whose blood glucose level was significantly depressed, the fasting insulin level and the peak which occurred between 2 and 5 minutes after glucagon administration was higher than in normal full-term infants [16]. In Le Dune's study the statistical comparison of the maximum insulin levels was meaningless, since the baseline concentration was different in the two groups of infants. As regards the mean plasma insulin levels at different times after the injection of glucagon, neither intra-, nor intergroup differences of statistical significance were observed. The hypoglycaemic SGA infants in Le Dune's material showed, however, large variations in plasma insulin concentration, and

part of them were in fact hyperinsulinaemic. Thus, by applying additional hormonal or metabolic parameters, hypoglycaemic SGA infants can be divided into further subgroups.

The assumption that a lacking or diminished insulin response can be a factor responsible for the unopposed hyperglycaemic effect of glucagon is supported by observations made on adults that hypoglycaemia, or 2—4 days starvation may abolish the insulinotropic effect of glucagon [17]. An increased sympathetic nervous activity associated with glucose deficiency may also contribute to the suppression of glucagon-promoted insulin release [8]. Other factors such as the plasma substrate profile should also be considered in the interpretation of the different type of blood glucose response to glucagon in the hypoglycaemic and non hypoglycaemic intrauterine malnourished infants. In this respect the plasma amino acids might be important [13]. In view of observations in vitro it is not unreasonable to assume that altered plasma concentrations of some amino acids such as e.g. leucine, arginine and alanine may profoundly modify the glucagon-insulin relationship either by potentiating, or by suppressing glucagon or glucose-mediated insulin release.

Besides an altered glucagon promoted insulin response a decreased sensitivity of the liver to insulin may also account for the sustained glycaemic effect of glucagon infusion. In this case, as it has been observed by Le Dune [9] and Reisner et al. [16], a normal or increased insulin secretion leaves the hepatic action of glucagon unopposed. As a consequence, glucagon can bring about an increased rate of glucose production. The sensitivity to insulin of the different glucagon-activated metabolic processes can be different, one may require higher insulin levels for suppression than the other. Such a modification of the interaction on the liver of the two hormones is also a reasonable working concept for further studies to explore the underlying mechanisms of the different metabolic behaviour of the normo- and hypoglycaemic SGA infants.

The unopposed action of glucagon on hepatic glucose release in hypoglycaemic infants suggests an essential difference between normoglycaemic and hypoglycaemic SGA infants as far as the influence and the interaction of hormones in the control of glucose homeostasis is concerned. An altered glucagon-insulin relationship might be at the root of the different metabolic behaviour.

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K. Schultz, M. D.
József A. u. 11.
H-7623 Pécs, Hungary