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ROLE OF INSULIN ON THE CONTROL OF POSTNATAL INCREASE IN ORNITHINE TRANSCARBAMYLASE ACTIVITY IN RAT LIVER

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Repeatedly supplementing glucose after birth abolishes postnatal increase in ornithine transcarbamylase activity and increases the plasma insulin level. A single administration of actinomycin D does not affect this activity. When glucose and actinomycin D are administered in association at birth or 13 hours after birth, actinomycin D overcomes the inhibitory effect of glucose on ornithine transcarbamylase activity and decreases insulinemia at the level found in normal neonate. Insulin supply 20 hours after birth to neonates, which previously received glucose and actinomycin D, decreases the enzyme activity and increases plasma insulin level. It is concluded that the postnatal increase in ornithine transcarbamylase activity is bound to the normal postnatal decrease in insulinemia. Furthermore, the paradoxical effect of actinomycin D on enzyme activity might be due to an inhibition of insulin synthesis or a decrease in insulin release.

Ornithine transcarbamylase (EC.2.1.3.3.) is one of the five hepatic enzymes involved in urea synthesis. It is located in the mitochondrial matrix of ureotelic animals (1) and catalizes the second step of the urea cycle. Its activity appears on day 16,5 of gestation, regularly increases during the late fetal period (2-6) and presents a marked increase 24 hours after birth (7).

It is known from previous work (8-10) that the activity of ornithine transcarbamylase needs the presence of the adrenals in the neonate and adult rat. Our recent studies have demonstrated that the appearance and increase of this enzyme activity in the foetus were under the control of glucocorticosteroids (2-3). An administration of actinomycin D to 18.5 days old foetuses increased the level of enzyme activity as determined 24 hours later, while a simultaneous administration of cortisol abolished this paradoxical effect (11). After birth, the rise in ornithine transcarbamylase activity is associated with the appearance of new fonctions in the newborn, particularly that of nitrogen excretion. In addition, it is known that birth is associated with a

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major endocrine crisis, i.e. an abrupt increase of glucagon secretion, associated with a decrease in insulin release. This alters the insulin/glucagon ratio. This hormonal crisis and the rise in ornithine transcarbamylase activity do not correlate with the age of the rat, but are associated with birth itself (7-12).

Moreover, postnatal increase in ornithine transcarbamylase activity is abolished by repeatedly supplementing glucose (25 mg every two hours), while a single administration of actinomycin D does not affect the activity. When glucose and actinomycin D are administered in association at birth or 13 hours after birth, actinomycin D overcomes the inhibitory effect of glucose (13). It was concluded that the inhibitory effect of glucose on the rise of enzyme activity might be caused by the inhibitory effect of resulting insulin release.

We decided to investigate further the role of insulinemia in the mechanism of the postnatal enzyme rise by altering the hormonal status. This was attained by measuring the enzyme activity and plasma insulin level after insulin supply in association with glucose and actinomycin D 20 hours after birth.

MATERIALS AND METHODS

Animals and experimental procedure.

All studies were carried out on pregnant Wistar rats fed "ad libitum". Gestational age was calculated from the ovulation and fertilization dates which occurred around 1 a.m. Fertilization was confirmed by vaginal smear. Pregnancy in this strain lasts 21.5 days. On day 21.5 at 9. a.m. pregnant rats were killed by a blow on the head and the whole fetuses were removed from the uterus with the amniotic sac intact. The pups were then excised from the sac and the ombilical cord tied and cut. The entire litter was delivered within 5-10 min after the mother's death and the newborns were held in a humidicrib at 37°C without feeding for the duration of the experiments.

Newborns delivered by caesarian section received immediately or 13 hours after birth a single administration of actinomycin D (3 µg in 0.05 ml i.p.) (Calbiochem, La Jolla, Ca) or 0.05 ml i.p. of a glucose solution (25 mg) repeatedly every two hours. Insulin (40 mU in 0.05 ml i.v.)(Novo, Paris) is administered 20 hours after birth to neonates which previously received repeadly glucose and actinomycin D 13 hours after birth. Control newborns received according to the same time schedule 0.05 ml of a 0.9 g/l NaCl solution.

Ornithine transcarbamylase assay.

At the choosen time, newborns were rapidly weighed, decapited and bled. Freshly excised livers were immediately weighed and homogenized in a solution of N-acetyl-N,N,N-trimethyl ammonium bromid (CTB) 0.1% in Tris-HCl buffer (0.05 M, pH = 7.4), (8 ml of CTB solution per gram of liver). After two successive centrifugations for 15 min at 4000 g at 4°C, the enzyme activity was

assayed on the supernatant solution according to the method of Brown and Cohen (13) with some modifications as previously described (11). A unit of enzyme was the amount which catalized the production of 1 µM citrulline/hour at 37°C under assay conditions. The activity was defined as units per milligram of proteins. Proteins were determined by mean of the biuret procedure (14) with bovine serum albumin as standard.

Plasma insulin determination

Neonates at the suitable stage were killed by decapitation and blood was collected in heparinized plastic centrifuge tube for the preparation of plasma insulin measurement. 100 µl of plasma were obtained from 2 or 3 neonates for each determination. Plasma insulin concentration (µUi/ml plasma) was determined by radioimmunoassay according to the method of double antibody. The insulin assay employed guinea pig antiinsulin serum and porcine ^{125}I-insulin as standard (Bio Merieux, France).

Statistical analysis.

Statistical analysis was performed using Fisher's t test.

RESULTS

Effect of a glucose administration at birth.

Glucose administration (25 mg every two hours) to neonates delivered by caesarian section on day 21.5 prevented for 24 hours the marked increase of ornithine transcarbamylase activity which normally occurs during this period (Fig. 1 and Table 1). The enzyme activity remained comparable with that of

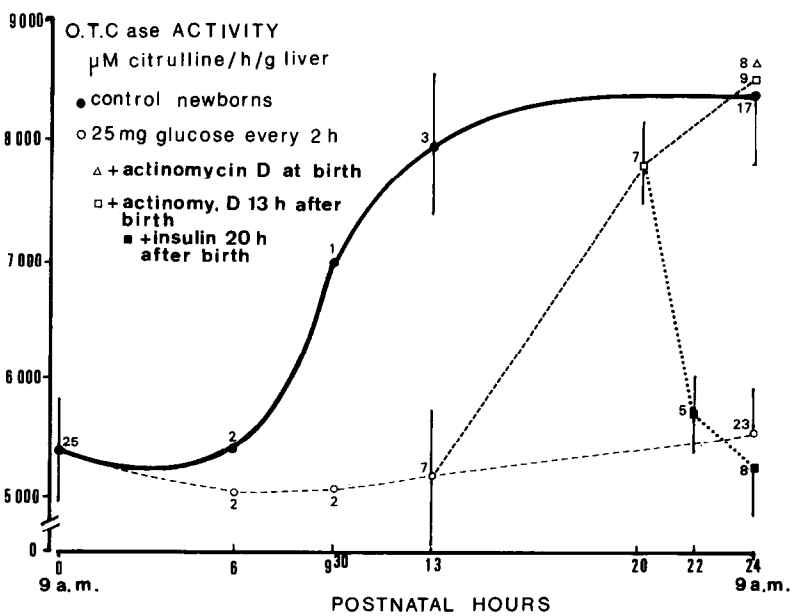

Fig. 1 : Time course of postnatal ornithine transcarbamylase activity. Effects of a repeated postnatal administration of glucose, with or without actinomycin D and insulin. (Values are expressed as the mean ± SEM. The numbers indicate how many enzyme assays were performed on the pooled livers of the same litter).

Table 1 : Ornithine transcarbamylase activity (OTC) and plasma insulin level attained 24 hours after birth. Influence of a repeated glucose postnatal administration with or without supplementing actinomycin D.

TREATMENT	OTC		Plasma Insulin
	activity/g liver	specific activity	µUI/ml
Control foetuses at 21.5 days pregnancy	5408 ± 948 (25)	67.0 ± 11.8 (25)	96.8 (3)
Control newborns 24 hours "post-partum"	8449 ± 1229* (17)	93.5 ± 17.3* (17)	18.0 ± 1.6 (4)
Glucose at birth	5553 ± 764* (23)	69.7 ± 9.3* (22)	215.0 ± 5.2* (3)
Glucose + Actinomycin D at birth	9430 ± 117● (8)	76.7 ± 9.3 (9)	- -
Glucose at birth + actinomycin D 13 hours later	8848 ± 1661● (9)	81.5 ± 14.7 (9)	19.8 ± 3.5* (4)

The values are expressed as the mean ± confidence interval. The number of assays on the pooled neonates of the same litter is given in parentheses.
● * Statistically different.

21.5 days old foetuses. In control neonates, the plasma insulin level (Table 1, and Fig. 2) decreased regularly from 96.8 µUi to 18.0 µUi/ml at the 24th

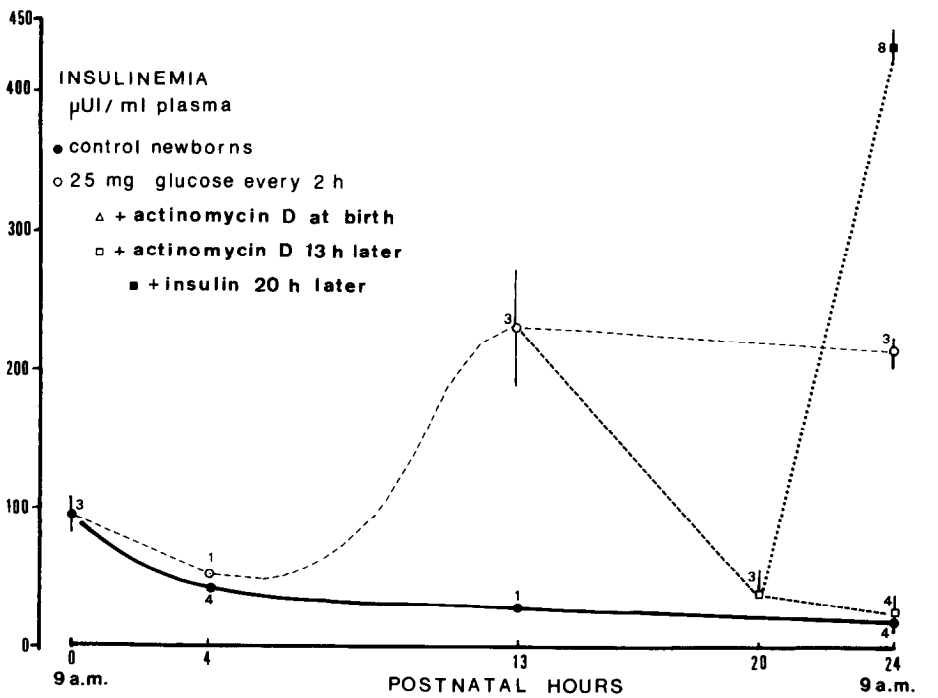

Fig. 2 : Time course of postnatal plasma insulin level. Effects of a repeated postnatal administration of glucose, with or without actinomycin D and insulin. (Values are expressed as the mean + SEM. The numbers indicate how many insulin assays were performed on pooled plasma of neonate of the same litter).

hours of postnatal life. Glucose supply at birth prevented this decrease. Ornithine transcarbamylase activity decreased in coordination with plasma insulin increase.

Effects of an administration of glucose at birth and actinomycin D 13 hours later.

The inhibitory effect of glucose at birth on ornithine transcarbamylase activity was abolished by a single administration of actinomycin D either at birth or 13 hours after birth (Table 1 and Fig. 1). On the other hand the increase in plasma insulin level associated with glucose administration is abolished after an actinomycin D supply, and remained at the level found in the standard neonates (Table 1 and Fig. 2). Ornithine transcarbamylase activity increases when plasma insulin level decreases.

Effect of supplementing insulin 20 h after birth to neonates which received glucose and actinomycin D.

A single administration of insulin 20 hours after birth to neonates with supplementary glucose and actinomycin D decreased ornithine transcarbamylase activity 2 and 4 hours later to the level found in the neonates with supplementing glucose. (Table 2 and Fig. 1). This decrease in enzyme activity is associated with a important rise in plasma insulin level (440 µUi) (Table 2 and Fig.2).

DISCUSSION

The main increase in ornithine transcarbamylase activity in newborns delivered by caesarian section on day 21.5 takes place between the 6th and the 13th hour after birth. This postnatal increase is not abolished by supplementing actinomycin D at birth. In addition, cycloheximide administration (10 µg) to newborns significantly overcomes this postnatal increase (16). These results suggest that the postnatal rise in ornithine transcarbamylase activity is not associated with a new RNA synthesis, but might involve a protein synthesis. The postnatal glucose administration has a major consequent : it increases the insulin secretion (17). It is known that this glucose administration does not

Table 2 : Time course of ornithine transcarbamylase activity (OTC) and plasma insulin level attained 24 hours after birth. Influence of an administration of insulin 20 hours after birth in association with glucose and actinomycin D.

	Hours after insulin supply					
	0		2		4	
	OTC activity/ g liver	insulin µUi/ml	OTC activity/ g liver	insulin µUi/ml	OTC activity/ g liver	insulin µUi/ml
Control newborns 24 hours "post partum"	-	-	-	-	8449 ± 1229* (17)	18.0 ± 1.6* (4)
Glucose at birth	-	-	-	-	5553 ± 764** (23)	215.0 ± 5.2** (3)
Glucose at birth later + Actinomycin D 13 h	7712 ± 1316 (7)	35.7 (3)	-	-	8848 ± 1661* (9)	19.8 ± 3.5* (4)
Glucose at birth + Actinomucin D 13 h later + insulin 20 h later	7712 ± 1316 (7)	35.7 (3)	5831 ± 923 (5)	-	5304 ± 454** (8)	440 ± 14** (8)

All values are expressed as the mean ± confidence interval. The number of assays on the pooled neonates of the same litter is given in parentheses.
** Statistically different.

alter the postnatal secretion of glucagon (18). The inhibitory effect of glucose on the rise of enzyme activity might be caused by the inhibitory effect of insulin. We can notice from our results that a low level in enzyme activity is bound to a hight insulinemia (neonate with supplementing glucose) and on the contrary, a high level in enzyme activity corresponds to a low insulin level (neonates with supplementing glucose and actinomycin D). Furthermore, insulin supply to neonates which previously received supplementing glucose and actinomycin D decreases the enzyme activity. It is concluded from our results that the postnatal rise in ornithine transcarbamylase activity is bound to the decrease in insulinemia, which normally occurs after birth. If this was the case, our work specifies the mechanism of paradoxical effect of actinomycin D on ornithine transcarbamylase activity. Actinomycin D supply at birth or 13 hours after birth overcomes the inhibitory effect of glucose on enzyme activity and decreases significantly the plasma insulin level. The effect of actinomycin D might be explained by an inhibition of insulin synthesis or insulin release. This explanation could be extended to the case of the foetus in which insulin was found to be high (19) and the activity of ornithine transcarbamy-

lase low (unpublished data). The administration of actinomycin D to the foetus might inhibit the foetal insulin secretion or release and this would lead to the paradoxical effect described in reference 11. Concerning the mechanism of glucose repression on enzyme activity, glucose or a metabolite may act directly on the liver (20) or induce changes in hepatic cyclic AMP. In further work, we intend to specify if supplementing cyclic AMP, anti-insulin serum and glucagon overcome the inhibitory effect of glucose. Moreover, the quantification of ornithine transcarbamylase by immunologic methods would be carried out on neonates after supplementing glucose, alone or in association with actinomycin D.

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