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REGULATION OF ORNITHINE TRANSCARBAMYLASE ACTIVITY IN NEONATAL RAT LIVER.

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SUMMARY.
Postnatal increase in ornithine transcarbamylase activity is abolished by repeatedly supplementing glucose after birth, while a single administration of actinomycin D does not affect this activity. When glucose and actinomycin D are administred in association at birth or 13 hours after birth, actinomycin D overcomes the inhibitory effect of glucose. Moreover, hepatic citrulline and urea concentrations remain low after glucose administration. The inhibitory effect of glucose on the postnatal rise in ornithine transcarbamylase activity might be due to the resulting 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 fetus were under the control of glucocorticosteroids (2-3). An administration of actinomycin D to 18.5 days old fetuses 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).

We decided to investigate further the mechanisms of the postnatal enzyme rise by altering the hormonal status. This was attained by a glucose administration. In addition, we studied the effect of actinomycin D, administered in association or not with glucose at different time 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. 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.

Determination of hepatic citrulline and urea concentrations.

Hepatic urea concentration is determined by mean of urease on an aliquot of the supernatant fraction of the homogenate (15-16). Citrulline concentration is determined spectrophotometrically after the whithdrawal of all the interfering urea (15-17).

Statistical analysis.

Statistical analysis was performed using Fisher's t test.

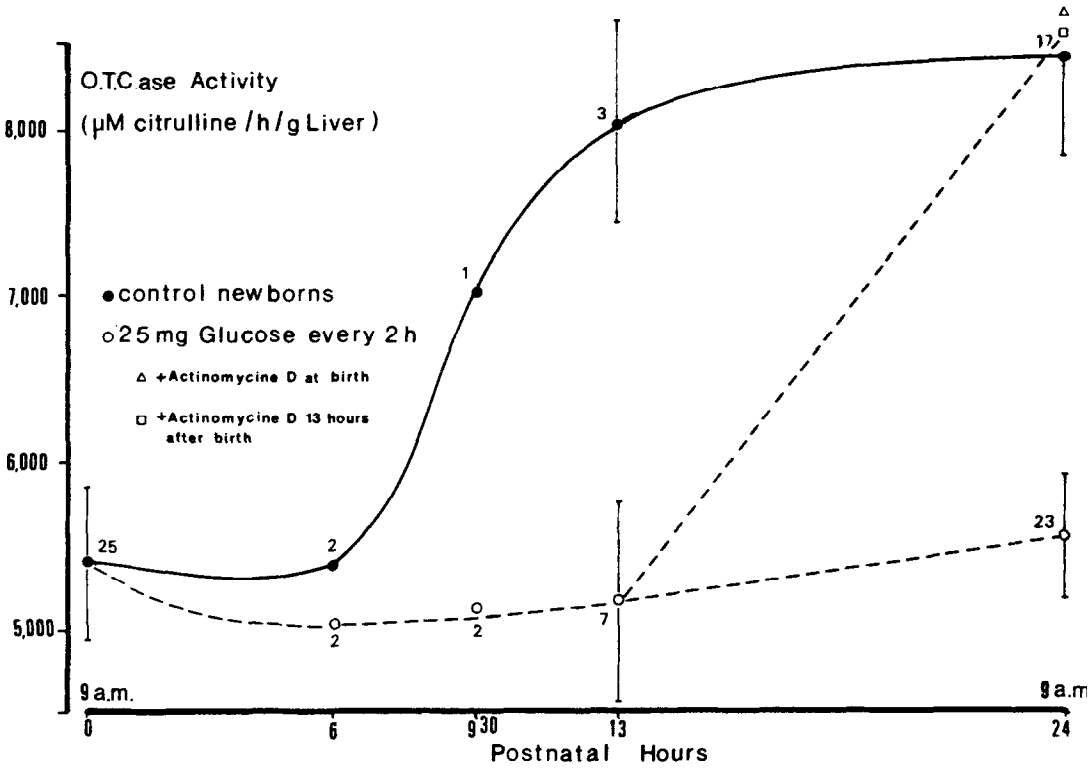

Fig. 1 : Time course of ornithine transcarbamylase activity. Effects of a repeated postnatal administration of glucose, with or without an administration of actinomycin D. (Values are expressed as the mean ± SEM. The numbers indicate how many enzyme assays were performed on the pooled livers of the same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

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 21.5 days old fetuses. In control neonates, liver citrulline (Fig. 2A) and urea (Fig. 2B) concentrations increased about three fold between the 13th and 24th hour after birth, attaining about 1.5 µM and 11 µM/g of liver respectively. Glucose supply at birth prevented this increase. Hepatic citrulline and urea concentrations increased in coordination with the activity of ornithine transcarbamylase.

Table 1 : Ornithine transcarbamylase activity attained 24 hours after birth. Influence of a repeated glucose postnatal administration with or without an administration of actinomycin D.

TREATMENT	Activity /g liver	Specific Activity	Proteins mg/g liver	Citrulline µM/g liver	Urea µM/g liver
Control fetuses at 21.5 days pregnancy	5408 ± 948 (25)	67.0 ± 11.8 (25)	79.2 ± 4.4. (18)	0.39 ± 0.34 (5)	4.0 ± 0.50 (22)
Control newborns 24 hours"post partum"	8449 ± 1229* (17)	93.5 ± 17.3* (17)	95.5 ± 9.2 (16)	1.41± 0.48* (22)	10.50 ± 1.33* (15)
Glucose at birth	5553 ± 764*• (23)	69.7 ± 9.3* (22)	83.3 ± 9.0 (21)	0.67 ± 0.42* (18)	5.53 ± 0.99*• (20)
Actinomycin D at birth	8872 ± 2301• (7)	96.8 ± 29.4 (7)	91.7 ± 11.9 (7)	–	8.83 ± 1.17 • (7)
Glucose + Actinomycin D at birth	9430 ± 1117• (8)	76.7 ± 9.3 (9)	122.5 ± 20.3 (8)	0.37 ± 0.20* (6)	5.80 ± 1.03* (8)
Glucose at birth + Actinomycin D 13 h later	8848 ± 1661• (9)	81.5 ± 14.7 (9)	109.6 ± 10.2 (9)	0.72 ± 0.67 (9)	4.10 ± 2.47* (9)

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

Effects of an actinomycin D administration at birth.

A single administration of actinomycin D (3 µg) at birth did not prevent the rise in ornithine transcarbamylase activity and liver urea concentration, as determined 24 hours later (Table 1).

Effect of a simultaneous administration of glucose and actinomycin D at birth.

The inhibitory effect of glucose at birth was abolished by a single administration of actinomycin D either at birth or 13 hours after birth (Fig.1 and Table 1). On the other hand, hepatic citrulline and urea concentrations remained at the level found in the neonates.

DISCUSSION.

The main increase in ornithine transcarbamylase activity in newborns delivered by caesarian section on day 21.5 takesplace between the 6th and

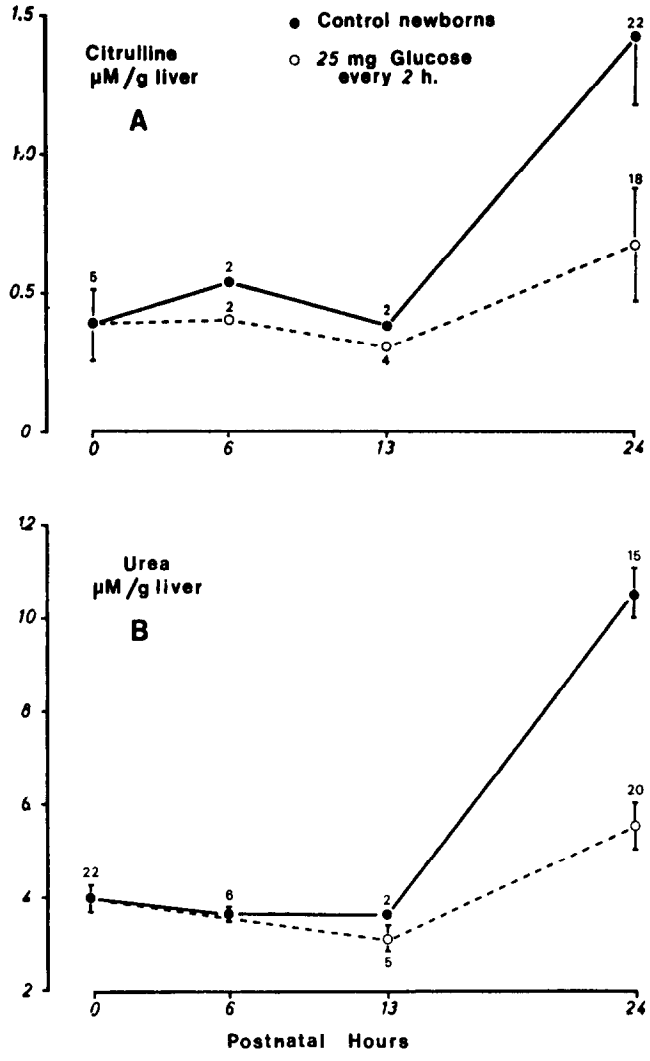

Fig. 2 : Time course of citrulline and urea liver concentrationsafter birth. Effects of a repeated postnatal administration of glucose during the first 24 hours of life. (Values are expressed as the mean ± SEM. The numbers indicate how many assays were performed on the pooled livers of the same litter).

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 partially overcomes this postnatal increase (unpublished data). These results suggest that the 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 (18). It is known that this glucose administration does not alter the postnatal secretion of glucagon (19). The inhibitory effect of glucose on the rise of enzyme activity might be caused by the inhibitory effect of insulin . If this was the case, the effect of actinomycin D might be explained by an inhibition of the insulin release. This explanation could be extended to the case of the fetus in which insulin was found to be high (20) and the activity of ornithine transcarbamylase low. The administration of actinomycin D to the fetus might inhibit the fetal insulin secretion and this would lead to the paradoxical effect described in reference 11.

The postnatal administration of glucose, however, has other effects. It is known to inhibit gluconeogenesis (which occurs after the marked postnatal hypoglycemia) (21), thus depriving the urea cycle of its priming metabolites. This might explain the occurrence of low citrulline and urea liver concentrations after actinomycin D injection, although the enzyme activity has been brought back to the control level. Ornithine transcarbamylase activity might be regulated by the insulinemia or by the supply of ureogenesis metabolites. Insulinemia and blood glucagon are correlated. An important glucagon release (22) and a high AMP_c concentrations (23) are known to be associated at birth with the insulinemia decrease. In further work, we intend to specify the effects of administring inhibitors of gluconeogenesis at birth (for instant 3-mercaptopicolinate) and the effect of supplementing insulin , glucagon and AMP_c to the fetus.

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