N-Acetylglutamate in rat liver during foetal development

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(Received 6 July 1984/Accepted 17 July 1984)

N-Acetylglutamate is present in foetal rat liver at 17 days' gestation. The tissue content (approx. 50 nmol/g wet wt.) remains constant during later foetal life. The appearance of *N*-acetylglutamate does not parallel the developmental pattern of the urea cycle.

The activities of the enzymes of the urea cycle, especially of argininosuccinate synthetase (EC 6.3.4.5), are extremely low in foetal rat liver until immediately after birth (see Raiha, 1976). The low activities of the enzymes correlate with the low capacity of the liver for urea synthesis. If the primary function of *N*-acetylglutamate were to regulate activity of mitochondrial carbamoyl phosphate synthetase (EC 6.3.4.16), as generally accepted (see Meijer & Hensgens, 1982), it would be reasonable to expect that appearance of *N*-acetylglutamate would parallel the appearance of carbamoyl phosphate synthetase. In the present paper we show that foetal liver has a relatively high *N*-acetylglutamate content at 17 days' gestation, which remains constant until birth. This indicates that *N*-acetylglutamate may have some function in addition to that as activator of carbamoyl phosphate synthetase.

Materials and methods

Time-mated virgin female Wistar rats were obtained from OLAC (1976) Ltd., Bicester, Oxon, U.K. and were fed on Rat and Mouse maintenance diet No. 1 (B.P. Nutrition, U.K.). Suckling rats and adult males were also Wistar strain, but bred in our animal house.

Foetuses were removed by caesarian section, one at a time, and their livers were quickly cut out and dropped into liquid N₂. Suckling rats were lightly anaesthetized with diethyl ether, and their livers were freeze-clamped as described by Wollenberger *et al.* (1960). Foetal livers from the whole

litter, or two or three livers from suckling rats, were pooled and ground in a mortar under liquid N₂. The weighed powder was deproteinized with 2 vol. of HClO₄ (5%, v/v), mixed, and left on ice for 30 min. After centrifugation (2000g for 5 min), the supernatant was weighed, neutralized with KOH, and re-weighed to obtain the dilution factor. Extract (1 ml) was treated by two-step elution on small Dowex 1-X8 columns as described previously (Lund & Wiggins, 1984); first with 0.15 M-formic acid (6 ml) to elute glutamate, then with 2 M-formic acid to elute *N*-acetylglutamate. The complete separation of *N*-acetylglutamate from glutamate was checked with marker L-[1-¹⁴C]glutamate or *N*-acetyl[1-¹⁴C]glutamate; the latter was prepared as described by Lund & Wiggins (1984). Further treatment of the eluate containing *N*-acetylglutamate and determination of *N*-acetylglutamate as glutamate were also as described by Lund & Wiggins (1984).

Results and discussion

As shown in Table 1, *N*-acetylglutamate is present in liver at 17 days of gestation, the earliest stage at which sufficient tissue can be obtained for the assay. The amount per g of tissue remains relatively constant during later foetal life. After birth (at 22 days' gestation) the *N*-acetylglutamate content appears to increase, reaching the adult value at 10 days. All values refer to whole tissue and are uncorrected in any way for the water content of the tissue, or for the high proportion of haematopoietic cells in foetal rat liver. The proportion of haematopoietic cells falls from about 46% at 3 days before birth to about 28% at birth (Finck & Theil, 1964) and then to about 7% by day 7 after birth (Oliver *et al.*, 1962). If one assumes that *N*-acetylglutamate is not present in haemato-

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Table 1. *N*-Acetylglutamate content of rat liver during pre- and post-natal development

Experimental details are given in the Materials and methods section. Values are expressed as means $\pm$ S.E.M., with the numbers of observations given in parentheses. The value for freeze-clamped adult liver from fed male rats was provided by D. Wiggins (unpublished work).

	<i>N</i> -Acetylglutamate (nmol/g wet wt.)
Foetal liver (days gestation)	
17	57 $\pm$ 8.8 (5)
18	50 $\pm$ 12.0 (3)
19	50 $\pm$ 6.7 (5)
20	49 $\pm$ 6.2 (5)
Neonatal liver (days after birth)	
3	74 $\pm$ 8.5 (4)
5	61 $\pm$ 12 (5)
10	101 $\pm$ 9.1 (6)
Adult liver	79 $\pm$ 6.3 (10)

poietic tissue, the foetal-liver content is close to the adult value, and bears no relation to the development of the enzymes of the urea cycle or to the capacity of the liver to synthesize urea. Urea production in rat liver slices is negligible before day 20 of gestation, but increases gradually towards birth, with a rapid rise to the adult rate 1 day after birth (see Raiha, 1976). The early appearance of *N*-acetylglutamate in foetal life, and its high concentration in the mitochondrial matrix at all stages of development, when haematopoietic tissue and other assumptions concerning its intracellular distribution are taken into account (see Lund & Wiggins, 1984), make it tempting to suggest that *N*-acetylglutamate has more than one function in the liver.

Our conclusions differ from those of Rabier *et al.* (1983), who measured *N*-acetylglutamate in rat liver mitochondria after incubation for 15 min at 25°C. A gestational age of 20.5 days was the earliest stage of development tested. Their results led them to suggest that *N*-acetylglutamate may be limiting for urea synthesis during the first 2–3 days after birth. The high values for *N*-acetylglutamate that we find when the compound is assayed as glutamate, as opposed to the kinetic assay used by others, have already led us (Lund & Wiggins, 1984) to question whether it can regulate urea synthesis under normal conditions. Cheung & Rajman (1980) came to the conclusion that decreases, rather than increases, in the activator are more important in determining the effective activity of carbamoyl phosphate synthetase because approx. 50% of the enzyme appears to be fully activated in the normal fed rat. There are a number of

situations in which availability of *N*-acetylglutamate could limit carbamoyl phosphate synthetase activity *in vivo*: in *N*-acetylglutamate synthase deficiency (Bachmann *et al.*, 1981) and other pathological states associated with hyperammonaemia in which CoA derivatives accumulate [methylmalonic- and propionic-acidaemias (Stewart & Walser, 1980); valproic acid treatment of epilepsy (see Coudé *et al.*, 1983); possibly also Jamaican vomiting sickness and Reye's syndrome]. There are various means by which acyl-CoA intermediates could bring about inhibition of urea synthesis: by competitive inhibition of the synthase (Coudé *et al.*, 1979), by depleting the CoA pool and hence the acetyl-CoA available for *N*-acetylglutamate synthesis, and/or by directly inhibiting carbamoyl phosphate synthetase (see Martin-Requero *et al.*, 1983).

We thank Dr. D. S. Parsons and Miss J. Hislop (Department of Biochemistry, University of Oxford) for providing foetal livers of known gestational age. M. van D. and the research were supported by a Grant awarded to Dr. B. D. Ross by the Muscular Dystrophy Group. P. L. is a member of the M.R.C. External Scientific Staff.

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