Studies on the Development of Ornithine-Keto Acid Aminotransferase Activity in Rat Liver

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1. During the normal development of the rat, the specific activity of liver ornithine-keto acid aminotransferase exhibits a transient elevation around term, and subsequently increases to adult activity levels during the third postnatal week. 2. The synthetic glucocorticoid triamcinolone, administered as a single injection, produces a marked elevation of the ornithine-keto acid aminotransferase activity within 24 hr. if given postnatally before the natural increase in ornithine-keto acid aminotransferase activity has occurred. In foetal and adult animals, triamcinolone does not induce any increase in this enzyme activity. 3. The repeated administration of puromycin completely prevents the rise in ornithine-keto acid aminotransferase activity that follows triamcinolone administration. 4. If adult rats are fed with a protein-free carbohydrate diet, or one free of arginine, the ornithine-keto acid aminotransferase activity diminishes to a fraction of the normal. When such diets are given, a single injection of triamcinolone does not increase the enzyme activity within 24 hr. 5. Partial hepatectomy, and repeated injections of growth hormone, depress the ornithine-keto acid aminotransferase activity in adult rats. 6. The findings are discussed in relation to the mechanisms concerned with developmental and adaptive changes in enzyme activities in the liver.

L-Ornithine plays an important role in mammalian metabolism; it functions as a component in the mechanism for urea biosynthesis in the liver, and as a precursor for polyamine synthesis (Raina, 1964). The catabolic pathway of ornithine to glutamic acid comprises two enzymic reactions, of which the first, a transamination of ornithine with α -oxoglutaric acid, yields glutamic γ -semialdehyde and is catalysed by OKT.*

Data on the distribution between different tissues, the intracellular localization (Peraino & Pitot, 1963) and the properties (Meister, 1954; Peraino & Pitot, 1963; Katunuma, Matsuda & Tomino, 1964; Strecker, 1965) of this enzyme have been presented in detail. OKT activity displays distinct adaptive characteristics to changes in the protein content of the diet (Waldorf & Harper, 1963; Civen, Brown & Trimmer, 1967), to oral supplements of ornithine and arginine (Civen *et al.* 1967) and to the administration of corticosteroids (Peraino, Lamar & Pitot, 1966; Peraino, 1967).

However, no data are available as yet on changes in the activity of this enzyme during mammalian

development. In view of the marked changes that occur in urea and polyamine biosynthesis around birth (Räihä & Suihkonen, 1968; Jänne, Raina & Siimes, 1964), it was decided to investigate the developmental course of this enzyme, and to study the factors that affect this process.

EXPERIMENTAL

Chemicals. L-Ornithine, α -oxoglutaric acid and 2-amino-benzaldehyde were obtained from Fluka A.-G. (Buchs SG, Switzerland). Puromycin was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.), triamcinolone acetonide (Kenacort-T; 9- α -fluoro-16 α -hydroxyprednisolone acetonide) from Squibb A.-B. (Lidingö, Sweden) and growth hormone (Somacton) from Ferring A.-B. (Malmö, Sweden).

Animals. Sprague-Dawley-strain rats were used. The birth dates of the litters were carefully recorded after frequent inspection. The gestational age of the foetuses was calculated from the day of mating. The animals were killed by decapitation, and the liver samples blotted and weighed in cold for enzyme assays.

Assay for OKT. Depending on the expected enzymic activity of the liver, a tissue homogenate of 0.5–5% was prepared in cold 0.1 M-potassium phosphate buffer, pH 8.0. The assay medium consisted of 150 μ moles of L-ornithine,

* Abbreviation: OKT, ornithine-keto acid aminotransferase (L-ornithine-2-oxo acid aminotransferase, EC 2.6.1.13).

150 μ moles of α -oxoglutarate and 1.0 ml. of tissue homogenate in a total volume of 1.2 ml. (Katunuma *et al.* 1964). The incubation was continued for 30 min. at 37° and stopped with 0.5 ml. of 10% (w/v) trichloroacetic acid. Then 0.5 ml. of saturated 2-aminobenzaldehyde in *N*-HCl was added to the homogenate, and the colour of the complex formed was developed for 5 min. at 100° (Strecker, 1960). After the homogenate had been cooled to room temperature, the cell debris was removed by centrifugation for 10 min. at 2000g. The E_{440} value of the clear supernatant was measured against a tissue blank containing trichloroacetic acid. The amount formed of Δ^1 -pyrroline-5-carboxylic acid (the spontaneous cyclic form of glutamic γ -semialdehyde) was calculated by using ϵ_{mM} 2.71 for the coloured complex (Strecker, 1960). The protein content of the original homogenate was measured by the method of Lowry, Rosebrough, Farr & Randall (1951). The specific activity of OKT was expressed in the units recommended by the Commission on Enzymes of I.U.B. (1961) per g. of soluble liver protein (1 unit catalyses the formation of 1 μ mole of Δ^1 -pyrroline-5-carboxylic acid/min.).

The kinetic constants of OKT for L-ornithine and α -oxoglutaric acid were determined by using five L-ornithine or α -oxoglutarate concentrations, ranging from 0.43 to 4.3 mM; with the values of the reaction velocity as a basis, the K_m values for both substrates were derived graphically from the double-reciprocal plot ($1/v$ versus $1/s$).

Diet experiments. Complete restriction of protein in the diet was accomplished by feeding the rats with solid cane sugar and water *ad libitum*. The standard laboratory rat food was supplied by Hankkija Oy (Helsinki, Finland) and contained 24% of protein. The arginine-free diet was Special Nutrico Commercial Diet from Nutritional Biochemicals Corp.

The various diets were given for 6 days. On the fifth day triamcinolone was injected intraperitoneally into one group of animals from a litter, and the enzyme activity of the liver was assayed on the sixth day after the diet had begun, 24 hr. after the injection of hormone.

Partial hepatectomy and growth hormone. Partial hepatectomy was performed under ether anaesthesia by the method of Higgins & Anderson (1931). Three litters of 4-week-old rats were divided and some of the animals subjected to operation; the remainder were subjected to sham-operation only. The assays for OKT activity were carried out 24, 48, 72, 96 and 144 hr. after this procedure.

Seven units of growth hormone were injected intraperitoneally three times at 12 hr. intervals into some animals of a 3-week-old litter. An equal volume of 0.9% NaCl was administered to the remainder of the litter. The assay of OKT activity was carried out 48 hr. after the first injection.

RESULTS

Development of OKT activity in rat liver. The development of the specific activity of OKT is indicated in Fig. 1. Slight enzyme activity was detectable even in the youngest foetuses studied at 5 days before term. Birth was accompanied by a distinct rise in activity; on the second postnatal day there was a diminution to values similar to those before birth. From 14 days after birth the

Fig. 1. Development of OKT activity in rat liver.

Table 1. *Effect of triamcinolone on the specific activity of liver OKT in developing rats*

Each experiment was made with one litter divided into two groups. One group was injected intraperitoneally with 5 mg. of triamcinolone/100 g. body wt. at the age indicated and the control group with an equal volume of 0.9% NaCl. Both groups were killed 24 hr. after the injection for assay of the liver enzyme activity. Four to seven animals were used in each experimental group, and each result presented is a mean. For further details see the text.

Age of animals (days)	OKT activity (units/g. of soluble liver protein)		Injected/controls OKT activity ratio
	Controls	Injected	
-1	2.1	2.9	1.4
0	1.9	5.6	2.9
1	0.6	7.9	13.2
4	0.5	8.0	16.0
12	1.2	14.1	11.8
Adult	15.1	15.4	1.0

enzyme activity steadily increased, to attain adult values about 25 days after birth.

The apparent K_m values of OKT for L-ornithine and α -oxoglutarate were similar for both foetal and adult tissue homogenates, being 2.1 and 2.4 mM for L-ornithine and 1.8 and 2.2 mM for α -oxoglutarate in foetal and adult liver homogenates respectively.

Effect of triamcinolone on the development of OKT activity. Table 1 shows that the parenteral administration of triamcinolone induced an increase in OKT activity in the animals when the hormone was injected after birth and before the specific activity of the enzyme had attained the normal adult level. Only a very slight elevation of the low foetal activity was caused by triamcinolone. Similarly in the adult animals a single dose of triamcinolone did not exercise any influence on the specific activity of OKT. After birth the relative effect increased, and

Fig. 2. Effect of puromycin on the triamcinolone-induced increase of liver OKT activity in developing rats. Repeated intraperitoneal injections of puromycin (5 mg./100 g.) were given every 2 hr. between 12 and 24 hr. after a single injection of triamcinolone (5 mg./100 g.) to 6-day-old rats (○). Control rats received equal injections of 0.9% NaCl (●).

Table 2. *Effect of diet and triamcinolone on the specific activity of liver OKT in the adult rat*

The various diets were administered for 6 days. On the fifth day 5 mg. of triamcinolone/100 g. body wt. was injected intraperitoneally into one group of animals. The liver enzyme activity was assayed on the sixth day after the diet had begun. Each result represents the mean $\pm$ s.d.; the numbers of animals studied are indicated in parentheses.

Diet	OKT activity (units/g. of soluble liver protein)
Standard food	15.1 $\pm$ 2.3 (7)
+ triamcinolone	15.4 $\pm$ 0.6 (7)
Cane sugar	0.5 $\pm$ 0.1 (7)
+ triamcinolone	0.9 (2)
Cane sugar + arginine	3.5 (2)
+ triamcinolone	3.7 (3)
Arginine-free diet	2.4 (3)
+ triamcinolone	1.5 (3)

was maximal on the fourth postnatal day. The highest absolute increment in the specific activity after glucocorticoid administration occurred in the 12-day-old group; in these animals the treatment caused the OKT activity to reach adult levels in 24 hr.

Repeated intraperitoneal injections of puromycin given to 6-day-old rats between 12 and 24 hr. after a single injection of triamcinolone almost completely inhibited the induced rise in enzyme activity (Fig. 2).

Effect of diet and triamcinolone on OKT activity in adult rats. The effects of the various diets are presented in Table 2. The feeding of protein-free carbohydrate diet for 6 days almost completely abolished the OKT activity of the liver. When 2% (w/v) L-arginine hydrochloride was added to the drinking water the diminution in the specific activity of the enzyme was less marked. A diet free of arginine also brought about a very considerable diminution in liver OKT activity. Table 2 indicates that a single injection of triamcinolone had no clear effect on the OKT activity during the administration of any of the diets tested.

Effect of partial hepatectomy and growth hormone on OKT activity. After partial hepatectomy the specific activity of OKT exhibits a more significant decrease in the remaining liver tissue than that observable in the sham-operated animals. Moreover, if a comparison is made with the non-operated group of animals, these exhibited significantly diminished values (Table 3). In both operated and sham-operated groups the effect of the procedure was maximal at 72 hr. after the operation.

The repeated administration of growth hormone induced a decrease in the specific activity of OKT from 9.8 ± 2.3 units/g. of liver protein (mean $\pm$ s.d.) to 5.6 ± 1.0 units/g. in 2 days. This effect was statistically significant ($P < 0.01$).

DISCUSSION

During the development of the rat two periods of increase are apparent in the specific activity of liver

Table 3. *Effect of partial hepatectomy and sham-operation on the specific activity of OKT in rat liver*

The time indicates that elapsed from operation to the assay of enzyme activity. Each result represents the mean $\pm$ s.d.; the numbers of animals studied are indicated in parentheses. The statistical significance of the results according to the Student's *t* test, for the operated group between the operated and sham-operated animals and for the sham-operated group between the sham-operated and control groups, is indicated as follows: * $P < 0.05$; ** $P < 0.001$.

Time (hr.) ... Treatment of rats	OKT activity (units/g. of soluble liver protein)				
	24	48	72	96	155
Partial hepatectomy	17.1 $\pm$ 4.4 (11)	11.1 $\pm$ 2.2 (9)**	5.9 $\pm$ 1.4 (5)**	9.4 $\pm$ 3.4 (6)*	15.6 (3)
Sham-operation	19.1 $\pm$ 4.8 (6)	15.2 $\pm$ 2.6 (7)	10.8 $\pm$ 2.4 (6)**	12.9 $\pm$ 2.9 (6)*	—
None (control animals)	16.2 $\pm$ 3.6 (28)				

OKT, a transient elevation around birth and a persisting increase to adult levels that begins 14 days after birth. During these first 2 weeks of postnatal life administration of the glucocorticoid triamcinolone exerted a marked inducing effect on the liver OKT activity, though the resultant activity did not exceed the normal levels of adult animals. By means very similar to those applied in these experiments, Doell & Kretchmer (1964) succeeded in bringing about a precocious development to adult levels of intestinal invertase activity in 3–9-day-old rats with hydrocortisone injections.

Nevertheless when the hormone was administered to foetuses before term it exerted no effect on the OKT activity. The lack of response of the enzyme in foetal animals suggests that the developmental increase around birth is not initiated by corticoids, and that such hormones are capable of influencing the regulatory mechanisms only when the competence to synthesize the enzyme has matured. Corticosteroid administration to foetal rats does not increase tyrosine aminotransferase activity (Sereni, Kenney & Kretchmer, 1959) or tryptophan pyrrolase activity (Nemeth, 1959) in the liver, though a marked increasing effect is obtained after birth.

The latter rise in OKT activity occurs during the rat's weaning period, during which time some other enzymes, e.g. liver argininosuccinate synthetase (Räihä & Suihkonen, 1968), liver glucokinase (Walker, 1966) and intestinal invertase (Doell & Kretchmer, 1964), exhibit a rapid increase in specific activity. Räihä & Suihkonen (1968) have suggested that the increase in some of these enzyme activities during the third week after birth is adaptive in character, and is triggered by a shift in the balance between the intake and utilization of protein and amino acids. This could occur when the diet of the rat is changed from a species-specific and comparatively low-protein diet to the standard laboratory food, which is richer in protein. The rapid increase in urea formation that occurs in the rat at this time (Illnerová, 1966) provides further support for such a concept. 'It is thus possible to interpret many of the enzymic adaptations that occur during the neonatal and weaning periods of the rat in terms of adaptations known to occur in the adult animal in response to dietary changes' (Vernon & Walker, 1968).

No change in the kinetic constants of OKT was observed during the development of the rat from a foetus to an adult animal, which suggests that the alterations in activity of this enzyme are attributable to quantitative changes in the same enzyme protein.

Feeding rats with pure carbohydrate resulted in a very marked diminution in the specific activity of OKT, which is in agreement with the data reported

by Peraino (1967). This repression was removed only partially by the addition of L-arginine to the diet (cf. Civen *et al.* 1967). A diet that supplied adequate amounts of protein constituents other than arginine was also accompanied by subnormal enzyme activity (Table 2). In the light of the quantitative effects of these diets on the activity of OKT, it is possible that arginine (ornithine) on the one hand, and other amino acids (protein) on the other, exert their regulatory influence on the enzyme synthesis in a manner that is at least partially synergistic. Triamcinolone was incapable of reversing the repressive effect of the protein-free carbohydrate diet on OKT, as has also been noted by Peraino *et al.* (1966). During administration of both the arginine-free diet and the arginine-supplemented carbohydrate diet, the glucocorticoid also did not exert any significant effect. The results obtained in earlier studies of the influence of glucocorticoid administration during different types of diets suggest that 'amino acids are primary effectors in the induction of ornithine transaminase (OKT)', and that possibly glucocorticoid hormones only 'alter the capacity of the regulatory systems for these enzymes to respond to amino acids' (Peraino, 1967).

Apparently the low OKT activity in the rat liver during feeding with an arginine-free diet has another implication; the rat is capable of growth during the withdrawal of exogenous arginine (Scull & Rose, 1930; Rose, 1938). Nonetheless the only reaction known to be capable of synthesis of the $\alpha\delta$ -diaminovaleric acid carbon chain of ornithine etc. is the reversed transamination catalysed by OKT (Womack & Rose, 1947; Sallach, Koeppe & Rose, 1951; Smith, Benziman & Strecker, 1967). However, the reaction balance of this enzyme is greatly in favour of Δ^1 -pyrroline-5-carboxylic acid formation (K about 50; Meister, 1954; Strecker, 1965). As it has now been found that the enzyme activity diminishes to a fraction of the normal level in the absence of arginine, considerable doubt exists as to whether this remaining activity can supply the organism with ornithine formed from Δ^1 -pyrroline-5-carboxylic acid. To start the reversed reaction of this enzyme, the intramitochondrial concentration of ornithine with respect to α -oxoglutaric acid should be very low (Smith *et al.* 1967). Under these circumstances the presence of another enzyme, with a function that is the reverse of that of OKT and that is repressed during the normal supply of arginine, provides a better hypothesis.

Partial hepatectomy and the administration of growth hormone both induced a diminution in the activity of OKT. Both these experimental conditions promote the utilization of ornithine and arginine for polyamine and protein syntheses respectively (Jänne *et al.* 1964; Jänne & Raina,

1966; Jänne, 1967). It is not surprising that this enzyme, probably of primary importance in control of the catabolism of arginine and ornithine, is repressed by these treatments. Presumably this repression is mediated through smaller stimulation by the substrates. Similar repression is also apparent in the activity of ornithine carbamoyl-transferase after partial hepatectomy and the administration of growth hormone (Räihä, Jänne & Suihkonen, 1967).

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