Effect of inhibition of fatty acid oxidation on neonatal liver carnitine content

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The hypoglycaemic agent 2-tetradecylglycidic acid (compound McN-3802) caused an increase in total liver carnitine content, this being due primarily to an increase in the free carnitine pool. In the neonatal animal, this may represent a mechanism to overcome the inhibitory effect of fatty acid oxidation by the drug.

Milk is the only source of food for the suckling rat until about day 15. The triacylglycerol content of milk is high, contributing 70% of the energy requirement of the neonatal animal (Hahn & Koldovsky, 1966). One of the results of this high-fat diet is elevated plasma concentrations of non-esterified fatty acids and ketones compared with those of adult rats (Aranda *et al.*, 1973; Girard *et al.*, 1973; Robles-Valdez *et al.*, 1976). Blood glucose concentration is maintained by synthesis *de novo* by the liver (Ballard & Oliver, 1963), this being initiated shortly after birth corresponding to the development of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) activity (Ballard & Hanson, 1967). In the starved adult rat, interference of fatty acid oxidation decreases the ability of the liver to produce glucose (Tutwiler & Dellevigne, 1979). This is true in the fed neonatal rat (Pégorier *et al.*, 1977; Frost & Wells, 1981*b*), and it establishes a relationship between ketogenesis and gluconeogenesis in the neonatal animal (Ferré *et al.*, 1981). However, in the neonatal animal, inhibition of oxidation of fatty acids causes a marked accumulation of triacylglycerol in the liver (Frost & Wells, 1981*b*). The inhibitor used in this later study was 2-tetradecylglycidic acid, a hypoglycaemic agent whose proposed site of action is carnitine palmitoyltransferase (EC 2.3.1.21) (Tutwiler & Dellevigne, 1979). This enzyme, located on the outer surface of the mitochondrial inner membrane, catalyses the synthesis of an acylcarnitine from an acyl-CoA, this reaction facilitating the transport of the acyl group across the mitochondrial membrane (for review see McGarry & Foster, 1980). Since L-carnitine is also a substrate for carnitine palmitoyltransferase, it was decided to investigate the effect of 2-tetradecylglycidic acid on the carnitine pool of the neonatal liver.

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Methods and materials

Animals

Suckling rats were obtained from a breeding colony maintained at the University of Arizona Division of Animal Resources and were handled as described previously (Frost & Wells, 1981*a*). Breeding pairs (Sprague–Dawley strain; Hilltop, Chatsworth, CA, U.S.A.) were allowed free access to food and water. Nine-day-old rats were given 2-tetradecylglycidic acid (10 µg/g body wt.; suspended in tragacanth) by gastric tube (PE-50, Intramedic). Control neonatal animals were left with the dam for another 18 h. At about 09:00 h the following morning, livers were excised, frozen with clamps pre-cooled in liquid N₂, pulverized at –40°C and processed as described by Brass & Hoppel (1978), except that the initial HClO₄ precipitate of the liver preparation was washed once with 3% (w/v) HClO₄.

Carnitine assay

Duplicate portions (0.1 ml) of these samples were assayed for free carnitine, acid-soluble carnitine and acid-insoluble carnitine by the Brass & Hoppel (1978) modification of the method of Cederblad & Lindstedt (1972), as modified by Böhmer *et al.* (1974), except that the [³H]acetyl-CoA was removed from the assay mixture with Dowex by the method of McGarry & Foster (1976). The radioactivity of a 0.5 ml portion of the supernatant (representing [³H]acetylcarnitine) was counted in Betaphase (WestChem Products, San Diego, CA, U.S.A.) in a Beckman LS 250 liquid-scintillation counter. The concentration of carnitine in the samples was calculated by comparison with authentic L-carnitine standards (0–5 nmol). The results were expressed as means ± s.d. and significance was calculated by the unpaired Student's *t* test.

Chemicals

Acetyl-CoA (lithium derivative) was purchased from P-L Biochemicals (Milwaukee, WI, U.S.A.).

[³H]Acetyl-CoA (>500mCi/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Carnitine acetyltransferase (EC 2.3.1.7) [5 mg/ml, >80 units (μmol/min)/mg] was purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Dowex 1-X8 anion-exchange resin (200–400 mesh; Cl⁻ form) was from Bio-Rad Laboratories (Richmond, CA, U.S.A.). 2-Tetradecylglycidic acid (compound McN-3802) and tragacanth were gifts from Dr. Gene Tutwiler (McNeil Laboratories, Fort Washington, PA, U.S.A.). L-Carnitine was provided by Dr. Merle Olson, Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX, U.S.A. All other chemicals were of the highest quality available.

Results and discussion

The dose of 2-tetradecylglycidic acid used in these experiments was previously shown to cause 50% inhibition of palmitic acid oxidation *in vivo* and 100% inhibition of palmitic acid oxidation in perfused liver of treated neonatal animals (Frost & Wells, 1981b).

Effect of 2-tetradecylglycidic acid on body weight and liver weight

There was no significant change in the body weight of 10-day-old rats as a result of 2-tetradecylglycidic acid treatment. In contrast, the liver weight of the 2-tetradecylglycidic acid-treated animals was significantly higher than that of controls (0.469 ± 0.055 g and 0.750 ± 0.035 g for livers of control and 2-tetradecylglycidic acid-treated rats respectively; $P < 0.001$). This increase in weight was primarily due to accumulation of lipid in the form of triacylglycerol (Frost & Wells, 1981b).

Effect of 2-tetradecylglycidic acid on the liver carnitine pool

The concentration of carnitine found in the liver of

control animals is given in Table 1. The total was slightly higher than published values for 10-day-old rats (652 nmol/g of liver; Yeh *et al.*, 1978). In comparison with adult liver, this concentration is 3 times that in the fed state and 1.7 times that in the starved state (2–6 days) (Brass & Hoppel, 1978). In the neonatal rat liver the ratio of free to esterified (i.e. acid-soluble plus acid-insoluble) carnitine is approximately 1.0:1. A similar ratio has been reported for adult liver by Kondrup & Grunnet (1973) and Brass & Hoppel (1978), although a higher ratio was reported for adult liver by Pearson & Tubbs (1967).

2-Tetradecylglycidic acid treatment of neonatal rats caused an increase in total liver carnitine of about 50%. This increase was primarily due to an increase in free carnitine, resulting in a free/esterified carnitine ratio of 1.7:1. This increase is actually underestimated, owing to the increase in liver weight caused by triacylglycerol accumulation. When carnitine concentration is calculated per liver (Table 1), the free carnitine content of livers of 2-tetradecylglycidic acid-treated animals is 2.3 times that of control livers. This compares with a 1.4-fold increase when the concentrations are expressed per g of liver.

The mechanism of action of 2-tetradecylglycidic acid is thought to be through its inhibition of carnitine palmitoyltransferase, thereby blocking oxidation of long-chain fatty acids (Tutwiler & Delleveigne, 1979). The accumulation of triacylglycerol in the liver is consistent with a block at carnitine palmitoyltransferase. Thus the lack of change in the pool of long-chain carnitine esters was surprising, since *in vitro* a decrease in long-chain carnitine esters as a result of 2-tetradecylglycidic acid treatment has been demonstrated (Tutwiler & Delleveigne, 1979). Although there is no experimental explanation for this discrepancy at the present time, the results clearly suggest that the action of 2-tetradecylglycidic acid *in vivo* in neonatal animals is not as simple as suggested for the situation *in vitro*.

Table 1. *Effect of 2-tetradecylglycidic acid on the concentration of liver carnitine in 10-day-old suckling rats*
For details see the Methods and materials section. The data represent means $\pm$ s.d. where $n = 5$. The data are expressed in part A as nmol/g of liver and in part B as nmol/liver. Abbreviations: Free, free carnitine; ASC, acid-soluble carnitine; AIC, acid-insoluble carnitine.

Conditions	Free	ASC	AIC	Total	Free/(ASC + AIC)
Part A					
Control	365.1 ± 78.7	231.7 ± 65.8	129.5 ± 29.6	726.2 ± 62.1	1.01
2-Tetradecylglycidic acid	$642.5 \pm 54.4^*$	257.2 ± 63.0	115.4 ± 22.6	$1015.1 \pm 84.1^\dagger$	1.72
Part B					
Control	168.3 ± 28.4	112.8 ± 42.3	60.5 ± 14.3	333.6 ± 53.4	0.97
2-Tetradecylglycidic acid	$482.3 \pm 51.2^*$	193.7 ± 51.4	86.2 ± 14.8	$726.2 \pm 81.5^\dagger$	1.72

* $P < 0.001$ compared with control.

† $P < 0.0001$ compared with control.

The increase in free carnitine is possible through several mechanisms. Although carnitine values for neonatal skeletal muscle have not been published, much of an adult rat's body carnitine is located in muscle mass (Brass & Hoppel, 1978). Thus an increase in flux of carnitine from muscle to the liver might account for an increase in liver carnitine. Alternatively, a decrease in efflux from the liver or a decrease in degradation by the liver might also account for an increase in the carnitine pool. Perhaps the most interesting hypothesis is an increase in synthesis of carnitine *de novo*, which might represent a compensatory mechanism by which the body attempts to overcome the inhibition of the transferase. If this could be demonstrated experimentally, then potentially 2-tetradecylglycidic acid could be used to study the regulation of carnitine biosynthesis in the neonatal animals. It must be noted that until day 7 after birth neonatal rats obtain carnitine via mother's milk (Robles Valdez *et al.*, 1976). After this time the liver is able to catalyse the conversion of γ -butyrobetaine (the immediate precursor of carnitine, synthesized primarily in the kidney of the adult; Carter & Frenkel, 1979) into carnitine via γ -butyrobetaine hydroxylase (EC 1.14.11.1) (Hahn, 1981).

Despite the different sources of carnitine (exogenous as opposed to synthesis *de novo*) and different synthetic capabilities of the liver, the concentration of plasma carnitine does not change during the neonatal period on into the adult stage (Robles-Valdez *et al.*, 1976). This indicates that an active-transport system must exist that transports carnitine against a concentration gradient, buffering the plasma against any change. We have determined that at physiological concentrations of circulating carnitine (50 μ M; Hahn *et al.*, 1980) the net rate of uptake by the perfused liver from a 14-day-old rat is 2.7 ± 0.2 nmol of carnitine/min per g of liver and that in the range 50–500 μ M the uptake is linear and concentration-dependent (S. C. Frost & M. A. Wells, unpublished work). Thus in less than 1 h the liver could clear the entire circulating carnitine pool if no further supply were available. The effect of 2-tetradecylglycidic acid on this process may provide additional information on the mechanism of elevated carnitine concentration in the neonatal rat liver.

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