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PEROXISOMAL β-OXIDATION AND SODIUM VALPROATE

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Abstract—The influence of sodium valproate on peroxisomal β-oxidation was investigated in rats, by evaluating *in vivo* changes in hepatic H_2O_2 production, using a combination of the catalase inhibitor 3-amino-1,2,4-triazole, and methanol. In rats starvation causes an increased flux of fatty acids through the peroxisomal β-oxidation pathway. Valproate inhibits the formation of 3-hydroxybutyrate but not increased H_2O_2 production during starvation. There is no inhibitory effect of valproate on the peroxisomal oxidase. At low valproate concentrations it is possible that peroxisomes partially take over impaired mitochondrial function.

Sodium valproate (sodium *n*-dipropylacetate, sodium 2-propylpentanoate) is a widely used anti-epileptic drug. Especially in children and in young adults, it may lead to severe toxic liver disease, the mechanism of which is not understood. Hepatic coma is often preceded by a period of starvation [1, 2].

The inhibition of fatty acid β-oxidation by valproate [3] has become well documented in recent years. Isolated hepatocytes from fed and starved rats were used to demonstrate the influence of valproate on β-oxidation *in vitro* [4–6]. *In vivo* studies of the valproate effect demonstrate decreased concentrations of ketobodies in urine [7] and blood [6, 8] from rats and patients. Becker and Harris [5] demonstrate a significant lowering of liver 3-hydroxybutyrate concentration and the 3-hydroxybutyrate/acetoacetate ratio in valproic acid treated intact rats. Papers on this subject do not make a distinction between β-oxidation in mitochondria and peroxisomes. The data relate to total fatty acid oxidation and conclusions only take the mitochondrial pathway into consideration.

The purpose of this study is to investigate the influence of valproate on peroxisomal β-oxidation. During the first step of peroxisomal β-oxidation H_2O_2 is produced. In this laboratory a method was developed to evaluate *in vivo* changes of H_2O_2 production in the liver of unanaesthetized mice, using a combination of the catalase inhibitor, 3-amino-1,2,4-triazole, and methanol. It was shown that increased H_2O_2 production in starved mice reflects an increased flux of fatty acids through the peroxisomal β-oxidation system [9]. We have studied the influence of valproate on peroxisomal β-oxidation by evaluating changes in H_2O_2 production in the liver of rats, in which β-oxidation in the peroxisomes was increased by starvation. Because drug metabolism by the cytochrome P-450 monooxygenase system can lead to H_2O_2 production [10], the influence of phenobarbital pretreatment was also examined.

MATERIAL AND METHODS

Male Wistar rats (250–300 g) were used in our experiments. Aminotriazole (1 g/kg in 0.9% NaCl), methanol (3.5 mmoles/kg), sodium valproate (in 0.9% NaCl) and sodium octanoate (in 0.9% NaCl) were administered by intraperitoneal injection. Aminotriazole† and methanol were administered simultaneously. Rats were killed by decapitation 1 hr after administration of valproate or octanoate and 1 or 3 hr after administration of aminotriazole–methanol. Some rats were pretreated with phenobarbital, by intraperitoneal injections (80 mg/kg) on three consecutive days prior to the experiments. Fed rats received standard laboratory diet (AO3-UAR, France). Starved rats were deprived of food (water *ad lib.*) for 24 or 48 hr.

Catalase activity was assayed at 0° in total liver homogenate by the titanium oxysulphate method [11]. 1 U_B is the amount of catalase which breaks down 90% of the substrate (1.5 mmoles H_2O_2/l) in a volume of 50 ml at 0° in 1 min; maximal reaction time is 10 min. For each animal the catalase activity calculated is the mean of ten measurements after 1–10 min reaction time. Residual catalase activity (RCA) is the catalase activity that remains after inhibition by aminotriazole alone or in combination with methanol. A lower RCA reflects a higher H_2O_2 production [9].

3-Hydroxybutyrate in plasma was determined according to Williamson *et al.* [12].

Each experimental group consisted of at least 5 animals. All results are presented as the mean ± S.E.M. For statistical analysis, the Mann–Whitney test was used [13].

Sodium valproate was a gift from Labaz (Belgium). Aminotriazole and sodium octanoate were obtained from Sigma and Merck respectively.

RESULTS

Catalase activity in the liver of untreated, standard diet fed rats is 85.12 ± 4.78 U_B/g of wet liver. After administration of aminotriazole, RCA declines ex-

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† Abbreviations: aminotriazole (AT), 3-amino-1,2,4-triazole; RCA, residual catalase activity.

ponentially with time [14], reaching a value of 4.63 ± 1.18 U_B/g of liver after 1 hr. Simultaneous administration of aminotriazole and methanol during 1 hr causes RCA to rise to a value of 70.83 ± 4.30 U_B/g of liver. Administration of aminotriazole and methanol during 3 hr brings down RCA to 36.07 ± 4.16 U_B/g of liver.

The influence of starvation and valproate administration on rat liver RCA and on plasma 3-hydroxybutyrate concentration is summarized in Table 1.

As could be expected, 3-hydroxybutyrate concentrations are increased after 24 and 48 hr of fasting; RCA is significantly decreased after 24 hr of starvation and decreases even more after 48 hr of starvation.

Valproate never causes a significant increase of RCA in the liver of 24 and 48 hr starved rats. There are even two cases in which significant decrease of RCA is noted: after administration of 200 mg/kg of valproate (aminotriazole–methanol, 1 hr) and of 100 mg/kg of valproate (aminotriazole–methanol, 3 hr). This stimulatory effect of valproate on H_2O_2 production is not increased by phenobarbital pretreatment. Phenobarbital pretreatment itself does not change liver RCA after 24 hr of starvation.

In fed animals valproate does not change liver RCA.

Administration of octanoate in amounts comparable to valproate (200 and 400 mg/kg) does not significantly increase plasma 3-hydroxybutyrate concentration and causes no significant decrease of liver RCA.

Administration of valproate causes a significant decrease of plasma 3-hydroxybutyrate concentration in 24 and 48 hr starved rats, but not in fed animals.

Sole administration of valproate (200 mg/kg, 1 hr) has no influence on total catalase content in rat liver.

DISCUSSION

In 24 hr starved mice increased H_2O_2 production in the liver reflects an increased flux of fatty acids through the peroxisomal β-oxidation system [9]. The present results demonstrate a similar phenomenon in rats: after 24 and 48 hr of starvation H_2O_2 production in rat liver is significantly increased. Plasma 3-hydroxybutyrate concentration is strongly increased after a 24 hr starvation period; it does not significantly rise between 24 and 48 hr of fasting. These results are in agreement with literature data [15, 16]. In rats, all hallmarks of the ketotic state which develop during prolonged fasting are fully present after only 12 hr of starvation [15]. We still measure a significant increase of H_2O_2 production between 24 and 48 hr of starvation. Activation of the peroxisomal β-oxidation system therefore seems to be a rather slow response to the higher supply of fatty acids resulting from starvation.

Administration of valproate during 1 hr to 24- and 48 hr-starved rats decreases 3-hydroxybutyrate concentration in plasma. Liver RCA is not increased; this means that H_2O_2 production from peroxisomal β-oxidation is not diminished. Although overall β-oxidation is inhibited by valproate there is no effect on the peroxisomal contribution. The inhibitory effect of valproate is restricted to the mitochondrial system.

Under some experimental conditions we notice a significant increase of H_2O_2 production due to valproate treatment. Metabolism of valproate itself might elicit H_2O_2 production, either through the β-oxidation pathway in the peroxisomes or by the P-450 dependent drug metabolizing system. Valproate is an eight carbon branched fatty acid. It is known that valproate can be β-oxidized in the peroxisomes [17]. In general the affinity of the peroxisomes for C8 fatty acids (octanoate) is low [18, 19]. Oshino *et al.* [20] however demonstrated an increased H_2O_2 production in perfused rat liver after addition of octanoate. When different doses of valproate are given to rats we notice no increase of H_2O_2 production with higher doses. On the contrary H_2O_2 production is only stimulated at the lower doses. Administration of octanoate in comparable doses causes no increased H_2O_2 production. Valproate is known to be ω-oxidized [21]. This metabolic pathway is under the influence of cytochrome P-450 [1]. To examine the possibility of valproate producing H_2O_2

Table 1. Influence of starvation and valproate administration on RCA values in total rat liver homogenate and on plasma 3-hydroxybutyrate concentration.

Conditions	RCA (U_B/g of liver)			3-hydroxybutyrate (mM)
	AT + methanol, 1 hr	AT + methanol, 3 hr	AT alone, 1 hr	
24 hr of starvation	53.15 ± 4.27 (a)	21.07 ± 2.38 (c)	2.89 ± 0.27	1.27 ± 0.10 (e)
24 hr of starvation + valproate:				
100 mg/kg		15.10 ± 1.60 (d)	3.17 ± 0.21	0.49 ± 0.05 (f)
200 mg/kg	34.65 ± 2.24 (b)	17.97 ± 2.40	3.49 ± 0.20	0.48 ± 0.07 (g)
400 mg/kg	49.76 ± 2.24	20.01 ± 1.59		0.18 ± 0.05 (h)
48 hr of starvation	41.72 ± 1.45			1.78 ± 0.21 (i)
48 hr of starvation + valproate:				
200 mg/kg	37.62 ± 3.29			0.37 ± 0.08 (j)
400 mg/kg	33.70 ± 4.64			0.27 ± 0.02 (k)
Fed	70.83 ± 4.30	36.07 ± 4.16	4.63 ± 1.18	0.03 ± 0.01
Fed + valproate 200 mg/kg	74.77 ± 5.96	53.51 ± 14.11		0.04 ± 0.01

Aminotriazole (AT) alone or aminotriazole in combination with methanol are administered 1 or 3 hr before killing the animals. Statistical significance of differences: RCA values: a vs b and c vs d, $P \leq 0.05$; 3-hydroxybutyrate: e vs f, e vs g, e vs h, i vs j and i vs k, $P \leq 0.01$.

through the P-450 dependent drug metabolizing system, we treated fed rats with valproate. Our method is able to detect extraperoxisomal H_2O_2 production caused by the metabolization of drugs (*N*-demethylation of aminopyrine) in rats [22]. In fed rats we see no decrease of RCA indicating supplementary H_2O_2 production following valproate administration. Induction of the drug metabolizing system by phenobarbital does not enhance the valproate effect in 24-hr starved animals. It therefore seems unlikely that increased H_2O_2 production following valproate treatment results from an extraperoxisomal source. Both valproate treatment [7] and fasting [23] are factors that stimulate the formation of dicarboxylic acids, which can be β-oxidized in peroxisomes [24, 25]. Perhaps more dicarboxylic acids are passing through the peroxisomal oxidase when mitochondrial β-oxidation is impaired, thus giving rise to increased amounts of H_2O_2. This hypothesis, however, does not explain why this effect is not seen with high doses of valproate. It is also possible that after valproate treatment peroxisomes take over part of the impaired mitochondrial β-oxidation function for all fatty acids. This was already suggested by Mortensen [24, 25] in general for a number of pathological situations, such as inborn enzymatic defects located at acyl-CoA dehydrogenase (non-ketotic dicarboxylic aciduria and glutaric aciduria type II), hypoglycin A intoxication, systemic carnitine deficiency and during valproate treatment. Peroxisomes seem to take over part of the mitochondrial function at low valproate concentrations only. The valproate concentrations we use are based on those used in literature to study its metabolic effects [5, 6, 26]. These concentrations are higher than therapeutic doses (20–60 mg/kg day) [8]. According to Turnbull *et al.* [6] β-oxidation is also inhibited at therapeutic concentrations. It seems possible that under these conditions peroxisomal contribution to overall β-oxidation becomes maximal.

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