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Mechanism of Zone-Specific Hepatic Steatosis Caused by Valproate: Inhibition of Ketogenesis in Periportal Regions of the Liver Lobule

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SUMMARY

Microvacuolar steatosis in periportal regions of the liver lobule was produced by injection of fasted rats with a single dose of valproate (500 mg/kg, subcutaneously). In livers perfused in the absence of exogenous fatty acids, ketone body (acetoacetate + β-hydroxybutyrate) production was decreased by valproate (500 μM) maximally by 67%. Concomitantly, NADH fluorescence detected from the liver surface declined about 30% with a time course similar to that of the inhibition of ketogenesis. Valproate had little effect on oxygen uptake but caused an elevation of the steady state level of catalase-H_2O_2 corresponding to an increase in H_2O_2 production of about 6 μmol/g/hr. In addition, valproate decreased the rate of oxidized glutathione release into bile by 45% but had little effect on bile flow. In the presence of oleate (250 μM), valproate inhibited ketone body production by 46% and decreased NADH fluorescence by 39%. Rates of ketogenesis in periportal and pericentral regions of the liver lobule were calculated from changes in NADH fluorescence detected with micro-light guides during infusion of valproate in the presence and absence of fatty acids. In the absence of valproate, endogenous ketogenesis was about 35 μmol/g/hr in both regions of the liver lobule. In the presence of oleate, however, rates were significantly higher in pericentral regions (89 ± 2 μmol/g/hr) than in periportal areas (71 ± 3 μmol/g/hr). In the presence of added oleate, valproate decreased rates of ketogenesis to 34 ± 4 μmol/g/hr in periportal regions and 51 ± 3 μmol/g/hr in pericentral areas. We conclude, therefore, that fat accumulates in periportal areas because valproate depresses ketogenesis to a greater extent in hepatocytes localized around the portal triad.

The increasing clinical use of the anticonvulsant valproic acid has been accompanied by reports of hepatic dysfunction and death due to liver failure (1–3). The histological appearance of human liver in cases of valproate-induced toxicity is similar to that found in Reye's syndrome and hypoglycin poisoning (2, 3). The primary visible change in these three conditions is microvesicular steatosis, appearing first in periportal regions of the liver lobule (2, 3). Rats treated acutely with large doses of valproate exhibit changes in hepatic morphology similar to those observed in cases of valproate toxicity in humans, although the parenchymal cell necrosis observed in some humans is absent in rats (4).

Valproate is therapeutically effective only at high plasma concentrations (>200 μM) (5). It presumably enters the mitochondria in a carnitine-independent manner and undergoes partial β-oxidation (6). Valproate inhibits ketogenesis from palmitate in isolated hepatocytes (7), the metabolism of decanoate in liver homogenates (8), and has been shown to decrease circulating ketone bodies in fasted rats *in vivo* (7). No specific site of action has been identified for the valproate-induced inhibition of ketogenesis. Kesterson *et al.* (9) have suggested that valproate might diminish the availability of CoA as well as inhibit an unspecified enzyme of β-oxidation. Hypoglycin A and 4-pentenoate, which yield metabolites structurally similar to those of valproate, have been shown to inhibit β-oxidation and ketogenesis by inactivating short-chain acyl CoA dehydrogenase and 3-ketothiolase, respectively (10, 11). In addition, in the presence of abundant α-glycerophosphate, inhibition of β-oxidation by valproate and the availability of large quantities of both valproate and endogenous fatty acids would be expected to lead to an increased rate of triglyceride synthesis and fatty liver (12).

The recent development of a technique to quantitate rates of ketogenesis in individual periportal and pericentral regions of the liver lobule based on the detection of NADH fluorescence with micro-light guides (13) now makes studies of alterations of ketogenesis in specific regions of the liver lobule by hepatotoxins possible. This study was initiated to determine whether

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ABBREVIATION: GSSG, oxidized glutathione.

selective inhibition of fatty acid oxidation in periportal regions of the liver lobule by valproate is involved in the mechanism of zone-specific accumulation of lipid observed clinically and experimentally. The data indicate that valproate indeed inhibits ketogenesis to the greatest extent in periportal regions of the liver lobule. Preliminary accounts of this work have appeared elsewhere (14, 15).

Materials and Methods

Chemicals. Valproate, as the free acid, was obtained from Saber Laboratories, Inc. (Morton Grove, IL). The sodium salt was prepared by dissolving the acid in a small volume of 95% ethanol and adding sufficient 1 N NaOH to adjust the pH of the solution to 10.0. The solvent was evaporated under a stream of N_2 and the dry sodium salt was dissolved in 0.9% sodium chloride and neutralized with 1 N HCl. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Liver perfusion. Fasted female rats (200–250 g) were used in all experiments. Livers were perfused via the portal vein with Krebs-Henseleit bicarbonate buffer, saturated with 95% O_2/5% CO_2, and maintained at 37° (16). The effluent perfusate flowed past a Teflon-shielded, Clark-type electrode for measurement of oxygen concentration. Rates of O_2 uptake were calculated from influent minus effluent oxygen concentration differences, the flow rate, and the liver wet weight.

Bile was collected via a cannula (PE-10 polyethylene tubing) placed in the common bile duct. Sodium valproate was infused into the perfusate in a solution of defatted (17) bovine serum albumin (molar ratio of valproate:albumin was 6:1). The sodium salt of oleate was bound to defatted albumin solution prior to infusion (oleate:albumin ratio was 4:1).

Metabolite determinations. Acetoacetate and β-hydroxybutyrate in the effluent perfusate and GSSG in bile were assayed by standard enzymatic techniques (18, 19). Rates of metabolite production were calculated from influent minus effluent concentration differences, the flow rate of perfusate or bile, and the liver wet weight.

Measurement of NADH fluorescence and catalase-H_2O_2. NADH fluorescence (366 → 450 nm) from the liver surface was detected with a large-tipped (tip diameter 2 mm) fiber optic light guide as described previously (20). The steady state level of catalase-H_2O_2 was determined spectrophotometrically (660–640 nm) with an air-turbine dual wavelength spectrophotometer (20) as described by Sies and Chance (21) and the rate of H_2O_2 generation was quantitated by the method of Oshino *et al.* (22).

Quantitation of ketogenesis in periportal and pericentral regions of the liver lobule. Micro-light guides used for the determination of NADH fluorescence from the liver surface were constructed from two 80-μm diameter optical fibers as described elsewhere (23). The tip diameter of the light guides is approximately 170 μm, much smaller than the diameter of an average hepatic lobule (24). One strand of the light guide was illuminated with 366 nm light and fluorescence was collected via another strand connected to a photomultiplier filtered to detect 450 nm light. The output currents from two sets of micro-light guides were equalized by adjusting the high voltage on the photomultiplier tubes. Micro-light guides were then placed carefully on periportal or pericentral regions of the liver lobule with micromanipulators using differential pigmentation to identify the regions (23). The signal was filtered, amplified, and recorded as described elsewhere (20). The steady state of NADH fluorescence was recorded during infusion of fatty acids or valproate; ketone bodies were determined in samples of effluent perfusate taken at 1–2-min intervals. NADH fluorescence was expressed as a percentage of basal fluorescence observed prior to valproate or fatty acid addition. Fluorescence changes were expressed as a percentage of basal values since basal fluorescence in pericentral areas is consistently less than in periportal regions, possibly due to quenching of fluorescence in pericentral regions by cytochrome P-450 (23, 25). Because the ratio of fluorescence signals from periportal/pericentral areas was essentially constant when a nonmetabolizable fluorochrome was infused, all fluorescence signals were expressed as a percentage of basal values. Rates of ketogenesis in periportal and pericentral regions were calculated from changes in NADH fluorescence in a given region, the rate of ketone body formation at the time of maximal fluorescence change, the flow rate, and the weight of each region, given that 50% of the liver is periportal and 50% pericentral (24).

In previous work, rates of ketone body production were highly correlated with changes in NADH fluorescence detected with a large-tipped light guide during inhibition of ketogenesis with bromooctanoate or stimulation with oleate (13, 13a, 14). A similar, strong correlation also exists between [β-hydroxybutyrate]/[acetoacetate] in the effluent perfusate and NADH fluorescence from the liver surface. We assumed that the correlation of NADH fluorescence signals and rates of ketone body production by the whole liver could be used as a calibration curve to convert local changes in fluorescence measured with micro-light guides to rates of ketogenesis.

Tissue preparation for histochemistry of lipid. At intervals following subcutaneous injection of valproate, saline, or octanoate, rats were anesthetized with pentobarbital and their livers were perfused briefly with Krebs-Henseleit buffer (pH 7.4, 37°). When all blood was removed from the liver, 1% paraformaldehyde in Krebs-Henseleit buffer was infused for 8 min. Tissue was taken from the left lateral lobe of the liver for routine histological preparation and sections were stained with hematoxylin and eosin. Tissue used for determining lipid distribution was washed extensively with Krebs-Henseleit buffer and post-fixed in 1% osmium tetroxide/2.5% potassium dichromate in distilled water for 4 hr (26). After fixation, the tissue samples were washed for 2 hr in running water and processed for light microscopy.

Results

Effect of valproate on hepatic lipid content *in vivo*. Valproate was administered once by subcutaneous injection to groups of fasted (24 hr) rats 1–20 hr before sacrifice. Livers were fixed by perfusion and the tissue was treated with osmium to stain lipid. Visible increases in hepatic lipid content were detected as early as 2 hr after valproate administration and reached maximal levels in about 4 hr (Fig. 1, B and C). Fat content was increased in periportal regions of the lobule to a greater extent than in pericentral regions although osmium-positive material was also increased slightly in pericentral regions when the interval between valproate treatment and sacrifice was greater than 8 hr. Treatment with either saline (Fig. 1A) or sodium octanoate (500 mg/kg, subcutaneously, not shown) had virtually no effect on hepatic lipid content.

The effect of valproate on ketogenesis from endogenous substrates in the perfused liver. Since rates of ketogenesis are maximal in fasted rats, the effects of valproate on ketogenesis and related parameters such as oxygen uptake were determined in perfused livers from fasted rats in the absence of added fatty acids (Fig. 2). Preliminary experiments demonstrated that 500 μM valproate decreased these parameters maximally. Before addition of valproate, oxygen uptake and ketone body (acetoacetate + β-hydroxybutyrate) production were 133 ± 7 and 42 ± 4 μmol/g/hr, respectively (Table 1). Within 10 min of valproate infusion, rates of ketone body production declined by 67% (Fig. 2, Table 1). NADH fluorescence detected from the liver surface was decreased maximally by about 30% within 10 min. During the first 20 min of valproate infusion, decreases in the rate of ketone body production and NADH fluorescence correlated well ($r = 0.78$). The effect of valproate on oxygen uptake was biphasic (Fig. 2). A brief (2–4 min)

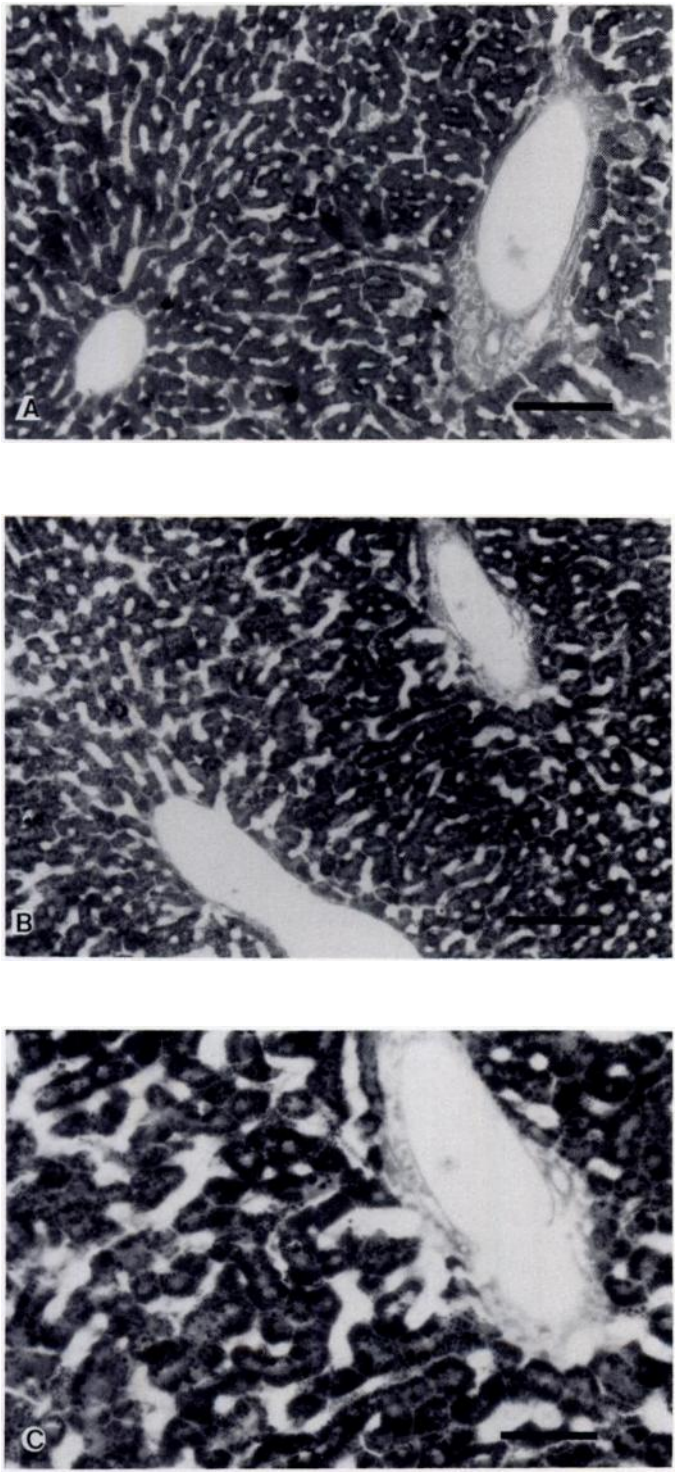

Fig. 1. Osmium staining of lipid in the livers of fasted rats treated with valproate. A. A representative view of the liver lobule in a 24-hr fasted rat receiving saline subcutaneously 4 hr before sacrifice. B. The hepatic lobule in a fasted rat treated with 500 mg/kg sodium valproate for 4 hr. C. Periportal region of liver shown in B. A and B, ×125 magnification, *horizontal bar* = 80 μm; C, ×250, *horizontal bar* = 40 μm. Osmium-positive material determined as described in Materials and Methods appears as black vesicles in the cytoplasm.

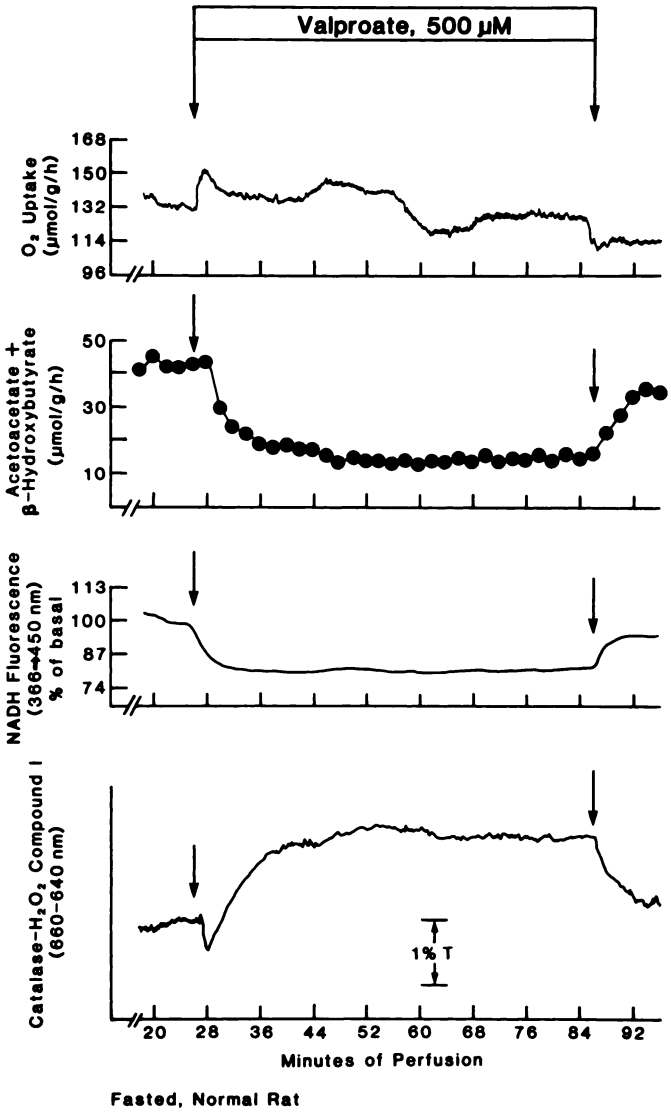

Fig. 2. The effect of valproate on oxygen uptake, ketone body production, NADH fluorescence, and the level of catalase-H_2O_2 in the perfused liver. Oxygen concentration in the effluent perfusate was determined polarographically and NADH fluorescence and catalase-H_2O_2 were measured continuously as described in Materials and Methods. The perfusate contained 100 μM methanol. Samples of the effluent perfusate were collected at 2- or 4-min intervals for determination of ketone bodies. Valproate (500 μM) was infused bound to defatted bovine albumin (final concentration, 225 mg/dl) as depicted by the *horizontal bars* and *arrows*. A typical experiment is shown.

increase in O_2 uptake of less than 5% was followed by a gradual decrease over the next 40 min of 12 ± 3% of basal values (Table 1). All parameters returned toward basal values after valproate infusion was terminated (Fig. 2).

The steady state level of catalase-H_2O_2 was first decreased and then increased upon infusion of valproate (Fig. 2). Catalase-H_2O_2 increased steadily over the next 60 min of infusion, corresponding to an increase in H_2O_2 generation of about 6 μmol/g/hr. The level of catalase-H_2O_2 decreased when valproate infusion was discontinued.

Effects of valproate on ketogenesis in the presence of oleate. The effect of valproate was also studied in the presence of oleate (250 μM) (Fig. 3). As expected, the measured parameters related to ketogenesis increased with similar kinetics

TABLE 1

The effect of valproate on ketogenesis from endogenous fatty acids or oleate in the isolated, perfused rat liver

In the top half of the table, data are from experiments of the type shown in Fig. 2 using livers of fasted rats, perfused in the absence of methanol. Values are expressed as the mean ± standard error, $n = 7$ livers. Oxygen uptake, ketone body production, and NADH fluorescence were determined as described in Materials and Methods. For the bottom half of the table, the experimental protocol is depicted in Fig. 3 ($n = 4$).

Condition	Oxygen uptake	Ketone body production	NADH fluorescence (366→450 nm)
	μmol/g/hr		% of basal
Basal[a]	133 ± 7	42 ± 4	100 ± 3
Valproate (500 μM)			
5 min	128 ± 7	14 ± 2[b]	76 ± 2[b]
10 min	125 ± 7[b]	14 ± 2[b]	71 ± 2[b]
Basal	97 ± 7	37 ± 3	100 ± 6
Oleate (250 μM)	120 ± 5[b]	85 ± 4[b]	132 ± 3[b]
Oleate (250 μM) + valproate (500 μM)			
5 min	117 ± 4[b]	57 ± 4[b,c]	104 ± 2[c]
10 min	112 ± 3[b]	46 ± 3[b,c]	93 ± 3[c]

[a] Basal values were taken during 8 min immediately before addition of valproate.
[b] Significantly different from corresponding basal value at $p < 0.001$ (matched pairs t test).
[c] $p < 0.001$ as compared to corresponding value during infusion of oleate alone.

during addition of oleate and steady-state values were reached within 4 min (Fig. 3). Infusion of oleate produced simultaneous increases in oxygen uptake (24%), ketone body production (130%), and NADH fluorescence (32%) (Table 1). During oleate infusion, changes in the rate of ketone body production correlated well with increases in both NADH fluorescence and the rate of oxygen consumption ($r = 0.89$–0.94). Upon infusion of valproate, rates of ketogenesis and NADH fluorescence decreased immediately and declined to basal levels within 20 min (Fig. 3, Table 1). Rates of ketogenesis were inhibited irreversibly by valproate under these conditions.

The steady state level of catalase-H_2O_2 was first decreased and then increased by infusion of oleate (250 μM, Fig. 3). Subsequent addition of valproate produced an increase in the steady state level of catalase-H_2O_2. When oleate infusion was stopped, catalase-H_2O_2 increased further. The steady state level of catalase-H_2O_2 did not change significantly upon termination of the infusion of valproate (Fig. 3).

Influence of valproate on bile flow and biliary GSSG production. During the course of valproate addition to the perfused liver, the rate of efflux of GSSG into bile was decreased by approximately 60%. In contrast, the rate of bile flow was virtually unaffected by valproate infusion (Table 2).

Inhibition of ketogenesis in periportal and pericentral regions of the liver lobule by valproate. Because valproate diminished ketogenesis in the perfused liver (Figs. 2 and 3) and caused preferential accumulation of lipid in periportal regions *in vivo* (Fig. 1), experiments were performed to determine whether selective inhibition of ketogenesis in periportal regions was involved in the zonal accumulation of lipid. Previous studies demonstrated that changes in NADH fluorescence from the liver surface correlated well with changes in rates of ketogenesis during maximal inhibition or stimulation of β-oxidation with bromooctanoate or oleate, respectively (13). During infusion of valproate in this study, a similar correlation between inhibition of ketogenesis and decreases in NADH fluorescence was also observed ($r = 0.78$). Accordingly, fiber optic micro-light guides were placed on periportal and pericentral regions of the liver

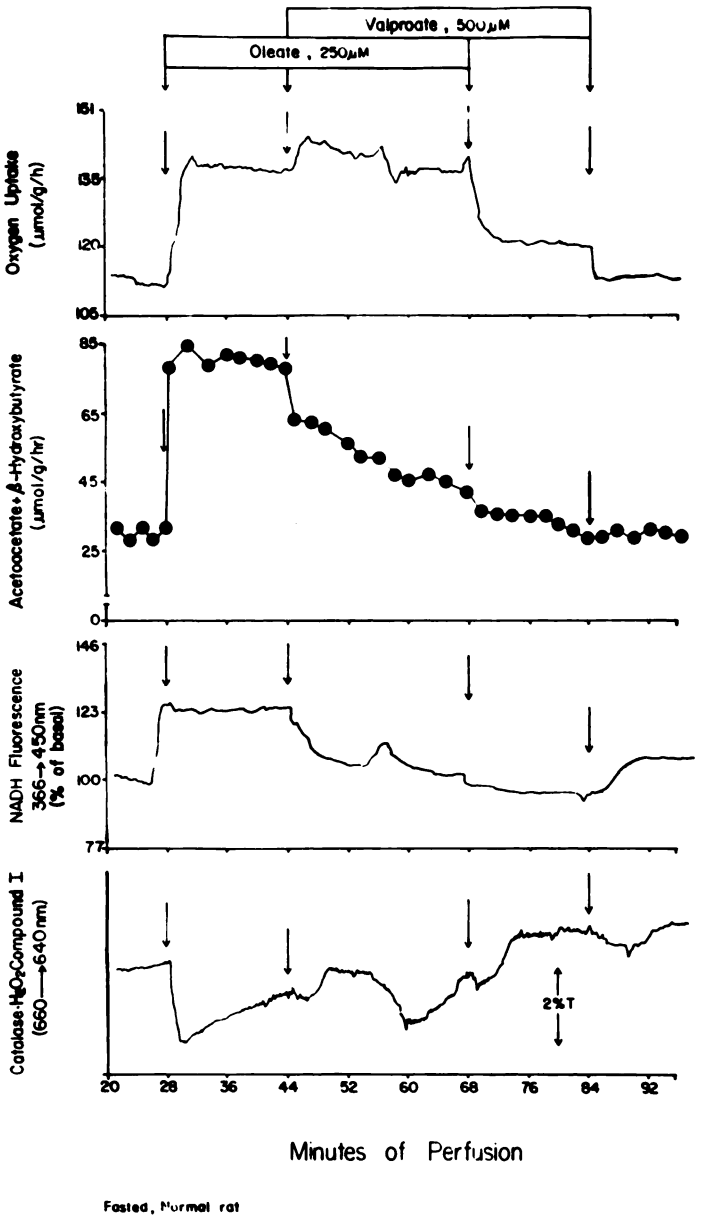

Fig. 3. Effects of oleate and valproate on hepatic ketogenesis. Conditions are as in Fig. 2. Prior to the addition of valproate, oleate bound to defatted albumin (final albumin concentration, 420 mg/dl) was infused into the perfused liver of a fasted rat. Oleate and valproate infusions are shown by the *horizontal bars* and *arrows*. Oxygen uptake, optical data, and ketone body production were determined as in Fig. 2. A typical experiment is shown.

lobule to measure NADH fluorescence which was converted into rates of ketogenesis (see Materials and Methods) when valproate alone or oleate followed by valproate was infused (Fig. 4). In the absence of added substrates, rates of ketone body production were identical in periportal and pericentral regions (Table 3) (13). In the presence of valproate, endogenous rates of ketogenesis were decreased in both regions; however, rates in periportal areas were diminished by 68%, whereas values in pericentral regions declined by only 47% (Table 3). When valproate was added to livers perfused in the retrograde direction, qualitatively similar control rates and decreases with valproate were observed (Table 3).

Upon addition of oleate, NADH fluorescence increased 13 ±

TABLE 2

The effect of valproate on the steady state level of catalase-H_2O_2, production of bile, and GSSG efflux into bile in the perfused liver

Catalase-H_2O_2 was detected spectrophotometrically (660–640 nm) through a lobe of the liver in the presence of methanol (100 μM). The common bile duct was cannulated with polyethylene tubing (PE 10) and bile was collected into 50 μl of 5% metaphosphoric acid on ice at 4-min intervals for 12 min before initiating valproate infusion. Bile samples were taken at 4-min intervals during valproate addition. Data are expressed as mean ± standard error, $n = 4$ livers.

Condition	Catalase-H_2O_2 compound I (increase in % transmittance) from basal	Bile flow	GSSG efflux
		μl/g/hr	nmol/g/hr
Basal	0	78 ± 4	119 ± 4
Valproate (500 μM)			
5 min	0.5 ± 0.2	95 ± 9[a]	104 ± 12
10 min	1.1 ± 0.3[a]	88 ± 13	60 ± 10[a]
20 min	1.3 ± 0.3[a]	77 ± 12	59 ± 13[a]
40 min	1.4 ± 0.2[a]	70 ± 10	41 ± 3[a]
60 min	1.5 ± 0.1[a]	71 ± 12	49 ± 11[a]

[a] Significantly different from basal value at $p < 0.001$ (matched pairs t test).

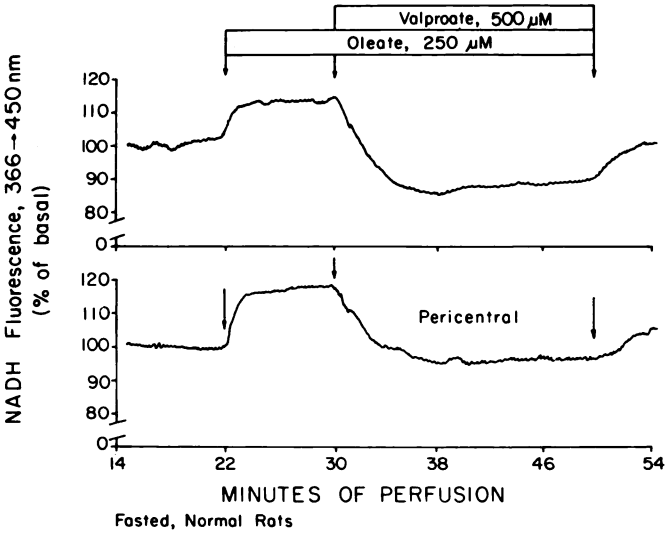

Fig. 4. NADH fluorescence in periportal and pericentral regions of the liver lobule during infusion of oleate and valproate. Micro-light guides were placed on periportal and pericentral regions of the liver lobule and the steady state fluorescence of NADH was recorded. Oleate and valproate infusions are depicted by *horizontal bars* and *arrows*. Effluent perfusate was sampled at 2-min intervals throughout the experiment for the determination of ketone bodies.

3% in pericentral regions and 8 ± 1% in periportal areas indicating greater ketogenic and β-oxidizing activity in pericentral areas of the liver lobule (Fig. 4, Table 3). The greater increase in NADH fluorescence in pericentral areas is not due to centrilobular hypoxia since venous pO_2 during oleate infusion was 133 ± 11 mm Hg. Previous work has shown that NADH fluorescence does not begin to increase in pericentral areas until venous pO_2 is below 50 mm Hg (23). Valproate decreased NADH fluorescence and the calculated rate of ketogenesis in both regions of the liver lobule when added after oleate (Fig. 4, Table 3). Residual rates of ketogenesis in the presence of oleate and valproate were 50% greater in pericentral than periportal regions of the liver lobule (Table 3). Thus, it is concluded that valproate inhibits ketogenesis predominantly in periportal regions of the liver lobule.

TABLE 3

Inhibition of ketogenesis in periportal and pericentral regions of the liver lobule by valproate

Rates of ketone body production were calculated as described in Materials and Methods. Data are means ± standard errors of five to eight livers.

Experimental condition	NADH fluorescence		Ketone body production	
	Periportal	Pericentral	Periportal	Pericentral
	% of basal		μmol/g/hr	
No addition, anterograde perfusion	100 ± 4	100 ± 3	37 ± 2	36 ± 4
Anterograde perfusion with 500 μM valproate	76 ± 2	83 ± 3[a]	12 ± 3	19 ± 2[a]
No addition, retrograde perfusion	100 ± 2	100 ± 2	34 ± 1	36 ± 3
Retrograde perfusion with 500 μM valproate	84 ± 4	91 ± 2[a]	8 ± 3	20 ± 4[a]
No addition	100 ± 2	100 ± 3	38 ± 4	35 ± 3
250 μM Oleate	108 ± 1	113 ± 3[a]	71 ± 3	89 ± 2[a]
Oleate (250 μM) + 500 μM valproate	94 ± 2	99 ± 1[a]	34 ± 4	51 ± 3[a]

[a] Significantly different from corresponding periportal value, $p < 0.05$ (matched pairs t test).

Discussion

One important clinical observation following valproate intoxication in humans is the development of fatty liver (2). In rodents, the accumulation of hepatic fat is quite rapid. In one study, triglycerides were increased by 50% in 3 hr with minimal evidence of mitochondrial degeneration (27). Total hepatic lipid was increased within 2 hr after valproate injection in our studies with fasted rats, an effect which was maximal in 4 hr and was confined nearly exclusively to periportal regions of the liver lobule (Fig. 1). In the fasted state, the liver relies primarily on the oxidation of fatty acids which are largely C_{16} and C_{18} compounds (28). In the perfused liver, valproate inhibited ketogenesis from both endogenous fatty acids (Fig. 2) and added oleate (Fig. 3) very rapidly (i.e., maximal effect within 10 min). Furthermore, valproate decreased circulating ketone bodies in fasted humans almost immediately (7). Since valproate does not appear to affect lipoprotein secretion or rates of peripheral lipolysis (7, 27), it is reasonable to propose that valproate causes hepatic steatosis by depressing the β-oxidation of endogenous fatty acids such as oleate.

Fatty acids are metabolized both in the mitochondria and in peroxisomes (29). Both systems produced NADH; however, only the peroxisomal system generates H_2O_2. It is possible to monitor H_2O_2 generation by measuring the steady state level of catalase-H_2O_2 (22). The observation that NADH fluorescence declined and catalase-H_2O_2 increased with a time course similar to that of the inhibition of ketogenesis following infusion of valproate suggests that both the mitochondrial and peroxisomal β-oxidizing systems are affected (Figs. 2 and 3) (30). The increase in both the steady state of catalase-H_2O_2 and in the measured rates of H_2O_2 production of about 6 μmol/g/hr with infusion of valproate in the absence of added oleate reflects either an increase in H_2O_2 derived from the oxidation of valproyl-CoA by peroxisomes or increased metabolism of endogenous fatty acids which are diverted from the mitochondrial to the peroxisomal β-oxidizing system (Fig. 2). Although we cannot distinguish between these alternatives, the H_2O_2 produced is most likely metabolized exclusively in the peroxisome, since GSSG efflux, which increases when cytosolic H_2O_2

is metabolized via glutathione peroxidase, was actually depressed by valproate (Table 2).

It has been proposed that an increase in acyl-CoA levels in the liver may be the signal for the proliferation of peroxisomes in the liver (31, 32). Accordingly, agents which decrease ketogenesis and increase acyl-CoA content would be expected to stimulate peroxisomal biogenesis. The observation that valproate inhibits ketogenesis and possibly increases acyl-CoA levels may be related to the increase in hepatic peroxisomal β-oxidation which has been observed after prolonged administration of valproate (33).

It is well established that intermediary metabolism occurs at different rates in periportal and pericentral regions of the liver lobule (34). For example, gluconeogenesis (35) and glycolysis (36) have been shown to occur predominantly in periportal and pericentral regions, respectively, in the perfused liver. Recently, a method to quantitate ketogenesis in periportal and pericentral regions of the liver lobule based on local changes in NADH fluorescence detected with micro-light guides was developed (13, 13a). In the presence of fatty acids, ketogenesis from β-oxidation was about 30% higher in pericentral than in periportal areas of the liver lobule (Table 3). Valproate inhibited ketogenesis more in periportal than in pericentral regions during submaximal ketone body production from endogenous fatty acids. It does not appear that this result can be explained by differential delivery of valproate, since ketogenesis was also inhibited predominantly in periportal areas during perfusion in the retrograde direction (Table 3). Valproate depresses ketogenesis about equally in both regions of the liver lobule in the presence of oleate (Table 3); however, because of different oleate-stimulated rates, residual rates of ketogenesis were 50% lower in periportal than in pericentral regions of the liver lobule in the presence of valproate (Table 3). It is known that impaired fatty acid oxidation leads to steatosis (12). Since ketogenesis and presumably β-oxidation occur at much lower rates in periportal than in pericentral areas in the presence of valproate (Table 3), it is concluded that inhibition of β-oxidation could be responsible for the accumulation of lipid in periportal regions of the liver lobule which occurs following addition of valproate.

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