Inhibition of mitochondrial $\beta$-oxidation and peroxisomal stimulation in rodent livers by valproate

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Valproic acid (3-d-n-propylacetic acid) is a branched-chain eight-carbon fatty acid in widespread use in the treatment of epilepsy. Although relatively free from side-effects, valproate therapy has been involved in several cases of a 'Reye's-like' syndrome, associated with hepatic steatosis which is indicative of defective fatty acid metabolism. It has been shown that treatment of hepatocytes with valproate in vitro inhibits the oxidation of fatty acids [1], and we have demonstrated that the $\beta$-oxidation of palmitoyl carnitine by isolated rat liver mitochondria is inhibited ($K_{\text{m}} \approx 125 \mu M$) [2]. Administration of valproate in the diet stimulates the hepatic peroxisomal $\beta$-oxidation capacity in rats and mice [3]. We have examined the effects of valproate in vitro on the oxidation of palmitoylcarnitine by liver mitochondria from rats, mice and guinea-pigs to compare with the effects of administration in vivo on the hepatic mitochondria and peroxisomal $\beta$-oxidation activities.

The purpose of this study was to determine whether the observed peroxisomal stimulation may be a compensatory response to decreased mitochondrial activity.

Mitochondrial $\beta$-oxidation was assayed polarographically with 16.7 $\mu M$-palmitoylcarnitine as substrate. Preincubation for 3 min with valproate markedly inhibited the activity in mitochondria from rat and guinea-pig ($K_{\text{m}} \approx 125 \mu M$ and 60 $\mu M$, respectively), but there was relatively little effect in mouse mitochondria, the inhibition being only 30% with 1 mM-valproate, which was the highest concentration tested. Oxidation rates with other substrates, 10 mM-fumarate and 10 mM-glutamate/1 mM-malate, were only slightly affected (10–20% inhibition at 1 mM) in all three species.

Rats and mice were fed valproate (0.25, 0.5 and 1% w/w) and guinea-pigs 1% (w/w) in the diet for 2 weeks. All three species displayed a valproate-induced hepatomegaly (dose dependent in rats and mice), with relative liver weights being 17, 19 and 15% larger than control in rats, mice and guinea-pigs, respectively, after 2 weeks of 1% (w/w) valproate. There was an increase in hepatic mitochondrial content as evidenced by an increase in the activity per g of liver of the mitochondrial marker enzyme, citrate synthase, which was confirmed by electron microscopy and cell morphometry. The capacity for peroxisomal $\beta$-oxidation was increased dose dependently in rats and mice, due to increased acyl-CoA oxidase activity, the effect in rats being twice as great as that in mice (270% and 130% increase in rats and mice, respectively, with 1% (w/w) valproate). The mitochondrial oxidative functions studied were essentially unaffected, activities being decreased by less than 20% with all the substrates tested. However, in guinea-pigs fed 1% (w/w) valproate, although there was an increase in citrate synthase activity per g of liver, the peroxisomal $\beta$-oxidation capacity was not increased (102% of controls), acyl-CoA oxidase activity being unchanged.

Therefore, although mitochondria isolated from guinea-pig livers were extremely sensitive to inhibition by valproate in vitro, administration of the drug in conditions in vivo which elicited a peroxisomal stimulation in rats and mice did not increase peroxisomal $\beta$-oxidation in the guinea-pig. Furthermore, although $\beta$-oxidation in mouse liver mitochondria was relatively insensitive to valproate, feeding of the compound still produced a marked stimulation of peroxisomal $\beta$-oxidation. The actual inhibition of mitochondrial $\beta$-oxidation by valproate has been suggested to be due to the sequestration of free CoA in the mitochondrial matrix in the form of valproyl-CoA [1], which is a poor substrate for medium-chain acyl-CoA hydrolase [4]. The peroxisomal response might therefore be part of a general stimulation of cellular mechanisms to deal with the increased medium-chain CoA ester fraction which the mitochondria are unable to metabolize. Indeed, CoA ester accumulation has been proposed to be a major factor in the stimulation of peroxisomal proliferation [5]. It remains to be seen whether the differences in responses observed in vitro and in vivo in the three species described may be due to differences in acyl-CoA hydrolase or acylcarboxyl transferase activities in these species.


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animals, which would allow a more efficient cycling of intra-mitochondrial CoA. It can be concluded, however, that the observation of an inhibition of mitochondrial function in vitro will not necessarily be accompanied by a peroxisomal response in vivo.


Peroxisomal and mitochondrial proliferation and increased alanine:glyoxylate aminotransferase activity in human liver after chlorpromazine-induced cholestasis

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The phenothiazine drugs such as chlorpromazine are known to cause cholestasis in a small proportion (<1%) of patients [1]. In addition, chlorpromazine has been shown to alter peroxisomal morphology in humans [2] and induce peroxisomal proliferation in experimental animals [3]. Whether this peroxisomal change is primary or secondary effects is not clear, as cholestasis from other causes has been reported to be associated with peroxisomal proliferation [4]. The present study concerns an investigation into the liver ultrastructure and biochemistry of a patient suffering from acute chlorpromazine toxicity.

The patient, a 52-year-old female, had been treated with the phenothiazines, chlorpromazine and trifluoperazine for psychotic depression. During the first 2 weeks of treatment she developed jaundice. The phenothiazines were withdrawn immediately and a percutaneous liver biopsy was obtained 2 weeks later. Histological examination at the light microscope level showed marked cholestasis, a mixed inflammatory infiltrate in the portal tracts and normal bile ducts, in keeping with chlorpromazine toxicity.

Ultrastructural analysis of the liver biopsy from this patient revealed abnormally swollen and damaged bile ducts, similar to the results reported on cultured rat hepatocytes following chlorpromazine treatment [8]. However, the most striking result was the marked proliferation of both peroxisomes and mitochondria (Fig. 1). Morphometric analysis demonstrated that the peroxisome frequency in this patient increased to 19.4/100 μm² of cytoplasm compared with an average of 8.3 (range = 5.9–10.1, n = 4) in control liver. The mitochondrial frequency also increased from 36 (range = 25.2–48.3, n = 4) in controls to 65.6/100 μm² in this patient. The activity of the liver specific peroxisomal marker enzyme AGT was twice the mean normal level [9.86 μmol/h per mg of protein compared with 4.79 μmol/h per mg of protein, range = 2.52–7.80, n = 13].

Protein A-gold immuno electron microscopy, using monospecific polyclonal rabbit anti-(human AGT) antiseraum,

Abbreviation used: AGT, alanine:glyoxylate aminotransferase.

P. peroxisomes: M, mitochondria. Bar=0.5 μm. Note the high density of immunolabelling for AGT in the patient's peroxisomes compared to control liver (inset).

Fig. 1. Hepatocyte of a patient with chlorpromazine toxicity after immunogold labelling for AGT

showed that the peroxisomal labelling density in the patient was greatly increased compared to controls (Fig. 1), the labelling density increasing from an average of 75 gold particles/μm² of peroxisomal area to 168/μm². These results suggest that a significant proportion of the chlorpromazine-induced AGT is in an inactive form.

Although these data show that chlorpromazine can induce peroxisomal proliferation in human liver in addition to livers from experimental animals [3], it is still not clear whether this is a primary effect of the drug or secondary to the liver pathology. The increase in peroxisome number and elevated

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