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Short Communication

Valproate inhibits the mitochondrial pyruvate-driven oxidative phosphorylation *in vitro*

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Valproic acid (2-*n*-propylpentanoic acid; VPA), a medium-chain branched fatty acid, has proved to be an effective antiepileptic drug. It has also been used widely in the symptomatic treatment of children for a variety of types of seizure. Hepatotoxicity is one of the adverse effects. Several groups have studied the *in vitro* deleterious effects of VPA on cellular intermediary metabolism. However, the exact mechanisms of VPA-associated hepatotoxicity are still unknown.

Despite the structural simplicity of valproate, its metabolic profile is quite complex. Glucuronidation and β -oxidation are quantitatively the most important routes of biotransformation, generating a complex pattern of intermediates (Baillie 1992) which could potentially interfere with mitochondrial metabolism at different levels. In the past, several papers have described inhibitory effects of VPA and its metabolites on mitochondrial oxidative phosphorylation, both *in vitro* (Haas et al 1981; Becker and Harris 1983; Ponchaut et al 1992) and *in vivo* (Rumbach et al 1983). However, the results are not easily comparable and the reported data are quite contradictory.

Although our primary interest was the effect of VPA on β -oxidation, we decided to study in parallel the interference of VPA with mitochondrial substrate oxidations. We therefore investigated the effect of this drug on mitochondrial oxidative phosphorylation, in concentrations similar to those found in man during therapy. The effects of two of its unsaturated metabolites, 2-propyl-2-pentenoic acid (1 mmol/L) ($\Delta^{2(E)}$ -VPA; mitochondrial β -oxidation intermediate) and 2-propyl-4-pentenoic acid (1 mmol/L) (Δ^4 -VPA; microsomal dehydrogenation product), were also studied. Two experimental approaches were used for this study: digitonin-permeabilized rat hepatocytes for the study of the rate of ATP synthesis, and rat liver mitochondria for the study of oxygen consumption by polarography, both in the presence of selected respiratory chain substrates.

METHODOLOGY

Synthesis of ATP: Measurement of ATP synthesis in digitonin-permeabilized hepatocytes was essentially based on the method described by Wanders et al (1993). Rat hepatocytes were isolated according to established procedures (Department of Biochemistry, University Hospital Amsterdam). Cell viability was routinely tested by trypan blue (0.4% w/v) exclusion and phase-contrast microscopy. The cells were suspended in Krebs Henselheit buffer (pH 7.4). Hepatocytes (final concentration 0.4mg/ml) were preincubated for 10min at 25°C, in the presence and absence (controls) of VPA. Then the incubations were allowed to proceed for 30min at 25°C in a cytosol-mimicking medium containing 1mmol/L ADP, 40µg/ml digitonin (optimal concentration found for hepatocytes; results not shown), 0.1% bovine serum albumin and the respiratory substrates. VPA was added to the reaction medium from a stock solution at pH 7.4. The ATP produced was measured fluorimetrically in the protein-free supernatants.

Pyruvate oxidation rate: Mitochondrial respiratory function was assessed by means of a polarographic technique in rat liver mitochondria. Adult male Wistar rats (<250g) starved for 18h were used for preparation of mitochondria. After killing by contusion, the livers were rapidly removed and rinsed into ice-cold homogenization medium (250mmol/L mannitol, 5mmol/L Tris-HCl, 0.5mmol/L EGTA; pH 7.4). After mincing and 2–3 washings, the chopped liver was homogenized in a precooled Teflon pestle glass homogenizer in 1:8 volumes of medium. The homogenate was centrifuged (2550rpm, 10min) and the obtained supernatant was spun down at 6200rpm for 10min (4°C). After one last round of centrifugation at 5500rpm for 10min, the mitochondria were finally resuspended in the homogenization medium (25–50mg/ml). Protein content was determined using the BCA assay and bovine serum albumin (BSA) as a reference substance.

Oxidation activity was measured at 25°C in a Clark-type membrane-coated oxygen electrode (Hansatech, Instr.) in a 0.5ml incubation reaction vessel. The medium had the following composition: 250mmol/L mannitol, 5mmol/L Tris-HCl, 0.5mmol/L EGTA, 10mmol/L potassium phosphate buffer (pH 7.4) and 0.5% BSA. Mitochondrial protein was used at a concentration of 0.4mg/ml, with 10mmol/L succinate (+3µmol/L rotenone) or 10mmol/L glutamate (+5mmol/L malate), and at 1.0mg/ml when 10mmol/L pyruvate was used as substrate. ADP was added to initiate state 3 oxidation. Evaluation of states 3 and 4, respiratory control ratio (RCR) and oxidative phosphorylation (ADP:O) were essentially done according to Estabrook (1967), assuming an oxygen concentration of 0.253µmol/ml (O₂) under the conditions described above.

RESULTS

Effect of VPA on ATP synthesis in rat hepatocytes: VPA and Δ^4 -VPA were found to inhibit the rate of ATP synthesis (76% and 92% inhibition with 0.1mmol/L and 0.5mmol/L of VPA, respectively) when pyruvate was used as respiratory substrate. No inhibitory effect was observed on succinate-driven oxidative phosphorylation and with glutamate/malate as substrate couple. $\Delta^{2(E)}$ -VPA induced a much milder inhibitory effect on ATP synthesis driven by pyruvate. Results are presented in Table 1.

Table 1 Rate of ATP synthesis^a obtained with different respiratory substrates in hepatocytes

	<i>Succinate/rotenone</i>	<i>Glutamate/malate</i>	<i>Pyruvate</i>
Control	41.1	49.8	13.7
+ VPA 0.1mmol/L	37.6 (92%)	40.4 (81%)	3.3 (24%)
+ VPA 0.5mmol/L	40.4 (98%)	38.4 (77%)	1.1 (8%)
+ VPA 1.0mmol/L	39.9 (97%)	35.2 (71%)	0.2 (2%)
+ VPA 2.0mmol/L	36.9 (90%)	34.1 (69%)	0.4 (3%)
+ Δ^4 -VPA 1.0mmol/L	39.7 (97%)	39.3 (79%)	0.3 (2%)
+ $\Delta^{2(E)}$ -VPA 1.0mmol/L	35.8 (87%)	37.5 (75%)	8.6 (63%)

^aResults are expressed in nmol ATP/min per mg protein; percentages in parentheses are percentages of control value

Table 2 State 3 oxidation rates^a (after ADP addition) of various substrates in rat liver mitochondria

	<i>Succinate/rotenone</i>	<i>Glutamate/malate</i>	<i>Pyruvate</i>
Control	52.1	40.9	11.9
+ VPA 0.01mmol/L	—	—	11.9 (100%)
+ VPA 0.02mmol/L	—	—	8.9 (75%)
+ VPA 0.05mmol/L	50.2 (96%)	35.3 (86%)	5.2 (44%)
+ VPA 0.1mmol/L	48.4 (93%)	35.3 (86%)	3.7 (31%)
+ VPA 0.5mmol/L	48.4 (93%)	37.2 (91%)	3.0 (25%)
+ VPA 1.0mmol/L	48.4 (93%)	29.8 (73%)	2.2 (19%)
+ VPA 2.0mmol/L	48.4 (93%)	31.6 (77%)	—
+ Δ^4 -VPA 0.1mmol/L	—	35.3 (86%)	4.5 (38%)
+ Δ^4 -VPA 1.0mmol/L	—	33.5 (82%)	3.7 (31%)

^aResults are expressed in nmol O₂/min per mg protein; percentages in parentheses are percentages of control value

Effect of VPA on oxygen consumption by isolated rat liver mitochondria: The effects of VPA and Δ^4 -VPA on the oxidation rate of succinate (+rotenone), glutamate (+malate) and pyruvate were determined in coupled mitochondria. In all experiments, the mitochondria were preincubated with the drug for 5min. This period is sufficient to give the maximum inhibitory effect for all substrates (Veitch and Van Hoof 1990), before initiation of state 3 oxidation. The addition of ADP was delayed for the same period in the controls. The results obtained are summarized in Table 2.

In agreement with ATP synthesis results, we also found a marked inhibitory effect of VPA on the rate of pyruvate oxidation (state 3), while the oxygen consumption with succinate/rotenone and glutamate/malate was not significantly affected. Δ^4 -VPA inhibited the oxidation of the same substrate to a similar extent. State 4 oxygen consumptions remained practically unchanged as compared to control values for all substrates.

DISCUSSION

The results described show that valproate inhibits the pyruvate-driven oxidative phosphorylation. Using succinate, in the presence of rotenone, as respiratory substrate, we

found that VPA does not affect the synthesis of ATP from ADP and phosphate. These results suggest that VPA is not affecting the electron transport chain *per se* (after complex II) or the phosphorylation system, since ATP synthesis is not affected by VPA when succinate/rotenone is used as substrate.

The observed inhibition by VPA and Δ^4 -VPA on ATP synthesis in the presence of pyruvate is therefore due to an inhibitory effect on mitochondrial pyruvate oxidation. The rate of oxygen consumption is decreased owing to the reduced NADH and possibly FADH₂ production in the mitochondrial matrix.

Several possible mechanisms may explain the observed results. A direct inhibitory effect of VPA and/or metabolites on PDH complex, a competitive inhibition on the pyruvate carrier, or the intramitochondrial depletion of free CoASH are hypotheses that need to be investigated further.

If the extrapolation to humans is legitimate, our results would support the contraindication of VPA in patients with compromised mitochondrial function such as respiratory chain defects, and, if CoASH-depletion would be the primary effect, carnitine deficiency states and genetic defects of fatty acid β -oxidation.

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REFERENCES

- Baillie TA (1992) Metabolism of valproate to hepatotoxic intermediates. *Pharmaceutisch Weekblad Scientific* **14** (3A): 122–125.
- Becker CM, Harris RA (1983) Influence of valproic acid on hepatic carbohydrate and lipid metabolism. *Arch Biochem Biophys* **223**: 381–392.
- Estabrook RW (1967) Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Methods Enzymol* **10**: 41–47.
- Haas R, Stumpf DA, Parks JK, Eguren L (1981) Inhibitory effects of sodium valproate on oxidative phosphorylation. *Neurology* **31**: 1473–1476.
- Ponchaut S, Van Hoof F, Veitch K (1992) In vitro effects of valproate and valproate metabolites on mitochondrial oxidations. *Biochem Pharmacol* **43**: 2435–2442.
- Rumbach L, Warter JM, Rendon A, Marescaux C, Micheletti G, Waksman A (1983) Inhibition of oxidative phosphorylation in hepatic and cerebral mitochondria of sodium valproate-treated rats. *J Neurol Sci* **61**: 417–423.
- Veitch K, Van Hoof F (1990) In vitro effects of eight-carbon fatty acids on oxidations in rat liver mitochondria. *Biochem Pharmacol* **40** (9): 2153–2159.
- Wanders RJA, Ruiter JPN, Wijburg FA (1993) Studies on mitochondrial oxidative phosphorylation in permeabilized human skin fibroblasts: application to mitochondrial encephalomyopathies. *Biochim Biophys Acta* **1181**: 219–222.