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The Early Effects of Valproic Acid in Low Doses in Liver Metabolism

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The effect of low doses of valproic acid (VPA), 0.6 mM in arterial blood, in liver metabolism was studied. Twenty four hour fasted rats were infused into the jugular vein with VPA at a dose of 4 mg/kg/min during 50 min. The right carotid artery was also catheterized in order to draw arterial blood samples for determining VPA concentrations and acid-base parameters. After VPA infusion, a tissue sample of liver was obtained and freeze-clamped. VPA did not change the arterial blood acid-base parameters. The liver tissue concentration of pyruvate and alanine increased in VPA group while lactate concentrations did not change. Concentration of glutamine, glutamate, malate, citrate and aspartate in the liver fell significantly. These results suggest that VPA in low doses may modify the hepatic metabolism of the rat *in vivo*.

Key words: Valproic acid, Hepatic metabolism, Glutamine, Pyruvate, Rat.

Valproic acid (VPA) is an eight carbon branched chain fatty acid frequently used as antiepileptic drug (17). It has been shown that its acute and chronic administration induces hyperammonemia in humans (6, 11, 17) and experimental animals (1, 7, 12, 16). This secondary effect could be explained by disturbances in hepatic detoxification of ammonia. Some authors have demonstrated that VPA provokes a decrease in hepatic N-Acetylglutamate levels, which is involved in the activation of the first enzyme of the urea cycle, Carbamoylphosphate-Synthetase (5). On the other hand, it has been demonstrated in brain and liver mitochondria that VPA inhibits the pyruvate oxidation affecting its intake by this organelle (2). This effect was also reported in dog kidney (12) and rat renal cortical tubules (7). These observations suggest that VPA has an inhibitory effect on the intermediary metabolism of the rat.

The present study was undertaken to evaluate the effects of VPA on hepatic metabolism in the rat. For this purpose, the liver concentration of relevant metabolites were assessed in normal rats after VPA administration.

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Materials and Methods

Seventeen male Sprague-Dawley rats, weighing 350-400 g (Control, n = 8) and (VPA-Infused, n = 9) were utilized. The animals were anesthetized with an i.p., dose of sodium pentobarbital (60 mg/kg). Then a PE-150 catheter was placed into the trachea to ensure ventilation, and a PE-10 catheter was placed in the jugular vein, for the administration of solutions. The VPA group received a solution of VPA (4 mg/kg/min, 50 min) in isotonic saline at the rate of 3 ml/min, while the control group received the same volume of isotonic saline. The right carotid artery was also catheterized in order to draw arterial blood samples for the determination of VPA concentrations and blood pH, PCO_2 and HCO_3-parameters.

Immediately afterwards, the peritoneal cavity was opened, and a piece of hepatic tissue was taken and immediately freeze-clamped (19).

The piece of tissue was pulverized in a N_2-colded ceramic mortar, deproteinized with one volume of perchloric acid 10 % and homogenized in a teflon pestle Potter. After centrifugation, the supernatant was collected and neutralized with KOH 20 % to pH 7.0 (14, 15). The measurements of glutamine, glutamate, α-ketoglutarate, pyruvate, lactate, alanine, aspartate, citrate, malate and ATP concentrations were made enzymatically (8). Oxaloacetate was calculated according to WILLIAMSON *et al.* (18).

Results

Arterial blood acid-base parameters (Control Rats: pH 7.40 ± 0.01, pCO_2 35.3 ± 1.3 mm Hg, HCO_3^- 21.9 ± 0.9 mM, Valproate 0 mM; VPA-Infused Rats: pH 7.43 ± 0.02, pCO_2 35.7 ± 1.6 mm Hg, HCO_3^- 23.8 ± 0.9 mM, Valproate 0.6 mM) did not change significantly by valproate infusion.

Table I. *Concentration of critical metabolites in the liver*

All values are expressed as μmol/g wet weight except for oxaloacetate (calculated value) expressed in nmol/g wet weight. Mean ± S.E. *p<0.05 vs control group. α-kg = α-ketoglutarate.

Metabolite	Control Rats n = 8	VPA-Infused Rats n = 9
Glutamine	5.95 ± 0.54	4.43 ± 0.38*
Glutamate	2.74 ± 0.31	1.73 ± 0.13*
α-kg	0.07 ± 0.01	0.06 ± 0.01
Malate	0.61 ± 0.05	0.37 ± 0.05*
Oxaloacetate (LDH)	3.15	4.99
Alanine	1.14 ± 0.22	2.19 ± 0.50*
Aspartate	1.44 ± 0.08	1.18 ± 0.09*
Citrate	0.18 ± 0.01	0.12 ± 0.01*
Lactate	2.72 ± 0.71	2.36 ± 0.48
Pyruvate	0.056 ± 0.006	0.127 ± 0.027*
ATP	0.66 ± 0.12	0.12 ± 0.30

The concentration of critical metabolites in the liver in both groups of rats is shown in table I. After the i.v. infusion of VPA, the tissue concentration of liver metabolites decreased in VPA-infused group with regard to those of control animals. One of the most striking findings was that pyruvate levels in VPA group showed a great increase, without a parallel change in the levels of lactate. Alanine concentration also showed a significant increase, reaching levels twofold those of control rats.

Discussion

The infusion of VPA in low doses provoked in the rats an important increase in hepatic concentration of pyruvate, reaching 227 % of the control. This increase, without parallel increase of lactate levels might result from two mechanisms: (a) A competitively VPA-inhibition of the pyruvate uptake by the mitochondria across its specific carrier in hepatic cells (2). This phenomenon would affect the rate of reduced pyridin-nucleotides (NADH) production in the tricarboxylic acid

(TCA) cycle provided that there are not enough amounts of other substrates coming from anaplerotic pathways in that cycle. (b) VPA could interfere with the pyruvate entry to the TCA cycle either by an inhibition of the pyruvate dehydrogenase (PDH) or from the unavailability of free CoASH. This could be based on its sequestration as Valproyl-CoA or any CoA-derivative of VPA. This second possibility is being supported by other studies (3, 4, 13). Similar observations were reported in dog kidney (12) and rat renal cortical tubules (7).

The hypothesis of an inhibition of PDH by VPA or any of its metabolites, may be supported by the fact that Succinyl-CoA dehydrogenase is inhibited by VPA (10). Furthermore, it has been reported that the oxidation of pyruvate is inhibited by a couple of short and medium chain fatty acid CoA-esters reducing the flux through the PDH pathway (9). This data could be taken as a support for the possibility of VPA acting in a similar manner.

Both mechanisms, (a) and (b), would involve an important impairment of the rate at which the TCA cycle operates. The decrease in the hepatic levels of citrate could be considered as reflecting such a phenomenon. Also the fall in glutamine and glutamate levels, even without any change in those of α-ketoglutarate, could be seen as an attempt to promote alternative routes for supplying to TCA cycle the substrates needed. The decrease in malate levels could be explained by the inhibition of the succinate-CoA dehydrogenase by VPA, previously reported (10).

From these results it could be inferred that the final effect of VPA infusion should be an increase of the [NAD]/[NADH] ratio in the mitochondrial compartment, thus making possible the increase of pyruvate levels without a relative increase of lactate levels, provided that there is an equilibrium in [NAD]/[NADH] ratio between mitochondrial and cytosolic compartment.

The high levels of alanine may be attributed to the availability of pyruvate, throughout the activity of alanine aminotransferase (ALAT). This activity would consume α-kg, but the glutamate so formed would be easily oxidized again to α-kg, because of the high [NAD]/[NADH] ratio, switching the glutamate dehydrogenase equilibrium towards α-kg and NADH formation.

From these results we conclude that, in the hepatic tissue of normal rats, VPA primarily affects the metabolism of pyruvate and other substrates of the TCA cycle as citrate, α-Ketoglutarate and malate, resulting in a general hepatic metabolic disorder.

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Resumen

Se infunde ácido valproico (VPA) a ratas en ayuno de 24 h, en dosis de 4 mg/kg/min durante 50 min a través de la vena yugular. La arteria carótida derecha fue cateterizada para extraer muestras de sangre, determinándose la concentración de VPA y los parámetros ácido-base. Tras la infusión se tomó un trozo de tejido hepático y se congeló instantáneamente a la temperatura del nitrógeno líquido. El VPA no modificó los parámetros ácido-base arteriales. En el grupo infundido con VPA aumentó la concentración tisular de alanina y de piruvato, mientras que la de lactato no cambió. La concentración de glutamina, glutamato, malato, citrato y aspartato disminuyeron significativamente. Estos resultados sugieren que el VPA en bajas dosis puede afectar el metabolismo hepático de la rata *in vivo*.

Palabras clave: Acido valproico, Metabolismo hepático, Glutamina, Piruvato.

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