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SHORT COMMUNICATIONS

Effect of valproate infusion *in vivo* on renal metabolism in fasted rats

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Since the description of the antiepileptic properties of sodium valproate (VPA) [1], several secondary effects of acute or chronic exposition of human and/or experimental animals to this branched short-chain fatty acid have been reported [2–5]. Among them, it has been observed that VPA administration may induce systemic hyperammonemia [2].

Systemic hyperammonemia following VPA administration might result from several mechanisms including: (1) a reduced urea synthesis in the liver, (2) an accelerated renal glutamine uptake and ammoniagenesis or (3) a combination of these mechanisms. The first mechanism was described using isolated rat liver mitochondria: valproate inhibited hepatic ureagenesis through a depression of the mitochondrial synthesis of *N*-acetyl-glutamate [6]. The second mechanism may be also involved since renal glutamine utilization and the released ammonia into the renal vein was shown to be increased in man [4, 7], and in the rat [3], but not in the dog [8] following VPA administration. However, other organs, especially the liver, the intestine, the muscle, and the brain are also important for the overall ammonia metabolism and may be affected by VPA [4].

The present study was undertaken in an attempt to clarify the role of the kidney in the increased renal ammoniagenesis in rats receiving VPA. To achieve this goal, the renal utilization of glutamine and the total renal ammonia production by the left kidney were measured in fasted rats before and after systemic infusion of sodium valproate elevating the arterial concentration to the level observed during valproate therapy in man. The renal concentration of metabolites relevant to the renal ammoniagenic pathway were also measured in the renal cortex of intact rats with or without infusion of VPA. Our data demonstrate that, in fasted rats, VPA changes modestly the rate of renal glutamine utilization and ammonia production *in vivo*.

Materials and methods

Twenty-four normal male Sprague–Dawley rats weighing 373 ± 9 g (control, N = 8), 382 ± 6 g (low VPA, N = 9), and 380 ± 7 g (high VPA, N = 7) were used. The rats were fed with the same commercial diet, and were fasted for 24 hr before starting the experiment (free access to water). Following pentobarbital anesthesia (60 mg/kg body weight) the trachea was cannulated. The rats were then allowed to breath spontaneously, except for the group infused, with VPA, that was ventilated with a mechanical respirator. The left jugular vein was then catheterized with a PE 10 catheter filled with heparinized saline. A solution composed of 0.9% NaCl containing adequate amounts of [^{14}C]-inulin and [^{3}H]-*p*-aminohippuric acid was continuously administered at 0.05 ml/min. The right carotid artery was also catheterized in order to draw arterial blood samples for the determination of blood metabolites and acid–base parameters. The abdominal cavity was opened, the bladder was cannulated and the left spermatic vein was ligated. A thin needle (gauge 25) was inserted in the left renal vein. The whole surgical procedure took less than 30 min. The temperature of the animal was monitored (rectal thermometer) and maintained around 38° with a heating lamp. The urine was collected under mineral oil in pre-weighed vials. Blood samples (1.5–2 ml) were drawn from the carotid artery and the left renal vein. The losses in blood volume were immediately replaced by equivalent volumes of arterial blood obtained from a matched normal fasted rat (control situation) or from a rat receiving a similar VPA load (experimental situation).

Blood samples were immediately mixed with ice-cold 10% w/v perchloric acid in preweighed tubes and centrifuged. The perchloric extract was used for the enzymatic determination of metabolites. Additional blood samples (total amount 150 μl) were obtained for hematocrit, blood pH, PCO_2, PO_2, as well as [^{14}C]-inulin and [^{3}H]-*p*-aminohippurate (PAH) determinations.

After the surgery, each rat received a bolus injection of isotonic saline equal to 1% of body weight. Then, isotonic saline (control group) or VPA [(4 mg/kg/min; low VPA infusion) or (8 mg/kg/min; high VPA infusion)] diluted in isotonic saline was infused through the jugular catheter at the rate of 3 ml/hr. After a 15 min equilibration period, two 20 min urine collections bracketed by two arterial and renal vein samples were obtained. The two kidneys were then pulled free of the vessels and immediately freeze-clamped according to the method of Wollenberger *et al.* [9]. The frozen tissues were pulverized and extracted with perchloric acid as previously described [10, 11]. Determination of relevant metabolites were performed on a neutralized aliquot of this extract.

Blood pH, PCO_2 and PO_2 were determined using a pH-Blood/Analyzer-Corning 168 (Corning Medical, Corning Glass Works, Medfield, MA, USA). Hematocrit was measured using 70 μl heparinized capillaries. Twenty μl plasma samples were placed (in duplicate) in scintillation vials containing 10 ml of scintillation cocktail and [^{14}C]-inulin and [^{3}H]-*p*-aminohippuric acid were counted with a Beckman-LS2800 Scintillation Spectrometer (Beckman Instruments, Inc., Nuclear System Operation, Irvine, CA, U.S.A.) and identified by the internal standard procedure. Glomerular filtration rate was calculated from the clearance of [^{14}C]-inulin. Renal blood flow was calculated from the clearance and renal extraction [^{3}H]-PAH and the hematocrit. The methods for determinations of glutamine, glutamate, α-ketoglutarate, malate, citrate, lactate, pyruvate, alanine, asparate, NH_4^+, and ATP are the same as previously described [12].

All results are expressed as the means ± SE. Statistical differences were determined by analysis of variances, followed by multiple mean comparison test (Scheffé test), choosing an α value of 0.05 as significant.

Results and discussion

The present study performed in fasted rats with normal acid base status (Table 1) shows that a modest VPA load (4 mg/kg/min, i.e. 200 mg in 50 min) does not increase glutamine utilization and ammonia production by the kidney. Only a higher load (8 mg/kg/min, i.e. 400 mg in 50 min) increases these parameters. These results presented in Table 2 are in good accordance with the data obtained in dogs [8] but disagree with some findings in man [4, 7] and in normal rats [3, 13]. These differences may be attributable to (1) the lack of measurement of total ammonia production (total release of NH_4^+ in urine + NH_4^+ in the renal vein) by the kidney in these reports; (2) the occur-

Table 1. Effect of valproate on blood and urine parameters in normal and VPA-infused rats

	Control N = 8	Low-VPA N = 9	High-VPA N = 7
Blood			
pH	7.40 ± 0.01	7.43 ± 0.01*	7.39 ± 0.01
PCO_2 mm Hg	35.3 ± 1.3	35.7 ± 1.6	41.8 ± 1.1*
PO_2 mm Hg	83.6 ± 3.8	80.3 ± 4.8	74.0 ± 4.0
HCO_3^- mM	21.9 ± 0.9	23.8 ± 0.9	25.3 ± 0.5*
Lactate mM	0.62 ± 0.10	1.71 ± 0.38*	1.47 ± 0.15*
Pyruvate mM	0.07 ± 0.01	0.17 ± 0.03*	0.11 ± 0.02*
Glutamine mM	0.56 ± 0.02	0.54 ± 0.02	0.46 ± 0.02*
Glutamate mM	0.19 ± 0.01	0.17 ± 0.01	0.14 ± 0.02*
NH_4^+ mM	0.13 ± 0.03	0.13 ± 0.01	0.15 ± 0.01
Valproate mM	0	0.6 ± 0.1	2.0 ± 0.2
Urine (One kidney)			
Flow ml/min	0.009 ± 0.002	0.016 ± 0.004	0.008 ± 0.002
$UNH_4^+ \cdot V$ (μmol/min)	0.58 ± 0.15	0.70 ± 0.24	0.38 ± 0.09
ULactate (nmol/min)	0	10 ± 5*	11 ± 4*
GFR, ml/min	1.52 ± 0.48	1.38 ± 0.14	0.89 ± 0.14
RPF, ml/min	4.12 ± 1.14	4.62 ± 0.76	3.03 ± 0.95
RBF, ml/min	7.48 ± 1.98	8.43 ± 1.39	5.49 ± 1.55

Values are mean ± SE. Low VPA = 4 mg/kg/min; High VPA = 8 mg/kg/min for 50 min.

* $P < 0.05$ vs control group.

Table 2. Effect of valproate infusion on extraction (−) or production (+) of metabolites by the rat kidney *in vivo*

	Control N = 8	Low-VPA N = 9	High-VPA N = 7
Total NH^+	+0.86 ± 0.22	+1.22 ± 0.21	+1.63 ± 0.27*
Glutamine	−0.35 ± 0.12	−0.51 ± 0.09	−0.96 ± 0.13*
Glutamate	−0.04 ± 0.03	+0.07 ± 0.04*	+0.12 ± 0.09*
Lactate	−0.72 ± 0.21	−1.26 ± 0.68	−0.90 ± 0.32
Pyruvate	−0.15 ± 0.05	−0.06 ± 0.1	+0.07 ± 0.04*

These data correspond to the left kidney and are expressed in μmol/min. Values are means ± SE.

* $P < 0.05$ vs control group. No differences between experimental groups were observed.

rence of respiratory acidosis following VPA administration [3]; (3) to differences on the doses of VPA administered as well as in the route of administration. For instance, intraperitoneal administration of VPA to rats gives a peak plasma concentration greater than the value found in humans during VPA therapy [3] but it does not provide a steady-state delivery of VPA to the kidney.

These modest effects of VPA on renal ammoniagenesis *in vivo* contrast with the reports by Gougoux *et al.* [14] and Doval *et al.* [15] of a dose-dependent increment of ammonia production induced by VPA in isolated dog or rat cortical tubules *in vitro*. The availability *in vivo* of alternative substrates displacing the oxidation of VPA, including fatty acids bound to circulating albumin, may explain these differences.

In agreement with the stimulation of the ammoniagenic flux observed at higher VPA concentrations, the renal tissue concentration of glutamine, glutamate and α-ketoglutarate were reduced by VPA, as it is shown in Table 3. This suggest that the increased ammonia production occurs through the usual metabolic pathway of glutamine utilization in the kidney. In contrast, the concentration of pyruvate was simultaneously increased, suggesting a reduced oxidation of this compound. However, Table 3 shows that lactate concentration in the whole kidney was not significantly changed. Systemic blood lactate and pyruvate concentrations were nevertheless increased, indicating a reduced utilization of these metabolites at extrarenal sites as shown in Table 1.

The inhibition of pyruvate metabolism may well be the primum movens explaining the ammoniagenic effect of VPA, and may be related to several synergistic mechanisms. First, VPA has been shown to interfere with the transporter for the lactate/pyruvate in the brush border membrane and therefore with the entry of lactate in proximal tubules [8]. Second, VPA is known to inhibit the pyruvate mitochondrial carrier [16]. Third, as reported by Turnbull *et al.* [17], VPA might also directly inhibit pyruvate (and fatty acids) oxidation in a dose-dependent manner. Benavides *et al.* [16] have reported that valproate can block the pyruvate dehydrogenase (PDH) complex. Indeed, it was previously shown that the metabolism of valproic acid produced various metabolites structurally related to 4-pentanoate [18] which could impair in this

Table 3. Tissue metabolite profile of kidneys of normal and VPA-infused rats

	Control N = 8	Low-VPA N = 9	High-VPA N = 7
Glutamine	1.27 ± 0.81	1.14 ± 0.11	0.63 ± 0.07*
Glutamate	3.63 ± 0.35	3.37 ± 0.18	2.08 ± 0.11*
α-KG	0.14 ± 0.02	0.13 ± 0.02	0.08 ± 0.01*
Malate	0.27 ± 0.02	0.18 ± 0.02*	0.14 ± 0.01*
Oxaloacetate (LDH)	2.12	3.05	2.52
Alanine	0.53 ± 0.03	0.64 ± 0.06	0.57 ± 0.04
Aspartate	1.04 ± 0.06	1.09 ± 0.07	0.64 ± 0.06*
Citrate	0.30 ± 0.04	0.18 ± 0.02*	0.20 ± 0.02*
Lactate	1.47 ± 0.27	1.42 ± 0.41	0.96 ± 0.15
Pyruvate	0.05 ± 0.004	0.10 ± 0.007*	0.07 ± 0.006*
ATP	1.09 ± 0.16	1.44 ± 0.13	1.15 ± 0.09

Values are means ± SE. All data are expressed as μmol/g wet weight except for oxaloacetate (calculated value) expressed in nmol/g wet weight.
* $P < 0.05$ vs control group.

fashion pyruvate utilization and oxidative phosphorylation [19]. A marked inhibition of pyruvate oxidation was demonstrated *in vitro* with kidney tubules from the dog by Gougoux *et al.* [14]. This inhibitory effect can be exaggerated by concomitant accumulation of valproyl-CoA in the tissue with secondary sequestration of free Coenzyme-A. Such an effect of VPA was also shown to occur in the liver of the mice by Thurston *et al.* [20].

If we assume that VPA inhibits the entry of pyruvate carbons into the Krebs cycle, the resulting NADH production will be decreased. This reduction in the flux of carbons entering through the PDH system in the Krebs cycle will be accompanied by a reciprocal, and partially compensatory, rise in the flux of NADH producing steps in the other part of the cycle, i.e. stimulation of the alpha-ketoglutarate dehydrogenase, and secondarily glutamate dehydrogenase, and glutaminase activities. This would directly stimulate the renal ammonia production together with the renal venous release of the skeletal carbon of glutamine under the form of an organic acid (since the local oxidation is reduced by PDH inhibition).

Valproic acid is a short chain fatty acid, and as such is able to affect mitochondrial functions and to impair ammonia production by the kidney [20]. In contrast with these observations, the renal ammonia production is increased and not reduced following administration of rather high dose of VPA. VPA thus affects ammoniagenesis in a specific and novel manner.

In conclusion, the present study demonstrates that a significant renal ammoniagenic response to VPA administration can be observed in the fasted rat *in vivo* only when large doses of VPA are administered. Our study confirms that VPA may lead to hyperammonemia in part through a renal effect.

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Induction of UDP-glucuronosyltransferase isozymes in male and female rat liver microsomes by an isoquinoleine derivative (52028 RP)*

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52028 RP, 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide, previously named PK 11195, is an antagonist of the "peripheral type" benzodiazepine binding sites [1], which presents several pharmacological properties on the cardiovascular [2], central [3] and immune systems [4]. 52028 RP was considered as a new model compound to investigate the physiological relevance of "peripheral type" benzodiazepine binding sites [5]. It has been tentatively used as a potential tranquilizer and anticonvulsant in man. Clinical trials and pharmacokinetic studies showed that 52028 RP was metabolized in rat and man very rapidly, and that the rate of metabolism increased upon repeated administration of the drug (A. Uzan, personal communication). This observation led us to the hypothesis that 52028 RP could induce the enzymes involved in drug biotransformation, especially the cytochromes P-450 and the corresponding monooxygenases and UDP-glucuronosyltransferases (UDPGT, EC 2.4.1.17). Indeed, we recently reported in rat that 52028 RP was a potent inducer of different cytochrome P-450 isoenzymes, P-450b (IIB1), P-450p (IIIA1) and P-450j (IIE1), with a different response according to the sex [6].

Concomitant induction of cytochrome P-450 and UDPGT is known to occur upon treatment with various drugs. For example, we found induction of cytochrome P-452 (IVA1) was significantly correlated with that of bilirubin UDPGT, after administration to rats of clofibrate and structurally related compounds [7].

Glucuronidation reactions are selectively enhanced by various inducers such as 3-methylcholanthrene, phenobarbital and hypolipidemic drugs related to clofibrate [8, 9]. Differential induction can be used to relate enzyme activities to several UDPGT isozymes [10]. Indeed after purification by electrofocussing, up to eight or nine isoforms of UDPGT have been isolated in rat liver microsomes [11].

In order to detect which UDPGT isozymes are affected by 52028 RP, the possible inducing effect of the drug was measured in male and female rats. For this purpose the activity of UDPGT towards mono-hydroxylated substrates such as planar and non-planar phenols, monoterpenoid alcohols, steroid hormones, and bilirubin was determined in liver microsomes. The variations in the biosynthesis of the corresponding enzyme proteins were revealed by immunoblots using polyclonal specific antibodies raised against purified UDPGT isoforms from rat liver and kidney.

Materials and methods

Animals. Male and female Sprague–Dawley rats (180–200 g) were obtained from Iffa-Credo (St Germain/l'Abresle, France); they had free access to food (U.A.R., Villemoisson, France) and tap water.

Treatment. 52028 RP (Rhône-Poulenc Santé, Centre de Recherches de Gennevilliers, France) was suspended in a sucrose solution (700 g/l) and then given at a daily dose of 500 mg/kg body wt for 5 days, by gavage to groups of five male and five female rats. For the immunoblot experiments, hepatic microsomes from male and female rats treated with phenobarbital were also used [6]. Control group received the vehicle, only. Microsomal fractions were prepared as previously described [12] and stored as aliquots at −80° until use.

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